Responsiveness of β-Adrenoceptors to Some Catecholamines in Chronic Cocaine Treated Guinea-Pig : A Study in Atria and Trachea

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ชื่อวิทยานิพนธ์	การตอบสนองของตัวรับอะดรีเนอร์จิคเบต้า ต่อยาแคทีโคลามีนบางตัว ในหนูตะเภาที่ได้รับโคเคนติดต่อกันเป็นเวลานาน: การศึกษาในเอเตรียและหลอดลม
ผู้เขียน	นางสาวพจนา ช่วยกูล
สาขาวิชา	เภสัชวิทยา
ปีการศึกษา	2541

บทคัดย่อ

การวิจัยครั้งนี้เป็นการศึกษาการตอบสนองของตัวรับอะดรีเนอร์จิคเบต้าต่อยาในกลุ่มแคทีโคลามีน 2 ตัว คือ norepinephrine (NE) และ isoproterenol (ISO) ในหนูตะเภาที่ได้รับโคเคนติดต่อกันเป็นเวลานาน โดยใช้หัวใจส่วนเอเตรียและหลอดลมที่แยกออกจากร่างกายของหนูตะเภา (*in vitro* study) และทำการวิเคราะห์หาความเข้มข้นของโคเคนในพลาสมาและเนื้อเยื่อหัวใจส่วนเอเตรียและเวนตริเคิล ด้วยวิธี High Performance Liquid Chromatography (HPLC) เพื่อนำมาหาความสัมพันธ์ระหว่างการตอบสนองต่อแคทีโคลามีน และความเข้มข้นของโคเคนในพลาสมาและเนื้อเยื่อหัวใจดังกล่าว

ในการทดลองหนูตะเภากลุ่มควบคุมได้รับ 0.9% w/v NaCl ในขนาด 1 มล/กก ส่วนหนูตะเภาอีก 3 กลุ่ม ได้รับโคเคนไฮโดรคลอไรด์ ในขนาด 1, 2.5 หรือ 5 มก/กก โดยวิธีฉีดเข้าทางช่องท้อง วันละ 2 ครั้ง เป็นเวลา 14 วัน หลังจากนั้นสัตว์ทดลองถูกฆ่าที่เวลา 1, 24 หรือ 72 ชม หลังหยุดให้โคเคน เพื่อแยกหัวใจส่วนเอเตรียและหลอดลมมาศึกษาการตอบสนองต่อ NE และ ISO บันทึกผลการเปลี่ยนแปลงของอัตราเร็วและความแรงในการหดตัวของเอเตรีย และการคลายตัวของกล้ามเนื้อหลอดลมที่ถูกชักนำให้หดตัวด้วย carbachol (1 มก/มล) จากนั้นสร้างกราฟแสดงความสัมพันธ์ระหว่างความเข้มข้นของยาแคทีโคลามีน กับการตอบสนองของเอเตรียและหลอดลม และหาค่า $[D]_{max50}$ (ความเข้มข้นที่ทำให้เกิดการตอบสนอง 50% ของการตอบสนองสูงสุด และ ค่า pD_2 (ค่า

ลบล็อค $[D]_{max50}$) ของยาทั้งสองตัว เพื่อนำมาเปรียบเทียบการตอบสนองของเอเตรียและหลอดลม ต่อ NE และ ISO ของหนูกลุ่มที่ได้รับโคเคนและกลุ่มควบคุม

จากการทดลองพบว่า ค่า pD_2 ของ NE และ ISO ทั้งในเอเตรียและหลอดลม สูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ ซึ่งแสดงให้เห็นว่าเกิดการตอบสนองไวเกินในกลุ่มหนูตะเภาที่ได้รับโคเคน โดยที่ความแรงในการตอบสนองไวเกินในเอเตรีย ต่อ NE จะมากกว่า ISO อย่างมีนัยสำคัญ ($p < 0.05$) แต่ในหลอดลมไม่พบความแตกต่างดังกล่าว นอกจากนี้ ยังพบว่า เอเตรียมีความแรงในการตอบสนองไวเกินต่อทั้ง NE และ ISO มากกว่าหลอดลม ดังนั้นอาจเป็นไปได้ว่าความแตกต่างในการตอบสนองไวเกินที่เกิดจากโคเคนระหว่างในเอเตรียและหลอดลมอาจเกิดเนื่องจากในเนื้อเยื่อทั้งสองมีตัวรับอะดรีเนอร์จิคเบต้าต่างชนิดกัน โดยตัวรับส่วนใหญ่ในเอเตรียเป็นชนิด β_1 และในหลอดลมเป็นชนิด β_2

นอกจากนี้ จากการเจาะเลือดและแยกหัวใจส่วนเอเตรียและเวนตริเคิลหลังหยุดให้โคเคนที่ 1, 24 และ 72 ชม เพื่อนำมาหาระดับความเข้มข้นของโคเคนที่เวลาต่างๆกัน พบว่ามีความสัมพันธ์กับการตอบสนองของเอเตรียดังนี้คือ หลังหยุดให้โคเคน 1 ชม ค่า pD_2 มีความสัมพันธ์โดยตรงกับความเข้มข้นของโคเคนในพลาสมาและเนื้อเยื่อหัวใจ ในขณะที่หลังหยุดโคเคน 24 และ72 ชม ค่า pD_2 ยังคงสูงกว่ากลุ่มควบคุม แต่ความเข้มข้นของโคเคนในพลาสมาและเนื้อเยื่อหัวใจลดลงที่ 24 ชม และไม่สามารถหาค่าได้ที่ 72 ชม

ฤทธิ์ของโคเคนในการยับยั้งกระบวนการ reuptake ของแคทีโคลามีนเข้าสู่ปลายประสาทซิมพาเธติค ไม่สามารถอธิบายการตอบสนองไวเกินที่กระตุ้นโดยโคเคนจากการทดลองนี้ได้ เพราะระดับของโคเคนในพลาสมาและเนื้อเยื่อหัวใจหลังหยุดให้โคเคนในหนูตะเภาทุกกลุ่ม ต่ำกว่าความเข้มข้นต่ำสุดของโคเคนที่สามารถยับยั้งกระบวนการ reuptake ของ NE ที่ได้มีผู้ทำการศึกษามาก่อนหน้า (ยกเว้นหลังหยุดโคเคน 1 ชม ในหนูตะเภาที่ได้รับโคเคนขนาด 5 มก/กก) นอกจากนี้ ยังไม่สามารถอธิบายการตอบสนองไวเกินของเอเตรียและหลอดลมต่อ ISO ซึ่งถูกนำกลับเข้าสู่ปลายประสาทซิมพาเธติคได้น้อยมาก และยังไม่สามารถอธิบายการเกิดการตอบสนองไวเกินในหลอดลม ซึ่งมีประสาทซิมพาเธติคมาเลี้ยงน้อยมาก ดังนั้น การตอบสนองไวเกินที่เกิดจากโคเคนอาจเกิดจากกลไกอื่นๆ เช่น การเพิ่มจำนวน หรือการเปลี่ยนแปลงโครงสร้างของตัวรับอะ

ดรีเนอร์จิคเบต้า หรือมีการรบกวน G protein และ/หรือระบบ adenyl cyclase ซึ่งเกี่ยวข้องกับการถ่ายทอดสัญญานภายในเซลล์

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ABSTRACT

The present study aimed to investigate the responsiveness of β-adrenoceptors to two catecholamines, norepinephrine (NE) and isoproterenol (ISO) in chronic cocaine treated guinea-pig using the isolated atria and trachea. The concentrations of cocaine in plasma, atrial and ventricular tissues were measured using High Performance Liquid Chromatography (HPLC) technique. The correlation between the responsiveness and cocaine concentrations in plasma, atrial and ventricular tissues was also performed. Guinea-pigs in control group received 1 ml/kg of 0.9% w/v NaCl and the three treated groups received cocaine HCL 1, 1.25 or 5 mg/kg, i.p., b.i.d. for 14 days. After cessation of cocaine, the animals were killed at 1, 24 or 72 hr. The atria and trachea were isolated for studying of their responses to NE and ISO. The change in rate and contractile force of atria and relaxation of trachea, which induced to contract by 1 μg/ml carbachol, was recorded. The concentration-response curves were then constructed, $[D]_{max50}$ (concentration which produced 50% of maximum responses) and pD_2 (-log $[D]_{max50}$) of both drugs were

determined. These values were used to compare the responsiveness of the atria and trachea to NE and ISO between the cocaine-treated and saline-treated groups.

The results showed that pD_2 values of NE and ISO in both atria and trachea of cocaine-treated groups were significant higher than those of control groups, which indicated that the supersensitivity occurred in the former groups. In atria, the degree of supersensitivity to NE was significant higher than that to ISO (p <0.05), but this significant difference was not observed in trachea. In addition, the degree of supersensitivity to both NE and ISO in atria was higher than those in trachea. Therefore, one possibility that might account for the differences in the cocaine-induced supersensitivity between atria and trachea was the distinction in the β-adrenoceptor subtypes containing in the atria and trachea, mainly β_1 and β_2-subtypes, respectively.

In addition, blood samples were collected and the atrial and ventricular tissues were isolated for analysis of cocaine concentration at 1, 24 and 72 hr after the cessation of cocaine. The cocaine concentrations were correlated with the responsiveness as follows: at 1 hr after cocaine cessation, the $[D]_{max50}$ ratio directly correlated with cocaine concentrations in plasma, atrial and ventricular tissues; at 24 and 72 hr of the cessation, the $[D]_{max50}$ ratio values were still higher than those of control groups, but the cocaine concentrations in both plasma and cardiac tissues declined after 24 of the cessation and could not be detected at 72 hr after cessation of cocaine.

The catecholamine uptake blocking action of cocaine could not describe the mechanism of cocaine-induced supersensitivity in this experiment because

the level of cocaine in plasma after cessation of all cocaine treated groups, except at 1 hr after cocaine cessation, was lower than the minimum effective concentration of cocaine that caused neuronal uptake blockade of NE according to previous studies of Trendelenburges *et al.* (1972) and Reiffenstein and Triggle (1974). This uptake blockade action still could not explain the supersensitivity to ISO, which is much less taken up to the nerve ending. Moreover, this uptake blockade mechanism could not describe the supersensitivity in trachea, which is almost entirely not innervated by adrenergic nerves. Therefore, other mechanisms e.g. increase in number, conformational changes of β-adrenoceptors or interference with G protein and/or adenyl cyclase system involved in the cellular signal transduction might involve in the supersensitivity induced by cocaine.

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This thesis is dedicated to my parents for their great love, kindness and patience.

Pojjana Chouykool

CONTENTS

LIST OF ABBREVIATIONS

b.i.d.	=	twice a day
°C	=	degree celsius
Cmax	=	maximum concentration
CNS	=	central nervous system
cocaine HCL	=	cocaine hydrochloride
Cont.	=	Continued
CV	=	coefficient of variance
$[D]_{max50}$	=	the molar concentration of agonist producing response which is 50% of maximum response
EC_{50}	=	effective concentration at 50% of maximum response
e.g.	=	exampli garatia (for example)
g	=	gram
kg	=	kilogram
mg	=	milligram
HPLC	=	High Performance Liquid Chromatography
hr	=	hour
i.p.	=	intraperitoneally
ISO	=	isoproterenol
l	=	liter
i.v.	=	intravenously
M	=	molar

ml	=	milliliter
min	=	minute
mM	=	millimolar
μM	=	micromolar
%	=	percent
NaCl	=	sodium chloride
n	=	number
NE	=	norepinephrine
ng	=	nanogram
nm	=	nanometer
p	=	*p* value
pD_2	=	negative logarithm of the molar concentration of agonist producing response which is a 50% of maximum response
r	=	correlation coefficient
rpm	=	round per minute
s.c.	=	subcutaneously
S.E.	=	standard error
Tmax	=	time to reach maximum concentration
UV	=	ultraviolet
w/v	=	weight/volume

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CHAPTER 1

INTRODUCTION

Long known as the popular drug which has sympathetic stimulant actions, cocaine is the principal alkaloid extracted from the leaves of *Erythroxylon coca*, a plant that cultivated abundantly in the Andean highlands and northwestern parts of the Amazon in South America. For long over a millennium, the small, elliptical leaves of the indigenous coca shrub had been in demand by the Andean Indian who chew them as a drug against cold and lassitude, a stimulation of general feeling of well-being and a prevention of hunger (Liljestrand, 1971). The ancient Indian legends described the origin and supernatural power of coca leaves as "a gift of the Sun God" and its use was especially served for ceremonial or religious purpose (see reviewed by Johanson and Fischman, 1989). Since its active constituent was chemically isolated by Albert Niemann in 1860, cocaine use has been escalated in Europe. One of the most distinguished psychoanalysts, Sigmund Freud used cocaine to cure his addictive patients, but soon he found that his patients added a dependence of cocaine. To prove the effect of cocaine, he used himself, a trained observer, as a subject. His data were published in 1855 in "A contribution to knowledge of the effect of cocaine". This report identified cocaine as both a central nervous system stimulant and a euphoriant drug (reviewed by Johanson and Fischman, 1989).

One of Freud's colleagues, Karl Koller, in 1884, demonstrated that the local applications of cocaine caused complete insensitiveness of the cornea, which lasted for ten minutes without disturbing the normal function of the eye. This local anesthetic effect of cocaine was valuable in the treatment of phlyctenular conjunctivitis with eruptions and ulcers of the cornea, as well as in the prevention for a few hour of pain from cauterization of eyelids with silver nitrate. He also mentioned that cocaine had been used with succession in several operations, such as removal of foreign bodies from the cornea and even in iridectomy and extraction of cataract without the experiencing of any pain (Liljestrand, 1971).

In 1923, Richard Willstater was the first to elucidate the structure of cocaine and accomplish its synthesis (reviewed by Jatlow, 1987).

Although its advantage as local anesthetics had been suggested, adverse effects of chronic use of cocaine were soon become worsen. Growing concern about its addiction and toxicity was one of the legislation of severe antidrug law. However, the widespread of its use continues to be one of the public problems in economics, social and health care systems (reviewed by Mouhaffel *et al.*, 1995).

Economic and Social Council of United Nation, in 1980, reported that the expansion of cocaine addiction had been increasing in North and South America and Europe. There was an increment of cocaine use in the Middle, East and Oceanea as well, but on a much smaller amount. Furthermore, the extensive use of cocaine among adolescent and young adults has rising mostly and has become a critical problem now.

In Thailand, there is no official report about cocaine users, but it is best known that cocaine is one of the most popular narcotic substances used in high societies and adolescent.

In the past, cocaine was so expensive, which it was called "the champagne of drugs". The high cost of cocaine served as a barrier to its widespread. Nowadays, cocaine not merely has become less expensive but increase in its availability and purity as well. Moreover, the misconception of cocaine as a benign, non-addicting substance, is widespread and as a result, cocaine has become one of the most popular abuse drugs (see reviewed by Cregler and Mark, 1986).

Cocaine is a substance with action in both central and peripheral nervous systems. It is a local anesthetic, a sympathomimetic agent and a strong stimulant of central nervous system (reviewed by Jatlow, 1987).

Cocaine as a local anesthetic produces conduction block without depolarizing the nerve membrane at low concentration. It diffuses through the membrane in its less prevalent non ionized form; once inside the cell, it is protonated to an ionized form, and then binding to the receptor sites on the surface of the membrane (see reviewed by Kloss *et al.*, 1984; Catterall and Mackie, 1996).

The subjective effects of cocaine as a psychostimulant, like those of all centrally active drugs, depend on the user, environment, dose and the route of administration. Cocaine users described the euphoric results of its use associated with irritability, dysphoria, depression and physical discomfort (O'Brien, 1996).

In the peripheral nervous system, the consequence of cocaine use is ordinary a combination of its local anesthetic and sympathomimetic actions. Cocaine increases the response of the adrenergically innervated organs such as heart, blood vessels, smooth muscle, etc. (reviewed by Kloss *et al.*, 1984).

Related to its pharmacological effects, cocaine induces various abnormalities and mortality in the experimental animals and humans. Drug Abuse Warning Network in the United States reported in 1990 about 79,398 emergency patients who had previous experience of cocaine. The most common complaint from patients suffering from acute and chronic cocaine-associated medical problems was concerned to the cardiovascular systems (Brody *et al.*, 1990). The cardiac responses to cocaine use are extremely complicated. The adrenergic activities are enhanced resulting in the increment of myocardial contractility, pacemaker activity and impulse conduction (reviewed by Mouhaffel *et al.*, 1995). These adverse effects cause many cardiovascular abnormalities, such as cardiac arrhythmia, myocardial ischemia, myocardial infarction and coronary thrombosis. However, the mechanism of its actions remains largely unresolved.

Two hypotheses have been proposed for the mode of action of cocaine enhancing supersensitivity of adrenergic innervated organs in response to norepinephrine and other catecholamines. Firstly, cocaine blocks the uptake process of norepinephrine at the presynaptic nerve terminals, increasing concentration and prolonging duration of action of this monoamine at the synaptic cleft (Trendelenburg *et al.*, 1972; Masuda *et al.*, 1980; Surprenant and Williams, 1986; Jain *et al.*, 1990). Secondly, cocaine may directly act at the

postsynaptic effectors (Kalsner and Nickerson, 1969; Shibata *et al.*, 1971; Alburges *et al.*, 1996).

According to the suggestion of Trendelenburg and colleagues (1972), the sensitizing effect of low concentration of cocaine, which has little effect on neuronal uptake may indicate some postsynaptic effects. Therefore, the purposes of the present study are to investigate the effect of low doses of chronic cocaine treatment on the responsiveness of β-adrenoceptors to the exogenous catecholamines, norepinephrine and isoproterenol, in the guinea-pig isolated atria and trachea, and to determine the concentrations of cocaine in plasma and cardiac tissues using HPLC method. The correlation between the response and the concentrations of cocaine in plasma and cardiac tissues is also performed. The results from this study may lead to more understanding of the actions of cocaine-induced supersensitivity in β-adrenoceptors and the mechanism of its related cardiotoxicity.

CHAPTER 2

REVIEW LITERATURE

COCAINE

Chemical and Physical Properties of Cocaine

Cocaine or benzoylmethylecgonine is an ester of benzoic acid and a nitrogen-containing base usually presented in a hydrochloride salt. The chemical structure of cocaine is shown in Figure 1.

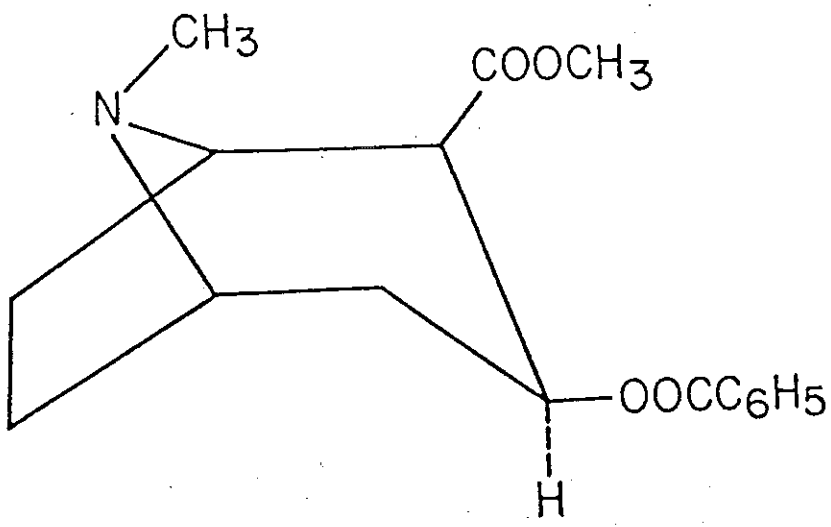


Figure1 The chemical structure of cocaine

Cocaine hydrochloride is prepared by dissolving the cocaine alkaloid in hydrochloric acid to form a water soluble salt, sold as a form of crystals, granules and powder, slightly bitter and numbs the tongue and lips. Cocaine hydrochloride decomposes on heating and melts at 195 °C. Its molecular weight is about 339.81. Another commercial cocaine, cocaine alkaloid or free base is the form smoked. It is soluble in alcohol, acetone, oil and ether but is almost insoluble in water. This form of cocaine is a colorless, odorless, transparent crystalline substance, which is not destroyed by heating. Cocaine base melts at 98 °C and vaporizes at higher temperature. Its molecular weight is bout 303.36 and aqueous solution of the substance is alkaline (see reviewed by Cregler and Mark, 1986).

Cocaine once was a popular constituent of stimulant, tonic, and sodas including Coca-Cola during the turn of the century. The potential of its abuse was recognized fairly and its sale was prohibited by The Harrison Narcotic Act in 1914 (reviewed by Johanson and Fischman, 1989).

Pharmacokinetic properties

Cocaine can be administered into the body by several routes such as sublingual, intravaginal, rectal, intramuscular, intravenous and respiratory routes. It is absorbed from all body mucous membrane, including nose, lung and gastrointestinal tract. Onset, duration of action, level of drug and its half life range from a few second to many hours depending on the route of administration and dose of drug taken (see reviewed by Mouhaffel *et al.*, 1995).

Route of administration and absorption

1. Oral route

After an oral administration of cocaine hydrochloride with a lag phase of about 30 minutes, absorption is rapid, reaching peak plasma concentration in about 60 minutes (see reviewed by Jatlow, 1987).

2. Nasal route

One of the most common nonmedical methods for administration of cocaine is the nasal insufflation (snorting) or inhalation as the white powder. The conversion of cocaine to the un-ionized free base at an alkaline pH facilitates its absorption across mucous nasal membrane. Absorption of cocaine by this route begins almost instantaneously but peak plasma level occurs approximately 30-60 minutes subsequent to inhalation. When cocaine is taken by the intranasal route, its absorption is probably limited by causing constriction of the nasal mucous membranes (see reviewed by Johanson and Fischman, 1989). Systemic bioavailability by this route has been estimated to be 20% to 60%, based on comparison of area under the time versus concentration curves for the nasal versus the intravenous route (reviewed by Jatlow, 1987). Administration of cocaine by nasal inhalation may lead to irritation of the nasal mucosa, sinusitis, and perforated septum (Bulster, 1994).

3. Pulmonary route

The increasing popularity of cocaine free base has been recognized in recent years. Free base is separated by adding a strong base (e.g. buffered ammonia) to an aqueous solution of cocaine hydrochloride and then extracting the alkaline precipitate or free base. This substance is much more volatile than

the hydrochloride salt and can be mixed into a cigarette and smoked or heated in a water pipe (Weiss *et al.*, 1981). Smoking free base provides effective respiratory absorption of cocaine and a rapid increase in its plasma concentration (reviewed by Cregler and Mark, 1986). The inhalation of cocaine free base may directly damage pulmonary gas exchange surface. The potent vasoconstrictor property of cocaine may cause an effect on the pulmonary vasculature (Weiss *et al.*, 1981).

4. Intravenous route

Administration of cocaine by the intravenous route produces nearly instantaneous access by the systemic circulation and brain with subjective and psychological effects similar to those produced by intranasal cocaine use (see reviewed by Jatlow, 1987). When cocaine is taken intravenously, its onset of action increase quickly, with an initial considerable result or intense "rush" reported within 1 or 2 minutes after administration (reviewed by Johanson and Fischman, 1989).

Doses, plasma concentration and effects of cocaine

The intense pharmacological effects of cocaine may be related to its pharmacokinetic properties including the route of administration, plasma concentration, metabolism and excretion, and other factors such as doses of administration and disease state. Rate of increase may be more important than the absolute peak plasma concentration. The rapid increase in plasma concentration appears to be associated with a greater response (Zahler *et al.*, 1982). Consequently, the effects of cocaine appear to be a function of route

and rate of administration as well as total doses (see reviewed by Jatlow, 1987). But there are controversial reports whether the effects and plasma concentration of cocaine will depend on the route of administration. Javaid *et al.* (1978) demonstrated that there was a correlation between the change in subjective and cardiovascular effects and plasma concentration of cocaine. In contrast, Van Dyck *et al.* (1976) showed that both the psychological and physiological effects did not correlate with cocaine blood level.

Resnick *et al.* (1976) determined the acute systemic effects of cocaine in 19 healthy volunteer subjects who regularly administered cocaine by themselves. Cocaine 10, 25 mg/kg, or placebo was administered intranasally and intravenously. Heart rate and blood pressure increased after cocaine administration compared with those observed in placebo. The onset of these effects occurred within 2 minutes after the administration, peaked at 5-10 minutes and 15-20 minutes when given intravenously and intranasally, respectively. The subjective effects, such as "high", pleasantness and hunger, of a 10 mg/kg dose were not different from placebo, but a 25 mg/kg dose produced the significant changes. An initial euphoria followed by dysphoric effect was reported in 4 subjects at 20 to 30 minutes after the 25 mg/kg intravenous dose and in 2 subjects at 45 to 60 minutes after the 100 mg/kg intranasal dose. This study noted that the subjective and cardiovascular effects of cocaine related to doses of cocaine and the route of administration.

Van Dyck *et al.* (1976) measured plasma cocaine concentration in 6 patients undergoing cardiovascular surgery compared with those in 4 patients undergoing dental surgery. A 10% solution of cocaine hydrochloride (1.5

mg/kg) was applied topically to the nasal mucosa prior to nasal intubation. Plasma concentration of cocaine increased rapidly for 15 to 20 minutes, peaked at 15 to 60 minutes, and decreased gradually over the next 3 to 5 hours. Maximum plasma concentrations ranged from 120 to 474 ng/ml calculated as the free base. The maximum plasma concentration of the cardiovascular surgery patients was significantly greater than those of the dental surgery patients. The authors suggested that, in the patients who had abnormal cardiovascular functions with lower cardiac output, liver function might decrease and resulted in a slower rate of cocaine metabolism.

Javaid *et al.* (1978) determined plasma cocaine concentrations in 10 healthy volunteer subjects with previous histories of cocaine use. Cocaine hydrochloride was administered intravenously or intranasally. Plasma cocaine concentration after intravenous injection of a 16 and 32 mg doses ranged from 86 to 309 ng/ml and from 216 to 409 ng /ml, at 5 minutes after dosing, respectively. Plasma cocaine concentration after different intranasal doses increased rapidly for the first 20 to 30 minutes after inhalation, reaching peak level before 60 minutes and then decreased slowly over the next hours. The maximum plasma concentrations of 16, 24 and 64 mg intranasal cocaine doses were 53 ± 12, 115 ± 21 and 266 ± 34 ng/ml, respectively. In addition, subjects replied that their maximum "high" were 15 to 20 minutes after inhalation and returned to the pre-drug level within 60 to 90 minutes. After intravenous administration, the cardiovascular and subjective effects occurred almost immediately, while the plasma concentration was also maximum. The onset of cardiovascular change paralleled to the increase in cocaine plasma levels, with

peak value at approximately the same time. These results showed that there was a good relationship between cocaine plasma level and the change in heart rate and subjective effects.

Van Duck *et al.* (1978) studied the central effect and plasma concentration after oral and intranasal administration of cocaine (2 mg/kg) in 4 healthy subjects with cocaine use histories. The plasma level of cocaine after oral administration was not found until 30 minutes later and increased swiftly for the next 30 minutes. The peak plasma concentration ranged from 104 to 424 ng/ml, occurred at 50 to 90 minutes and decreased gradually over the next 4.5 to 5 hours. After intranasal application, cocaine was detected in plasma by 15 minutes, reached peak concentration at 60 to 120 minutes and then lessened slowly over the next 2 to 3 hours. Maximum plasma concentration of intranasal cocaine ranged from 61 to 408 ng/ml. After oral administration, measurable effect on the "high" scale occurred within 15 to 75 minutes and peaked at 45 to 90 minutes. Peak effects lasted for 60 minutes and then decreased over the next 4 hours. After intranasal application, measurable effects on the "high" scale were noted within 15 to 30 minutes, lasted for 60 minutes and then decreased over the next 2 to 3 hours. Peak "high" after oral administration was significantly greater than that after intranasal application. The results demonstrated that the central effect of cocaine depended on route of administration.

Wilkinson *et al.* (1980) measured plasma cocaine level in 7 healthy subjects using cocaine for recreational purpose. Cocaine was administered intranasally and orally. The peak plasma concentrations after intranasal cocaine

(0.19, 0.38, 0.75, 1.5 and 2 mg/kg) were 12.9 ± 2.2, 31.3 ± 3, 44.9 ± 6.8, 107.4 ± 14.2 and 170.4 ± 62.5 ng/ml, respectively. The peak plasma concentrations of oral cocaine (2 and 3 mg/kg) were 242 ± 67.3 and 190.6 ng/ml, respectively. The plasma level after intranasal cocaine peaked at a mean time of 57.6 ± 5.8 minutes for all doses. Cocaine could not be detected in plasma until 30 minutes after oral administration and peaked at 64 ± 6.7 minutes. The relative bioavailability that determined by the area under the concentration–time curve for the 2 mg/kg dose by the intranasal and the oral route was not different.

Perez-Reyes *et al.* (1982) investigated the subjective and cardiovascular effects of cocaine in 6 healthy subjects with previous experience of cocaine use. Free base cocaine (50 mg) was administered by smoking. The subjective "high" induced by smoking of cocaine free base was slightly greater than that induced by injection. Peak value of subjective 'high" reached more rapidly after intravenous injection, but higher levels were reached after smoke inhalation. The overall magnitude and duration of subjective "high" were also slightly larger after smoking, but the differences between the mean values after the intravenous and smoke inhalation routes were not significant. Ten minutes after the initial of drug exposure, there was a decrease in depression and fatigue factors, and elevation in the vigor/activity, tension/anxiety and stimulant factors of the profile mood of state (POMS). Thirty minutes after initial of drug exposures, the rating of all these factors failed to level approaching baseline values for both routes. There was a slightly greater acceleration of the heart

and systemic blood pressure when free base cocaine was smoked than those when it was intravenous injected.

Chow *et al.* (1985) investigated the plasma concentration of intravenous cocaine in 5 healthy subjects with histories of drug abuse. Intravenous injection of cocaine increased heart rate in all subjects. Heart rate was maximally accelerated within 5 to 15 minutes after the start of the 2 minutes injection and returned to baseline within 60 minutes.

Boni *et al.* (1991) determined the pharmacokinetic and pharmacological effects of cocaine by inhalation route in rats. Maximum plasma concentrations, which were obtained 45 seconds after each exposure time, were 95 ± 24 and 205 ± 58 ng/ml for the 1.5 and 5 minutes exposures, respectively. The maximum cocaine concentration in plasma emerged rapidly and decreased nearly a full order of magnitude within the first 30 minutes. Mean changes in heart rate and arterial blood pressure appeared to correlate temporally with the plasma cocaine concentration. An increase in the duration of exposure led to a general increase in both magnitude and duration of cardiovascular responses. Data suggested that the cardiovascular effects of cocaine depended upon the plasma concentration and doses of cocaine administration.

Distribution and tissue disposition

Cocaine has a greater affinity for tissue than plasma (Hansson, 1971; Devane *et al.*, 1989). It can distribute into most parts of the body including genital organs and placenta because it has high lipophilic property to promote rapid membrane penetration (reviewed by Cregler and Mark, 1986; Yazigi and

Polakoski, 1992). Cocaine can cross the blood-brain barrier freely. The peak level of cocaine in brain was achieved within 10 minutes and remained relatively high over the first hour and then decreased with an elimination rate such that the levels at 4 hours after administration were undetectable in most of the brain regions (Javaid and Davis, 1993). In the experimental animals and in human cases of drug overdose, the brain concentration of cocaine ranged between 4 and 10 times higher than the concentration in plasma when measured from 0.5 to 2 hours after administration (see reviewed by Karch, 1991). They have low affinity binding sites in peripheral organs such as heart, lung, gut and kidney, except in blood and liver (see reviewed by Karch, 1991). Hansson (1971) reported that cocaine concentrations in the kidney and spleen were about 4 times those in cardiac and skeletal muscle, 3 times that in the liver and 2 times those in fat and pancreas. Alburges *et al.* (1996) noted that after chronic administration, cocaine was not accumulated in certain areas of the brain, only its major inactive metabolite, ecgonine methyl ester, was observed to be accumulated. The volume of distribution of cocaine is about 1.96 - 2 l/kg and the biological half-life ranges from 0.5 to 1.4 hours (Chow *et al.*, 1985; reviewed by Jatlow, 1987).

Benuck *et al.* (1987) determined levels of cocaine and benzoylecgonine, its inactive metabolite, in plasma and brain of mice injected with cocaine 10 and 25 mg/kg, intraperitoneally. Cocaine concentration in the brain reached peak value within 5 minutes after the administration. There was a positive correlation between the concentration of cocaine in brain and plasma at time points between 5 and 60 minutes after injection of cocaine. The concentration

of cocaine in brain was 7 folds higher than in plasma. Cocaine and benzoylecgonine disappeared from brain and plasma with half–life of 16 and 62 minutes, respectively.

Khan *et al.* (1987) studied the distribution of cocaine in 4 sheep received intravenous doses of 1, 2 and 4 mg/ kg. The mean values of the volume of distribution (Vd) and the steady state of volumes of distribution (Vdss) were 4 and 3.5 l/kg, respectively.

Devane *et al.* (1989) determined the tissue distribution of a single intraperitoneal dose of cocaine (30 mg/kg) in pregnant rats. Cocaine was rapidly absorbed and distributed to all tissues measured with the time to reach maximum concentration (Tmax) ranged from 0.5 to 1.5 hours. The order of the maximum concentration (Cmax) was placenta > fetal liver > maternal heart > whole fetus > fetal brain > maternal brain = maternal plasma.

Boni *et al.* (1991) investigated the tissue disposition of cocaine in rats by inhalation route. The concentrations of cocaine in brain, heart and lung after a 5 minutes of cocaine exposure were measured. Peak concentrations at the termination of exposure were 1500 ± 207 ng/g in brain, 513 ± 136 ng/g in heart and 2020 ± 589 ng/g in lung. The major metabolite of cocaine, benzoylecgonine, was appeared rapidly in lung. Maximum lung concentration of 848 ± 66 ng/g was found at 1.75 minutes, whereas the apparent of benzoylecgonine occurred more slowly in other tissues. The maximum concentration of benzoylecgonine, 84 ± 6 ng/g, was found in brain at 120 minutes and 162 ± 40 ng /g was found in heart at 30 minutes.

Metabolism

Cocaine is promptly cleared from the blood circulation. Plasma half-life of cocaine varies from 15 to 20 minutes (Javaid *et al.*, 1978; Bernuck *et al.*, 1987).

There are two distinct pathways that cocaine is metabolized in humans. The first, accounting for better than 90% of cocaine biotransformation, associated with various hydrolytic reactions. The main enzyme that converts cocaine into the inactive compounds is cholinesterase. Plasma, lung and liver cholinesterase rapidly hydrolyze cocaine to ecgonine methyl ester and benzoylecgonine, which are the water-soluble metabolites excreted in urine. These two metabolites are further converted to ecgonine, the last water-soluble metabolite by hydrolytic pathway (see reviewed by Kloss *et al.*, 1984 and Johanson and Fischman, 1989). The hydrolytic pathway of cocaine and its metabolites is shown in Figure 2.

Plasma cholinesterase activity is much lower in fetuses, infants, elderly men, patients with liver diseases, and pregnant women, so all of whom would be expected to be more sensitive to small doses of cocaine (see reviewed by Johanson and Fischman, 1989).

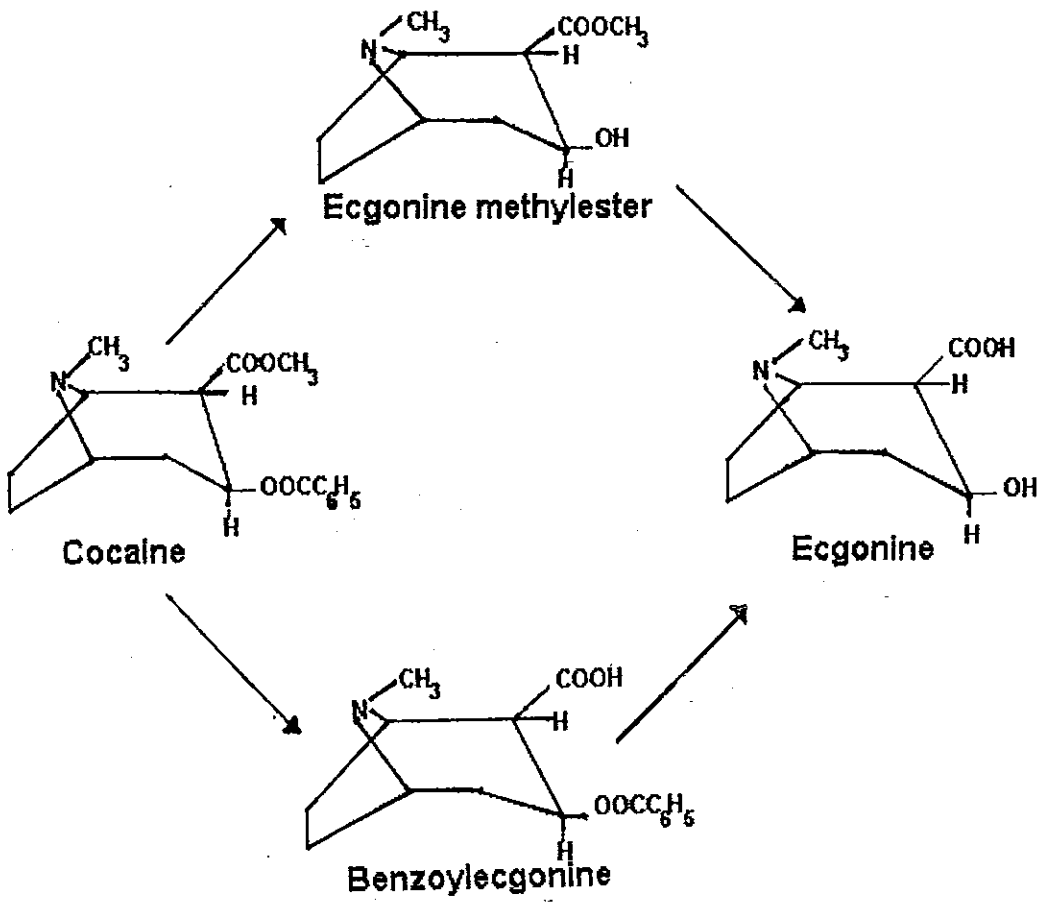
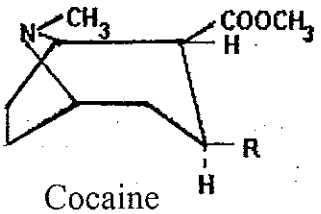


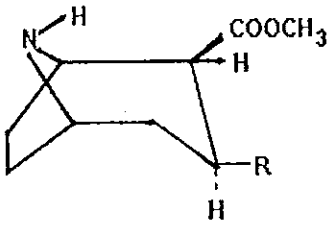
Figure 2 The hydrolytic metabolism of cocaine

The second pathway, the minor route of cocaine metabolism is an oxidation process centered around the propane nitrogen. This mechanism occurs via the cytochrome P-450 and the FAD-containing monooxygenase systems (see reviewed by Kloss *et al.*, 1984 and Karch, 1991). The most likely metabolic pathway of cocaine via the cytochrome P-450 mixed function oxidase systems is the N-demethylation, or N-oxidization followed by demethylation, to give norcocaine. Norcocaine has more potent pharmacological effect than cocaine but it is rapidly oxidized to N-hydroxynorcocaine. Many investigations revealed that the N-hydroxynorcocaine could be further metabolized to norcocaine nitroxide. The oxidative metabolism of cocaine is illustrated in Figure 3. When tested for their ability to induce liver damage by measuring the amount of serum glutamic-oxaloacetic transminase (SGOT), the hydrolytic metabolites of cocaine were not found to be hepatotoxic inducers but the oxidative metabolites were shown to produce marked hepatotoxicity (Thompson *et al.*, 1979; Freeman and Harbison, 1981; see reviewed by Kloss *et al.*, 1984).

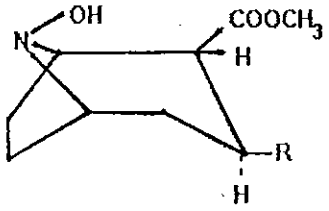
In the experimental animals, benzoylecgonine and ecgonine methyl ester are the major and persistent metabolites of cocaine. Sanberg and Olsen (1991) reported that norcocaine was the persistent metabolite after cocaine dose of 4 mg/kg or higher but only be detected during the first 2 hours after the administration of cocaine.



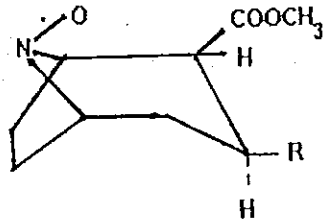
Cocaine



Norcocaine



N-Hydroxynorcocaine



Norcocaine nitroxide

Figure 3 The oxidative metabolism of cocaine

($R = OCOC_6H_5$)

Excretion

Only a very small amount (about 1-5 %) of cocaine appears unchanged in the urine and it is cleared in 4-6 hours. Ecgonine methyl ester accounts for about 30-50 % of cocaine urinary disposition and benzoylecgonine for most of the remainder (reviewed by Jatlow, 1987). Benzoylecgonine and ecgonine methyl ester may persist in the urine for a very long time, they are detectable for 24-60 hours after cocaine use. Recent evidence suggests that in the long term abuse subjects, they may continue to be excreted for up to 10-24 days after the last dose of cocaine (see reviewed by Karch, 1991)

The elimination half-life of cocaine is dose-dependent and ranges from 0.5 to 1.5 hours. The systemic clearance varies from 2 to 2.3 l/min (Wilkinson *et al.*, 1980; Chow *et al.*, 1985; Tisdale *et al.*, 1995). Both half-life and clearance of cocaine in pregnant and non-pregnant animals are not significantly different (Sanberg and Olsen, 1991). The biological half-lives of benzoylecgonine and ecgonine methyl ester, approximately 4 and 6 hours, respectively, account for their prolonged presence in urine as compared with that of cocaine (reviewed by Jatlow, 1987).

Pharmacodynamic properties

The principal actions of cocaine can be divided into three categories, local anesthetic, CNS stimulant and sympathomimetic actions. Cocaine is the only naturally occurring local anesthetic of high efficacy, although relatively high toxicity as well. It also has a powerful CNS stimulating effect of relatively short duration which manifests a relatively low margin of safely (Gay, 1975).

Furthermore, cocaine is a potent sympathetic stimulant, which enhances the cardiovascular functions, increases the body temperature and induces the release of endocrine hormone (see reviewed by Fleming *et al.*, 1990).

Local anesthetic action

Cocaine came into general use as local anesthetic in 1884, in the forms of topical application on mucous membrane and subcutaneous injection. However, several of the patients suffered from considerable postoperative effects, such as headache, vomiting and even death during the early use which led to a marked decline in the amount of cocaine applied without unduly decreasing the anesthetizing effect. Later, cocaine is substituted with other safer local anesthetics (De Jong, 1994). When applied topically, cocaine produces excellent surface analgesia, with intense vasoconstriction effect. Bowman and Rand (1980) noted that when applied to the eyes, cocaine anesthetized the cornea and produced the constriction of the conjunctival blood vessels, and caused mydriasis. The intraocular pressure was slightly increased but the effect was less than that of atropine. Cocaine has a deleterious action on the cornea, which may become clouded and pitted. This toxic effect is increased by the abolition of normal protective eyelid reflex. Because of this damage and the tendency to produce mydriasis, cocaine has been largely replaced by other local anesthetics in ophthalmology.

The primary sites of local anesthetic action of cocaine in blocking nerve conduction is binding to neuronal sodium ion channel, thus increasing the threshold for electrical stimulation, slowing the rate of rise of the action

potentials and the rate of the initiation and conduction of the nerve impulse (Ritchie, 1971; see reviewed by Fleming *et al.*, 1990).

Cocaine, similar to other local anesthetics, also causes a delayed increase in the membrane conductance to potassium ions, which consequently flow down their electrochemical gradient out of the axoplasm. Local anesthetics and calcium also have the same site of action. Local anesthetics inhibit the release of calcium from sites to which it is bound in the membrane. As a results, the changes in sodium and potassium permeability that normally follow calcium release do not occur and generation of the nerve impulse is prevented (Ritchie, 1971).

Cocaine and other local anesthetics directly affect other excitable tissues, including the myocardium. Local anesthetics decrease the rate of depolarization and amplitude of action potential, slow conduction speed and increase the effective refractory period of atrial, atrioventricular and ventricular tissues (see reviewed by Fleming *et al.*, 1990; Qiu and Morgan, 1993; Catterall and Mackie, 1996). In higher concentration, cocaine completely blocks conduction in the heart (reviewed by Fleming *et al.*, 1990)

Przywara and Dambach (1989) studied the effects of cocaine in isolated rabbit heart in the presence of propranolol, the β-adrenergic blocker. The actions of cocaine on cellular electrophysiology were concentration and time dependent and were reversibly. Cocaine (60 μM) produced a profound prolongation of the effective refractory period (ERP). The phase 0 depolarization of action potential was depressed by about 80% in paced right atrial and 53% in ventricular papillary tissue. Automaticity was moderately

depressed in sinus node, which led to a 2-fold increase in action potential duration during exposure to cocaine. This study suggested that cocaine interacted with sodium and potassium channels. Several effects of cocaine on isolated tissues of the heart were characteristic of local anesthetic (Class 1) antiarrhythmic drug.

Kimura *et al.* (1992) determined the electrophysiological effects of cocaine in isolated feline single myocytes. The action potential and membrane currents were recorded using the patch clamp technique. Cocaine (10 or 50 μM) exposure caused the depression of the plateau of action potential, prolongation of action potential duration and also the development of the spontaneous early afterdepolarization. Application of 1 nM isoproterenol, the β-adrenergic agonist, enhanced and induced sustained triggered activity arising from early afterdepolarization while application of 2 μM verapamil, the Ca^{2+} channel blocking drug, abolished the early afterdepolarization. Whole-cell voltage clamp experiment revealed that cocaine (50 μM) reduced the peak L-type Ca^{2+} current and also reduced the peak delayed rectifier K^{+} current but did not affect activation and inactivation kinetic of both channels. Cocaine could prolong action potential duration and induced early afterdepolarization and triggered activity by blocking the delayed rectifier K^{+} current. The mechanism by which cocaine could lengthen action potential duration and induce early afterdepolarization similar to those that might be responsible for classic proarrhythmia, which caused by local anesthetics used as antiarrhythmic drug.

Clarkson *et al.* (1993) investigated the electrophysiological effects of high doses of cocaine in the *in situ* heart. Cocaine (2-11 μg /ml) increased both

atrial and ventricular refractory periods and produced rate-dependent increases in atrial, atrioventricular, His-purkinje and ventricular conduction intervals. Cocaine produced a rate-dependent increase in QT interval, which was greatest at high heart rate yet produced no change in the ST (QT-QRS) interval. These results suggested that high plasma levels of cocaine caused a slowing of conduction without any effect on ventricular repolarization.

Central nervous system stimulating action

Immediately following inhalation or intravenous administration of cocaine, the users may experience an intense sensation (usually referred to as "rush" or "flash") that lasts only a few minute and is generally described as extremely pleasurable. Intranasal use of cocaine hydrochloride or oral use of amphetamine produces euphoria (a "high"), but not a sensation of a "rush" (O'Brien, 1996).

Cocaine affects both the neuronal conduction and the function at synapse by involving the presynaptic neurotransmitter transporters (reuptake pumps) of dopamine, norepinephrine and serotonin (see reviewed by Fleming *et al.*, 1990; Darmani *et al.*, 1992; reviewed by Woolverton and Johnston, 1992). The central stimulant action of cocaine is first manifested on the behavioral effects such as increased talkativeness, laughter, irritability, hypervigilance and disturbance of eating and sleeping (Gay, 1975; Bowman and Rand, 1980; see reviewed by Johanson and Fischman, 1989). It also changes the psychological effects so the users feel restlessness, excitement and produce feeling of well-being. The psychological effects of cocaine also include the lowering of

anxiety and social inhibition, heighten energy, self-esteem, hypersexuality and produce the emotion aroused by interpersonal experiences (Patrick, 1977; see reviewed by Gawin and Ellinwood, 1988). The sensation of fatigue is diminished and this may result in an increase in the capacity for muscular works, which probably due to a lessened sense of lassitude (Bowman and Rand, 1980). Subsequently, the users discover that high dose of cocaine produces disinhibition, impaired judgment, grandiosity, impulsiveness, hypersexuality, compulsively repeated actions, extreme psychomoter activation, delusion and vivid visual, auditory and tactile hallucination (reviewed by Gawin and Ellinwood, 1988 and Johanson and Fischman, 1989).

After a small amount of cocaine, the cortical stimulating action is shown largely by the increase in motor activity. Initially, the motor activity is well coordinated. However, at the higher dose, the lower centers are also affected. Stimulation of lower motor centers causes tremor, convulsive movement (Gay, 1975; see reviewed by Woolverton and Johnston, 1992) and eventually produces tonic-clonic convulsion (Bowman and Rand, 1980).

Post and Rose (1976) showed the electrophysiological effects of repetitive cocaine administration in rats. Eight rats received intraperitoneal injection of a 10 mg/kg cocaine once daily, with two drugs free each week for 28 days or saline in equal volume of injection. The animals showed the increase in horizontal and vertical hyperactivity and stereotype in response to the repeated cocaine. Data suggested that repetitive administration of cocaine produced the behavioral sensitization.

Kalivas *et al.* (1988) studied the behavioral and neurochemical effects of chronic administration of cocaine (15 mg/kg, intraperitoneally for 3 days) in rats. Cocaine significantly augmented the behavioral response to subsequent cocaine administration. The behavioral augmentation persisted for 2 weeks after the last daily injection of cocaine and was associated with decrease in dopamine metabolites.

Peris *et al.* (1989) exhibited the characteristic of behavioral sensitization in rats after receiving once daily injection of cocaine (10 mg/kg, intraperitoneally for 28 days). The behavior of the rat was rated in response to saline or cocaine injection on days 1, 2 and 8 of cocaine administration and 7 days after the last day of the repeated drug administration. Behavioral scores of 0 and 1 comprised the major rating, which was observed after saline injection regardless of how many injections an animals had previously received. Behavioral scores of 1, indicating an increase in locomotor activity, were more in rats receiving injection of cocaine than in the rats receiving injection of saline. On the 8^{th} consecutive day of cocaine injection, the behavioral increase in cocaine groups indicated that there were higher levels of locomotor activity and stereotype behaviors, and these behavioral effects still observed at 7 days after cessation of cocaine.

Sympathetic nervous system stimulating action

Cocaine potentiates the response of sympathetically innervated organs to both endogenous and exogenous catecholamines, norepinephrine, epinephrine, dopamine and other neurotransmitter, 5- hydroxytryptamine (serotonin). The

cardiovascular system, the organ first considerably affected by cocaine is the most important organ supplied with the adrenergic nerves.

The Cardiovascular actions of cocaine

The most important sympathetic action of cocaine is its effects on the cardiovascular system. Cocaine induces an increase in cardiovascular functions such as enhancing pacemaker activity, impulse conduction and myocardial contractility, result in positive chronotropic and inotropic response and increased blood pressure. The intense cardiovascular effects depend on dose of cocaine administration. The cardiovascular effects of cocaine have been studied for a long time but the mechanism of its action is still unclear (see reviewed by Fleming *et al.*, 1990 and Mouhaffel *et al.*, 1995).

Several mechanisms have been proposed for the response of cardiovascular organs to cocaine: (1) alteration of myocardial automaticity by direct action on the myocardial tissues; (2) autonomic dysregulation by potentiation of adrenergic and neurohumoral stimulation; and (3) direct alteration of ion flux at the cell membrane (reviewd by Mouhaffel *et al.*, 1995).

In small dose of administration, both an increase and a decrease in pulse rate and blood pressure are probably seen. Moderate dose of cocaine, heart rate increases due to the peripheral effects of cocaine on the sympathetic nervous systems. Tachycardia and vasoconstriction may appear. Large dose of cocaine can significantly produce ventricular fibrillation and promptly lead to sudden cardiac standstill (Gay, 1975; Catterall and Mackie, 1996).

Inoue and Zips (1988) studied the effects of cocaine (5 mg/kg, intravenously) on the cardiac electrical response to norepinephrine infusion in

canine hearts. Cocaine potentiated shortening of sinus cycle length, atrioventricular and ventricular effective refractory period induced by norepinephrine infusion.

Wilkerson (1988) determined the cardiovascular effects of intravenous cocaine in conscious dogs. All doses of cocaine (0.063–8 mg/kg) significantly increased both systolic and diastolic blood pressure but pulse pressure was not markedly changed with any cocaine dose regimen. Cocaine, dose below 2 mg/kg, could not produce inotropic response. However, cocaine dose of 2 mg/kg and above produced a considerably increase in heart rate in all experimental animals.

Hale *et al.* (1989) demonstrated that intravenous cocaine administration (1 mg/kg/min) for 30 minutes significantly produced the increase in heart rate, systolic and diastolic blood pressure compared to those of saline–control groups. After 3 hours of infusion, cocaine prolonged the cardiac conduction and repolarization, frequently induced atrial and occasionally ventricular arrhythmia. The prolongation of both intraventricular conduction and duration of repolarization by cocaine might be attributed to its depressant effects on action potential and led to cardiac arrhythmia.

Jain *et al.* (1989) showed the effects of cocaine (0.0625-2.0 mg/kg) on cardiac responses elicited by sympathetic nerve stimulation in anesthetized dogs. After intravenous bolus doses of cocaine, maximal potentiation of inotropic response elicited by nerve stimulation was observed with a 0.25 mg/kg dose. Cocaine also potentiated heart rate elicited by sympathetic nerve stimulation.

Tella *et al.* (1991) investigated the effects of repeated daily administrations of cocaine on blood pressure and heart rate in conscious rats. Cocaine (0.1, 0.3 and 3 mg/kg, i.v.) increased blood pressured and heart rate after the first injection. The magnitude and duration of the increases in blood pressure caused by cocaine (0.1-3 mg/kg) dramatically increased on day 2 and remained (except 1 mg/kg dose) at this level through day 5. Following two drug-free days (days 6 and 7), the increase in blood pressure caused by cocaine (0.1, 0.3 and 3 mg/kg) on day 8 was still elevated. Unlike blood pressure, no enhancement in the heart rate response to cocaine was observed.

Toxicity and complications

The toxicity of cocaine can be observed as an extension of its effects. Toxicity may occur with sudden onset and rapidly fatal courses. Assessment of a lethal dose is difficult because of the variability in the rate and route of administration, absorption, metabolism and individual tolerance. Postmorterm blood level of cocaine may relate to its lethal dose, but the limitation of this interpretation is the timing of sampling and the possibility of other factors contributing to death (see reviewed by Fleming *et al.*, 1990). Fatality victims had an average blood cocaine concentration of 6.2 mg/l (reviewed by Mittleman, 1994), and there was no apparent difference in the average blood concentration with respect to the route of administration. The patients with blood concentrations of more than 5 mg/l will probably die of seizure and respiratory arrest (reviewed by Karch and Stephens, 1991). Acute overdose may be manifested as an overwhelming stimulation of the central nervous

system, respiratory system and cardiovascular system, results in seizure and follows by profound depression and cardiopulmonary collapse. Alternatively, cardiac dysrhythmias may progress to ventricular fibrillation and finally, cardiac arrest (Jonsson *et al.*, 1983; see reviewed by Fleming *et al.*, 1990).

Brody and colleagues (1990) reviewed the incidences and characteristics of medical problems associated with cocaine use in 216 cocaine-using patients, with total visits of 233, entering emergency department during 6 months as demonstrated in Table 1.

The most common complain was chest pain though it was rarely believed to represent ischemia. Altered mental status was common and ranged from psychosis to coma. Acute mortality was less than 1%.

1. Gastrointestinal tract complications

Cocaine can cause gastrointestinal tract ulceration and perforation (see reviewed by Karch, 1991). In addition, as shown in Table 1, the cocaine use patients experienced nausea, vomiting and abdominal pain (Brody *et al.*, 1990).

2. Respiratory system complications

Free base cocaine smoking has been usually reported to produce pulmonary hypertension and damage of gas exchange surface of the lung (see reviewed by Karch, 1991). This damage may associate with a direct effect of drug on the pulmonary vasculature (Weiss *et al.*, 1981). Gay (1975) reported that chronic cocaine abuse might cause an early stimulation of respiratory system such as increase respiratory rate and depth, followed by an advanced state of respiratory stimulation, resulted in cyanosis, dyspnea and rapid irregular

respiration. Finally, the respiratory failure, ashen gray cyanosis and death could occur due to depressive effect of cocaine on the respiration center.

Table 1 Symptoms associate with cocaine use

Symptoms	Number (%)[a]
Cardiopulmonary	
Chest pain	93 (39.5)
Short of breath	51 (21.9)
Palpitation	48 (20.6)
Diaphoresis	15 (6.4)
Cardiac arrest	2 (0.9)
Psychiatric	
Psychotic	21 (9)
Hallucination	7 (3)
Depression	5 (2.1)
Neurologic	
Dizziness	30 (12.9)
Tremor	12 (5.2)
Seizure	10 (4.3)
Coma	8 (3.4)
Constitutional	
Weakness	14 (6)
Fever	8 (3.4)
Insomnia	2 (0.9)
Gastrointestinal	
Nausea	21 (9)
Abdominal pain	18 (7.7)
Vomiting	15 (6.4)

[a] Totals exceed 100 % because patients often had more than one symptoms

3. Neurotoxicity

One of the most significant consequences of cocaine abuse is the development of neuropathology. Complication of cocaine in the central nervous system is due to the behavioral and psychological stimulation. Cocaine can produce psychological changes characterized by paranoid, impaired reality testing, anxiety, emotional instability, tactile hallucination and delusion. Cocaine also produces extreme change in a stereotype compulsive competitive pattern of behavior, results in hyperactivity and follows by tonic-clonic convulsion, which resembles grand mal seizure. In severe cases, cocaine dramatically depresses the motor activity attributed to paralysis of muscles, loss of reflexes, unconsciousness, loss of vital function and lastly death (Gay, 1975; see reviewed by Gawin and Ellinwood, 1988).

4. Hepatotoxicity

Cocaine-induced acute hepatic necrosis in some sensitive strain of animals has been reviewed by some scientists, but the evidence that it can cause liver damage in humans is yet lacking.

Freeman and colleagues (1981) suggested that chronic cocaine treatment (10, 20 or 30 mg/kg, intraperitoneally, daily for 1, 2 or 3 weeks) in mice might cause an alteration of liver function, which indicated by an increase in serum glutamic-pyruvic transminase (SGPT) level. Chronic cocaine treatment produced dose-dependent alteration in hepatic cytochrome P-450 level. Significant depression of the cytochrome P-450 levels was observed in mice received a 30 mg/kg dose of cocaine either at 1, 2 or 3 weeks. Chronic cocaine treatment also produced hepatic necrosis.

Thompson *et al.* (1979) reported that liver damage following cocaine injection in mice was due to the action of a metabolite of cocaine rather than of cocaine itself. The bioactivation of cocaine to more toxic metabolites appeared to be a multi-step and was carried out by the cytochrome P-450 microsomal mixed oxidase systems. An inhibition of esterase activity increased the damage from both cocaine and norcocaine.

Cocaine, therefore, may induce hepatotoxicity in some patients who have lower esterase enzyme level or in subjects administered cocaine together with alcohol because there is evidence that alcohol can enhance the level of cocaine, norcocaine and other metabolites in plasma and liver tissue (Roberts *et al.*, 1991).

5. Fetal toxicity and teratogenic toxicity

Mahone *et al.* (1994) exhibited that cocaine and its metabolites were found in the amniotic fluid after maternal use and appeared in fetal and maternal blood within 24 hours. Therefore, cocaine may induce high risk of fetal abnormality and death in the cocaine–using women.

Woods and colleagues (1987) studied the alterations in the cardiovascular system of fetuses exposed to cocaine. Cocaine was given to the ewes or fetuses as 0.5 or 1.2 mg/kg intravenous bolus injection. Maternal injection of cocaine produced dose-dependent increase in blood pressure and decrease in uterine blood flow. Direct cocaine administration to the fetus produced smaller increase in heart rate and blood pressure than those observed following maternal injection. The conclusion was that (1) cocaine altered fetal oxygenation by reducing uterine blood flow and impairing the oxygen transfer

to the fetus and (2) fetal cardiovascular changes to maternal administration of cocaine might reflex fetal hypoxemia.

Chasnoff *et al.* (1983) reported that cocaine–using women had a significant higher rate of spontaneous abortion than control. In addition, infant exposed to cocaine had a significant depression of interactive behavior and a poor organization response to environment stimuli.

Cocaine useed by people on their reproductive years tends to induce teratogenesis. The rate of birth defect is approximately 2 or 3 folds higher than that observed in control population (Manson and Wise, 1991). Macgreger *et al.* (1987) noted that 60% of the 70 newborn of cocaine-subjects in a case-controlled study had congenital malformations of anus, kidney and limbs. Furthermore, Yazigi and Polakoski (1992) demonstrated that in the experimental animals, long-tern cocaine use could affect the spermatozoa, and resulted in a decrease of sperm count and mortality.

6. Cardiovascular toxicity

Most medical complications of cocaine appear to be concerned with the cardiovascular effects. Cocaine abuse has been associated with tachycardia, systemic hypertension, ventricular arrhythmia, myocardial infarction cerebrovascular accidents and sudden death (see reviewed by Cregler and Mark, 1986, Rezkalla *et al.*, 1990 and Mouhaffel *et al.*, 1995).

Isner and colleagues (1986) concluded the clinical and pathological finding in 26 patients who used cocaine that (1) the cardiac consequences of cocaine abuse were not unique to parenteral use of the drug, since nearly all patients took the drug intranasally; (2) the underlying heart disease was not a

prerequisite for cocaine–related cardiac disorder; (3) seizure activity, a well document noncardiac complication of cocaine abuse, was neither a prerequisite for nor an accompanying feature of cardiac toxicity of cocaine; and (4) the cardiac consequences of cocaine were not limited to massive dose of the drug.

6.1 Myocardial infarction

A major proportion of the case reports of cocaine relates to the cardiovascular toxicity involve myocardial infarction, which does not appear to be related to doses or route of administration (Isner *et al*, 1986). Acute myocardial infarction is the chief symptom in the patients who used cocaine. Many studies showed that acute myocardial ischemia and infarction were the most frequently reported of the cardiac consequences of cocaine abuse (Kossowski and Lyon, 1984; Schachne *et al.*, 1984; Cregler and Mark, 1985; Howard *et al.*, 1985; Pasternack *et al.*, 1985; Hollander *et al.*, 1995). Coronary thrombosis appears to be an important pathophysiologic factor in cocaine induced myocardial infarction (Rezkalla *et al.*, 1990). However, acute myocardial infarction patients with angiographically normal coronary artery have been reported (see reviewed by Johanson and Fischman, 1989 and Mouhaffel *et al.*, 1995; Hollander *et al.*, 1995). Several mechanisms have been proposed for cocaine–induced myocardial ischemia: (1) coronary thrombosis; (2) increased myocardial oxygen demand in the setting of limited myocardial oxygen supply; (3) coronary vasoconstriction; and (4) accelerated artherosclerosis (reviewed by Mouhaffel *et al.*, 1995).

6.2 Coronary artery vasoconstriction and thrombosis

Lange and colleagues (1989) reported the effect of cocaine on blood flow and dimensions of coronary arteries and on myocardial oxygen demand in 45 patients with cocaine use histories. Cocaine caused vasoconstriction of coronary arteries with a decrease in coronary blood flow and increase in oxygen demand. The coronary angiography occasionally showed occlusive coronary thrombi in patients with cocaine related acute myocardial infarction. In addition, autopsies following cocaine–related deaths also revealed complete thrombotic occlusion of normal and artherosclerotic coronary arteries. Coronary artery thrombosis and subsequent ischemia could be attributed to alteration in platelet and endothelial cell function (reviewed by Mouhaffel *et al.*, 1995).

6.3 Myocarditis

It has been suggested myocarditis is common with cocaine use (see reviewed by Karch, 1991). Virmani and associates (1988) performed the histologic examination of myocardial tissue from 40 patients with cocaine–associated death. The examination revealed active myocarditis in 20%. In addition, Bricker and associates (1991) reported the cardiac disorder in 40 chronic cocaine use patients. Left ventricular mass index and the posterior wall were significantly higher in the patients with a history of cocaine abuse than in controls.

6.4 Cardiac arrhythmia

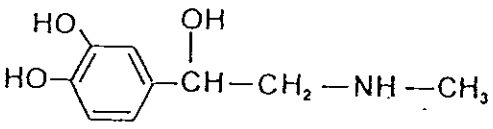
Sudden death and a wide variety of ventricular arrhythmia were reported in cocaine users (Clusin *et al.*, 1982; Inoue and Zips, 1988; Kimura *et*

al., 1992). Nanji and Filipenko (1984) reported asytole and ventricular fibrillation as the presenting sign of cocaine intoxication. Mouhaffel *et al.* (1995) reviewed that ventricular fibrillation, polymorphic ventricular tachycardia and supraventricular tachycardia had been documented in ischemic and myocardial infarction after cocaine use. Isner *et al.* (1986) described ventricular tachycardia and ventricular fibrillation in young patients following cocaine use who had no evidence of myocardial infarction, angiographically normal coronary artery and normal results of electrophysiologic study. Cregler and Mark (1986) proposed that cocaine was arrhythmogenesis, a characteristic that might be attributed either to a direct effect of drug or its effects on catecholamines, however, the mechanism of cocaine induced cardiac arrhythmia is still probably multifactorial.

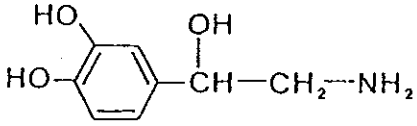
CATECHOLAMINES

Endogenous catecholamines such as norepinephrine, epinephrine and dopamine are the chemical mediators liberated by mammalian postganglionic sympathetic nerves. Norepinephrine is the neurotransmitter of most sympathetic postganglionic fibers and of certain areas in the central nervous system; dopamine is the predominant neurotransmitter of the mammalian extrapyramidal systems and of several mesocortical and mesolimbic pathways; and epinephrine is the principal hormone released from the adrenal medulla. Isoproterenol is the synthetic catecholamine. The chemical structures of the catecholamines are demonstrated in Figure 4.

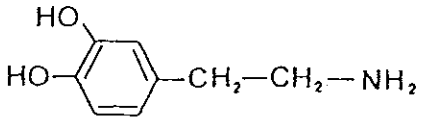
Epinephrine



Norepinephrine



Dopamine



Isoproterenol

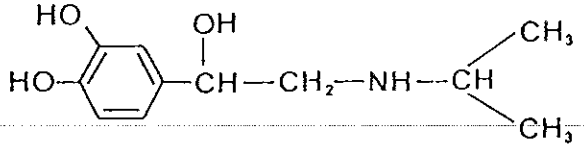


Figure 4 The chemical structures of catecholamines

Synthesis, storage, release and inactivation of catecholamines

The diagram of a generalized adrenergic junction, which demonstrates the synthesis, storage, release and reuptake into the nerve terminals of norepinephrine is illustrated in Figure 5.

1. Biosynthesis of catecholamines

Enzyme systems that catalyze the formation of catecholamines are present in sympathetic neurones, neurones in central nervous system, adrenal medulla and chromaffin cells in other tissues. In adrenergic neurones, the enzymes are synthesized in perinuclear region of cell body. However, the terminal axons are the important sites of transmitter synthesis and the enzymes are conveyed there by axon transport of cytoplasm and organelles (Bowman and Rand, 1980).

The precursor of biosynthetic catecholamines in the mammalian body is the essential aromatic amino acid, tyrosine. Tyrosine is present in the adequate amount in any balanced diet and may additionally by synthesis in the body from the related amino acid, phenylalanine. Tyrosine is actively transported into the cytoplasm of adrenergic nerves and chromaffin tissues, the cells that produce and store epinephrine by a sodium-dependent carrier. In adrenergic nerves, tyrosine is converted to dihydroxyphenylalanine (DOPA) by the enzyme tyrosine hydroxylase, which is synthesized in the endoplasmic reticular apparatus. DOPA does not accumulate in adrenergic neurones because it is formed very slowly, but rapidly converted to dopamine by L-dopa decarboxylase, an enzyme presenting in the cytoplasm.

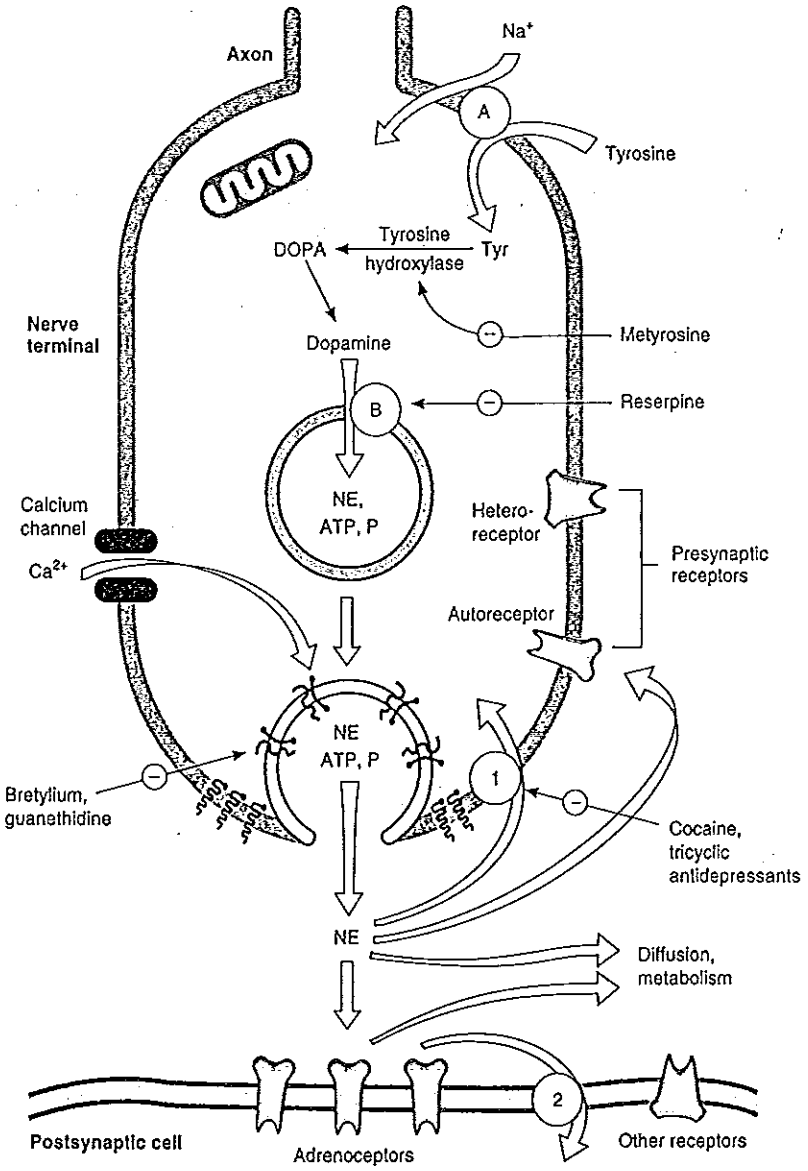


Figure 5 The diagram of generalized adrenergic junction. (A): a sodium-dependent carrier, which transports tyrosine into the adrenergic ending or varicosity. (B): a carrier, which transports dopamine into the vesicle. (1): the neuronal uptake (uptake 1), which can be blocked by cocaine. (2): the extraneuronal uptake (uptake 2) (Source: Katzung, 1998, pp. 78)

Dopamine is the first in sequence of the catecholamine synthesis. It is the end product of synthesis and the transmitter substance of dopaminergic nerves in the central nervous systems, the neurones that lack the enzyme for converting dopamine into norepinephrine (Bowman and Rand, 1980). In other catecholamines-synthesized cells such as sympathetic adrenergic nerves, dopamine is actively taken up into the vesicle of the varicosity and is catalyzed to norepinephrine within the vesicle in the presence of dopamine β-hydroxylase enzyme. In neurones in which norepinephrine is the neurotransmitter substance, catecholamine synthesis is complete at this step, the enzyme for the formation of epinephrine is being absent (Day, 1979; Rang and Dale, 1991; Nicholls, 1994; Katzung, 1998). In the chromaffin cells of adrenal medulla, phenylethanolamine N-methyl transferase (PNMT) catalyzes the N-methylation of norepinephrine to epinephrine (Rang and Dale, 1991; Nicholls, 1994). The process of catecholamine synthesis is shown in Figure 6.

2. Intraneuronal storage of catecholamines

There are some experiments supported that catecholamines are stored within the neurones by two ways. Firstly, some are diffusely distributed through out the cytoplasm of the cells in a loosely bound form to maintain normal physiological functions whereas the remainders are stored within both small and large synaptic vesicles (Nelson and Molinoff, 1975; Day, 1979; Nicholls, 1994). Both types of vesicles show the same electron-dense contents but for different reason. Small vesicle contains catecholamine and adenosine triphosphate (ATP) but no neuropeptide, the electron density is due to the catecholamine contents (Nelson and Molinoff, 1975). It requires a special

active carrier system to transport catecholamine across the vesicle membrane. Certain drugs such as reserpine interfere with this process and cause nerve terminals to become depleted of their norepinephrine stores (Rang and Dale, 1991; Nicholls, 1994).

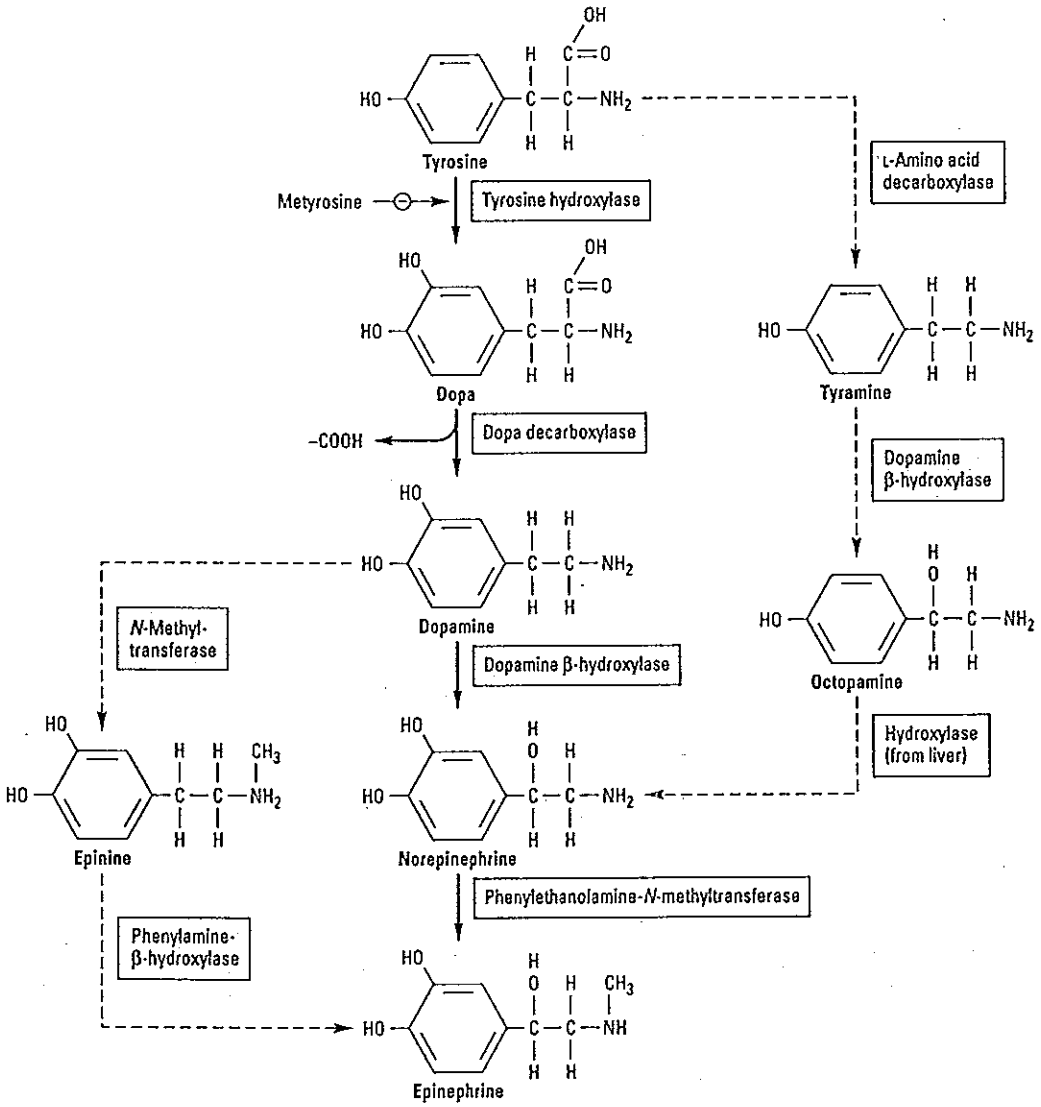


Figure 6 The process of catecholamine synthesis

(Source: Katzung, 1998, pp. 80)

There are some evidences to prove that many of the large catecholamine storage vesicles are formed in the cell body of sympathetic neurones and passed along the axon by a process involving structures called microtubules to the varicosities. Within the storage vesicle, norepinephrine is presented in very high concentration and it stores in the form of a molecular complex, probably with ATP. Norepinephrine and ATP are presented in the vesicle in constant stoichiometric proportion of 4 molecules of norepinephrine to 1 molecule of ATP (Day, 1979; Rang and Dale, 1991).

3. Release of norepinephrine on neuronal stimulation

The events which affect the release of norepinephrine from the terminal varicosity of the sympathetic neurones, are relatively unclear. Some evidences suggest that stimulus-secretion coupling in adrenergic fiber is primarily similar to those occurring in chromaffin cells and in cholinergic neurones (Langer, 1981; Hoffman and Lefkowitz, 1996). As in cholinergic nerves, at rest there is a small constantly random release of neurotransmitters from the nerve ending to maintain the electrophysiological functions of the postsynaptic cells. On the other hand, at adrenergic junction, when the sympathetic nerve is electrically stimulated, an action potential causes calcium influx into the nerve terminal which induces subsequent fusion of the vesicle with the plasma membrane of the postsynaptic cells and releases the content of norepinephrine into the synaptic cleft. This releasing process is called exocytosis (Day, 1979; Langer, 1981; Hoffman and Lefkowitz, 1996; Katzung, 1998).

4. Biological inactivation of catecholamines

Epinephrine and norepinephrine are inactivated by: (1) being reuptake into nerve terminals; (2) diffusion out of the junctional cleft and being uptake at the extraneuronal sites; and (3) metabolic transformation. There are two enzymes involving in the initial step of the metabolic transformation, monoamine oxidase and catechol-O-methyltransferase (COMT) (see reviewed by Iversen, 1967; and Trendelenburgs *et al.*, 1981; Kopin, 1985; Cryer, 1990).

5. The neuronal uptake process of catecholamines

The uptakes of catecholamine and other amines into the storage vesicles are an active process and can be inhibited by various drugs. The most important mechanisms for inactivation and turnover of norepinephrine and other catecholamines are their reuptake into the nerve terminal called uptake 1. This neuronal uptake process may account for the inactivation of 75-90% of norepinephrine released by sympathetic nervous system stimulation (Day, 1979; Bowman and Rand, 1980). Uptake 1 is an energy-dependent process, can operate against concentration gradient, is saturable and has a high affinity for norepinephrine. It requires the presence of both sodium and potassium ion for its function (Day, 1979; Parkinson, 1990; Nicholls, 1994).

All parts of the adrenergic neurones have the capacity to take up norepinephrine across the cell membrane; however, the process is quantitatively more important in the terminal axons because of the large surface area relative to the mass of tissues and it is functionally more important because the terminals are the site of release (Bowman and Rand, 1980; see reviewed by Trandelenburgs, 1981).

The norepinephrine that is taken up by the terminal axons and stored in the synaptic vesicles is available for re-use as transmitter. When neuronal reuptake is blocked, the responses to adrenergic nerve stimulation are first enhanced, but the norepinephrine content of the adrenergic axons becomes depleted with repeated stimulation (Bowman and Rand, 1980).

Extraneuronal uptake (uptake 2) becomes more important when neuronal uptake is blocked, the amines being diverted to the alternative site. It is also the important site of uptake when large concentrations of amines are applied and neuronal uptake is saturated. All of the amines that are taken up intraneuronally are also taken up extraneuronally. On the other hand, isoproterenol and other sympathomimetic amines with large substituent groups on the amine nitrogen are taken up by extraneuronal cells, but not by adrenergic neurones. The responses to isoproterenol are not affected by adrenergic denervation or by inhibitors of neuronal uptake (Bowman and Rand, 1980).

6. Inhibitor of the neuronal uptake process

Many drugs have been found to inhibit the neuronal uptake process (Table 2). Among the most potent inhibitors are the agents affecting amine-binding mechanism such as reserpine, the tricyclic antidepressant such as desmethylimipramine and imipramine, an adrenoceptor antagonist such as phenoxybenzamine, the sympathomimetic amine such as tyramine, the compounds related to phenylethylamine and guanethidine, the adrenergic neuronal blocking drug such as cocaine, the monoamine oxidase inhibitor such as tranylcypromine, etc. (see reviewed by Iversen, 1967; Bowman and Rand, 1980).

Cocaine is considered as the prototype drug for inhibition of neuronal uptake of amines. Cocaine does not deplete norepinephrine from adrenergic neurones; in fact, responses to nerve stimulation are enhanced by it (Bowman and Rand, 1980).

Table 2 The inhibitors of norepinephrine uptake in isolated rat heart

(Source: Iversen, 1967, pp. 12)

Drug	ID_{50} [a]
Desmethylimipramine	1.3×10^{-8}
Imipramine	9×10^{-8}
Cocaine	3.8×10^{-7}
Tyramine	4.5×10^{-7}
Reserpine	8×10^{-7}
Phenoxybenzamine	1.1×10^{-6}
Tanylcypromine	1.2×10^{-6}
Guanethidine	3.3×10^{-6}

[a] The molar concentration of drug required to produce a 50% inhibition of the uptake of ^{3}H-norepinephrine

Pharmacological action of catecholamines

In the peripheral nervous system, catecholamines mediate rapid communication between the sympathetic components of the autonomic nervous system and visceral tissues. Neural signals trigger prompt exocytic release of

large quantities of stored catecholamines; the resultant biologic expression of sympathoadrenal activation may be modulated by adrenergic regulation of catecholamine clearance and of adrenergic receptor functions (Cautrecasas *et al.*, 1974; Bowman and Rand, 1980). Most of the actions of catecholamines can be classified into seven broad types: (1) a peripheral excitation on certain types of smooth muscle such as vascular smooth muscle supplying skin and mucous membrane and on glands such as those in salivary and sweat glands; (2) a peripheral inhibitory actions on other certain types of smooth muscle such as the wall of intestinal tracts, bronchial smooth muscle and the blood vessels supplying skeletal smooth muscle; (3) a cardiac excitatory actions, responsible for an increase in heart rate and force of contraction; (4) metabolic actions such as an increase in rate of glycogenolysis in liver and muscle and producing lipolysis from adipose tissues; (5) endocrine actions such as modulation of the secretion of insulin, renin and pituitary hormones; (6) central nervous system actions such as respiratory stimulation and cardiovascular regulation; and (7) presynaptic actions with result in either inhibition or facilitation of the release of neurotransmitter such as norepinephrine and acetylcholine (Hoffman and Lefkowitz, 1996).

Norepinephrine (levarterenol, noradrenaline)

Norepinephrine is the chemical substance synthesized by the mammalian postganglionic adrenergic nerves. Norepinephrine acts as a neurotransmitter of the sympathetic nervous system. It differs from epinephrine only by lacking of the methyl substitution in the amino acid group (Hoffman and Lefkowitz,

1996). Norepinephrine and epinephrine have similar effects on β_1-adrenoceptor in the heart and similar potency at α-adrenoceptors but has relatively little effects on β_2-adrenoceptor (see reviewed by Bulbring and Tomita, 1987; Hoffman, 1998).

Actions on the cardiovascular system

The basis for the clinical use of norepinephrine is its ability to raise systemic arterial blood pressure by causing vasoconstriction resulting from its α_1-adrenoceptor stimulant activity. The pressor response to norepinephrine is due almost entirely to the increased peripheral resistance of blood vessels (Day, 1979; Bowkoski, 1988). The increase in peripheral vascular resistance in most vascular beds results in reducing blood flow through the important organs such as kidney, liver and usually skeletal muscle, however, the glomerular filtration rate is maintain unless the decrease in renal blood flow is significantly remarked. Norepinephrine also causes the vasoconstriction of mesenteric vessels and reduces splanchnic and hepatic blood flow in humans (see reviewed by Bulbring and Tomita, 1987; Rang and Dale, 1991; Hoffman and Lefkowitz, 1996).

Norepinephrine usually has little effects on cardiac output. In most subjects, usual clinical dose of norepinephrine produces bradycardia, which results from activation of the baroreceptor reflex leading to increased vagal (inhibitory) and decreased sympathetic (excitatory) in the heart (Day, 1979; Hoffman and Lefkowitz, 1996). In the patients with markedly reduced cardiac output, norepinephrine may increase it by causing the increase in venous return as a result of venoconstriction. Cardiac oxygen consumption is increased by

norepinephrine even in the absence of changes in cardiac output (Day, 1979; Bowman and Rand, 1980). Szakacs and Mehlman (1960) marked that high dose of norepinephrine could cause high mortality rate in the treatment of cardiogenic shock or shock-like state that might be due to its cardiotoxicity e.g. myocarditic effect.

Sato *et al.* (1995) demonstrated that norepinephrine induced calcium influx through voltage-sensitive calcium channel, which could be blocked by calcium channel blocker, nicardipine, and through receptor-operated calcium channel, which could not be blocked by calcium channel antagonist.

Isoproterenol (isoprenaline, isopropylnoradrenaline)

Isoproterenol is the synthetic catecholamine derived from norepinephrine by substitution of the isopropyl group on the nitrogen atom of the aliphatic chain. It is a potent agonist on β-adrenoceptors and is virtually without action on α-adrenoceptors (Bowman and Rand, 1980; Hoffman and Lefkowitz, 1996). Therefore, the pharmacological actions of isoproterenol are entirely due to its powerful β-adrenoceptor stimulant activity.

Actions on the cardiovascular system

Isoproterenol is more potent than norepinephrine in increasing the rate and force of the heart beat. It produces vasodilatation in all blood vessels, particularly in the vessels of skeletal muscle, and causes a fall in blood pressure with a mark increase in heart rate. The tachycardia is due to the direct action of isoproterenol on the heart, and it is reinforced by stimulation of the cardio-accelerator reflex induced by the fall in blood pressure (Bowman and Rand,

1980). It produces increase in cardiac output, as a result of stimulation of cardiac β_1-adrenoceptor subserving increase in heart rate and force of contraction and cardiac output, which lead to the increase in myocardial oxygen consumption (Bowman and Rand, 1980; Hoffman and Lefkowitz, 1991). The cardiovascular effects of isoproterenol may lead to cardiac hyperexcitability such as palpitation, sinus tachycardia and more serious cardiac arrhythmia (Hoffman, 1998).

Actions on the smooth muscle

Isoproterenol is considerably more active than norepinephrine in its relaxing action on smooth muscle (Bowman and Rand, 1980). Previous studies showed that isoproterenol produced tracheal muscle relaxation by inhibiting a receptor-operated pathway for calcium influx across the membrane which normally was activated by prostaglandins (Ito *et al.*, 1995). Because of its relaxing property, isoproterenol was once mostly designed for use in the treatment of asthma. However, with the clear evidence of cardiac toxicity that it may cause, the use of isoproterenol in asthma is now replaced with the more specific and selective β_2-adrenoceptor drugs such as terbutaline and salbutamol (Hoffman and Lefkowitz, 1996).

CLASSCIFICATION OF ADRENERGIC RECEPTORS

Adrenergic receptors are originally introduced into the pharmacology of the sympathetic nervous system to explain the different actions of the endogenous catecholamines, dopamine, epinephrine and norepinephrine. Alquist, in 1948, presented an evidence for the existence of the two different receptors in the sympathetic nervous system. He used five different catecholamines to determine their relative potencies in over 20 intact and isolated animal preparations from four different species. When the five test compounds were arranged in order of potency, the data segregated into two groups. In the first group, designated as α-receptor-mediated, the order of potency was epinephrine > norepinephrine > α-methylnorepinephrine > α-methylepinephrine > isoproterenol. In the second group, designated as β-receptor-mediated, the order of potency was reversed to isoproterenol > epinephrine > α-methylepinephrine > α-methylnorepinephrine > norepinephrine (Brodde, 1989; Parkinson, 1990).

Later, it becomes clear that at least two major subtypes of both α and β-adrenoceptors can be distinguished by a variety of methods of pharmacological criteria (Brodde, 1989). Furthermore, the recent method demonstrated the different of two distinct α-adrenoceptors, α_1 and α_2, and three distinct β-adrenoceptors, β_1, β_2 and β_3 (Cautrecasas *et al.*, 1974; Molenarr and Summers, 1987; Cruickshank and Prichard, 1994; Lefkowitz *et al.*, 1996). The distribution of adrenoceptor subtype varies, although one type may predominant

in an organ or tissues. The tissue distribution of adrenoceptors and responses they mediate are shown in Table 3.

Table 3 Tissue distribution and responses mediated by adrenergic receptors (Source: Parkinson, 1990, pp. 18; Lefkowitz *et al.*, 1996, pp. 125)

Type	Tissues	Response
α_1	Smooth muscle: vascular, iris, radial ureter, pilomotor, uterus sphincters (gut, bladder)	Relaxation
	Heart	Positive inotropic effect
	Salivary gland	Secretion
	Adipose tissue	Glycogenolysis
	Sweat glands	Secretion
	Kidney (proximal tubule)	Glucogenesis, Na^+ reabsorption
α_2	Presynaptic sites on sympathetic nerve ending	Inhibition of NE release
	Adipose tissue	Inhibition of lipolysis
	Platelets	Aggregation, granule release
	Endocrine pancreas	Inhibition of insulin release
	Smooth muscle (vascular)	Contraction
	Kidney	Inhibition of renin release

Table 3 Tissue distribution and responses mediated by adrenergic receptors (Continued)

Type	Tissues	Response
β_1	Heart	Positive inotropic effect; positive chronotropic effect
	Adipose tissue	Lipolysis
	Kidney	Renin release
	Liver	Glycogenolysis, gluconeogenesis
		Glycogenolysis, lactate release
β_2	Skeletal muscle	Relaxation
	Smooth muscle: bronchi, uterus, gut, vascular (skeletal muscle) detrusor, spleen capsule	Relaxation
	Endocrine pancreas	Insulin secretion
	Salivary gland	Amylase secretion
β_3	Adipose tissue	Lipolysis

The different subtypes of receptors are also classified by their relative responsiveness to agonists and antagonists, not only by their location (see reviewed by Tomita and Bulbring, 1987).

Receptor structure

The adrenergic receptors are integral membrane glycoproteins (Figure 7). The most striking feature of all of the adrenergic receptors is that each contains seven stretches of 20-28 hydrophobic amino acids that likely represent membrane spanning regions (see reviewed by Lefkowitz and Caron, 1988). The various adrenergic receptors share many amino acid sequences, particularly with respect to the membrane-spanning domains, and to the extent in the cytoplasmic loops, CI and CII. Cytoplasmic loop CIII, the cytoplasmic carboxyl terminus and the extracellular domain are the most divergent as regards amino acid sequence. The extracellular loops (EI, EII and EIII) connect the hydrophobic membrane-spanning amino acid sequences and the glycosylated, amino terminus, the function of which is not clear, but which does not appear to be involved in ligand binding. However, the cysteine residues within the extracellular loops may have some function in stabilizing ligand binding (Cruichshank and Prichard, 1994).

A major function that is likely associated with the cytoplasmic portions of adrenergic receptors is coupling to guanine nucleotide regulatory proteins (G protein). The CIII region and in particular the carboxyl terminus may be involved in determining the specificity of interactions of the various receptors

with different G protein (see reviewed by Lefkowitz and Caron, 1988; Cruichshank and Prichard, 1994).

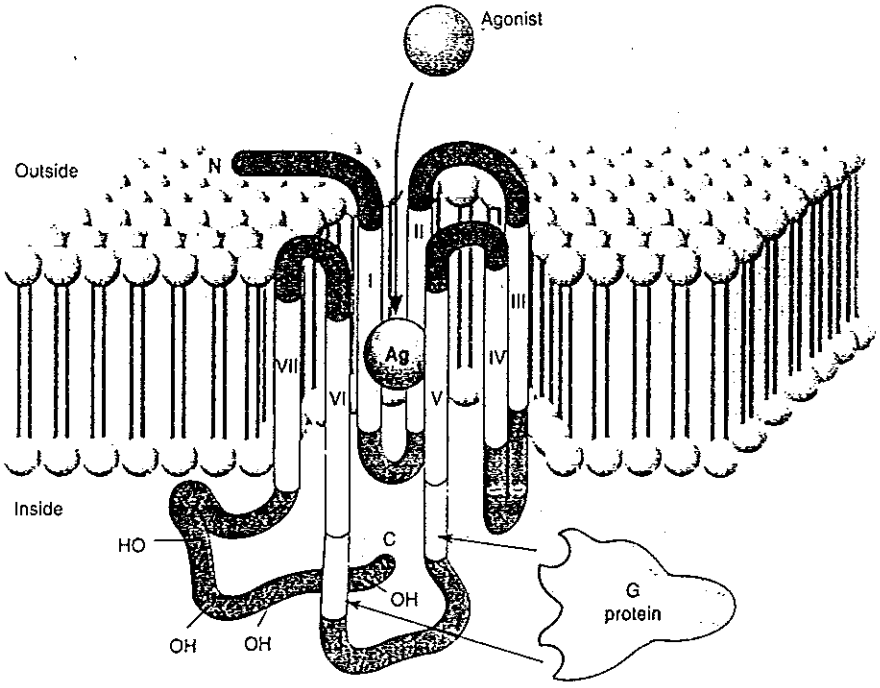
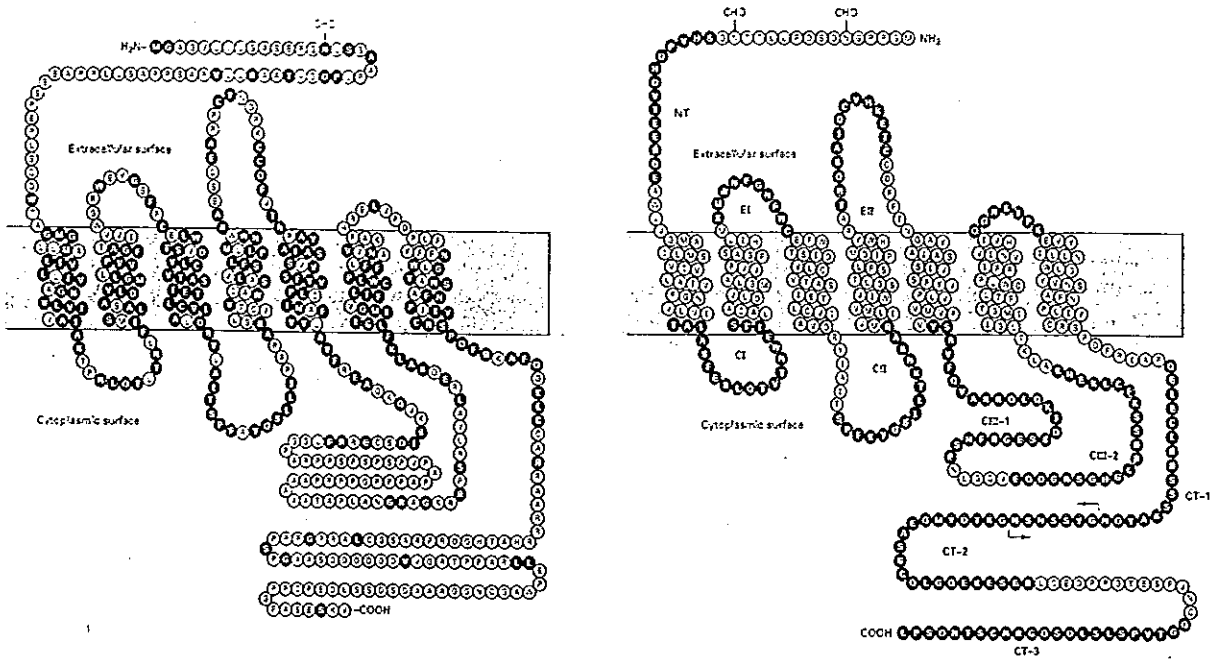


Figure 7 The schematic representation of the organization of G protein-coupled receptor within the plasma membrane. The receptor's amino (N) terminal is extracellular and its carboxyl (C) terminal is intracellular. The terminals are connected by a polypeptide chain that traverses the plane membrane seven times (*I-VII*). The agonist (Ag) approach the receptor from the extracellular fluid and binds to a site surrounded by the transmembrane regions of receptor protein (Source: Katzung, 1998, pp. 24)

The cytoplasmic portions of the β-adrenoceptor are probably crucial for G protein coupling, for phosphorylation reactions and desensitization (Cruichshank and Prichard, 1994). The proposed structures of human β_1- and the hamster β_2-adrenoceptors in the plasma membrane are shown in Figure 8.



(A) β_1-adrenoceptors (B) β_2-adrenoceptors

Figure 8 The proposed structures of the human β_1- (A) and the hamster β_2-adrenoceptors (B) in the plasma membrane (Source: Cruichshank and Prichard, 1994, pp. 13)

Molecular mechanisms of sympathomimetic action

The differences in responses to stimulation of various adrenergic receptors are brought about by alterations in activity of effector enzyme, such as adenyl cyclase or the phospholipidases, and various ion channels. The coupling between the adrenergic receptors to the effector enzymes and ion channels occurs via specific heterotrimeric proteins, which bind and hydrolyze guanine nucleotide triphosphate (GTP) and, thus, are termed G proteins. The G proteins comprise a large family with multiple members each of alpha (G_α), beta (G_β) and gamma-subunits (G_γ) (Cruichshank and Prichard, 1994). Different G proteins have distinctive alpha subunits; less variation has been found between the respective beta and gamma subunits of each of the G proteins. G proteins of particular important for the adrenoceptor functions include Gs, the stimulatory G protein of adenylate cyclase and Gi, the inhibitory G protein of adenylate cyclase (see reviewed by Lefkowitz and Caron, 1988).

As a result of the binding of agonist, the receptor undergoes a conformational change that allows it to bind to a G protein complex. In the absence of agonist, G_α will bind GDP. After the agonist-receptor complex binds to the G protein complex, the GDP is substituted by GTP and G_α dissociates from the complex. This activated form of G_α then binds to and stimulates the catalytic unit of adenyl cyclase to synthesize the cAMP from ATP. When the GTP is hydrolyzed to GDP, G_α dissociates and then reforms the G protein complex with the other G subunit to await further binding to the agonist-receptor complex (Parkinson, 1990). The general scheme for transmembrane signaling mediated by G proteins is shown in Figure 9.

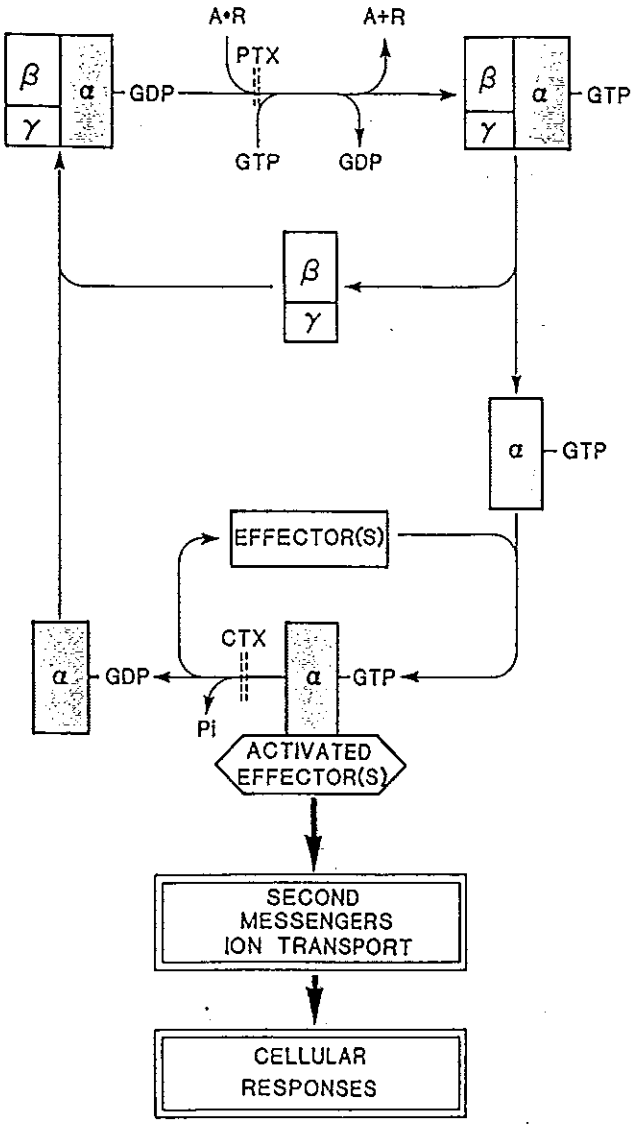


Figure 9 General scheme for transmembrane signaling mediated by heterotrimeric G proteins; PTX and CTX represent pertussis and cholera toxins, respectively (Source: Hollenberg and Severson, 1995, pp. 25)

The alpha-adrenoceptors

The adrenergic α-receptors or α-adrenoceptors can be distinguished into two main subtypes, α_1 and α_2.

1. Alpha$_1$-adrenoceptors

The primary response of activation of α_1-adrenoeceptor is the increase in phosphatidylinositol turnover, which often associated with calcium mobilization. After agonist-receptor binding, phosphatidylinositol-1,4,5-phosphate is hydrolyzed into diacylglycerol and inositol-1,4,5-triphosphate, the two potent second messengers (as shown in Figure 10). Inositol-1,4,5-triphosphate increases intracellular calcium released from endoplasmic reticulum. Elevated intracellular calcium can influence a range of intracellular event, including vesicle-mediated secretion and contraction (in muscle cells) (Parkinson, 1990).

Diacylglycerol on the other hand remains in the membrane and acts as anchored binding site for protein kinase. Protein kinase C activation requires calcium and phophatidylserine as cofactors (Ariens and Simonis, 1983; Parkinson, 1990). Activation of protein kinase C also seems to lead to phosphorylyzation of cytoplasmic protein such as myosin light chain of muscles (Lefkowitz *et al.*, 1996).

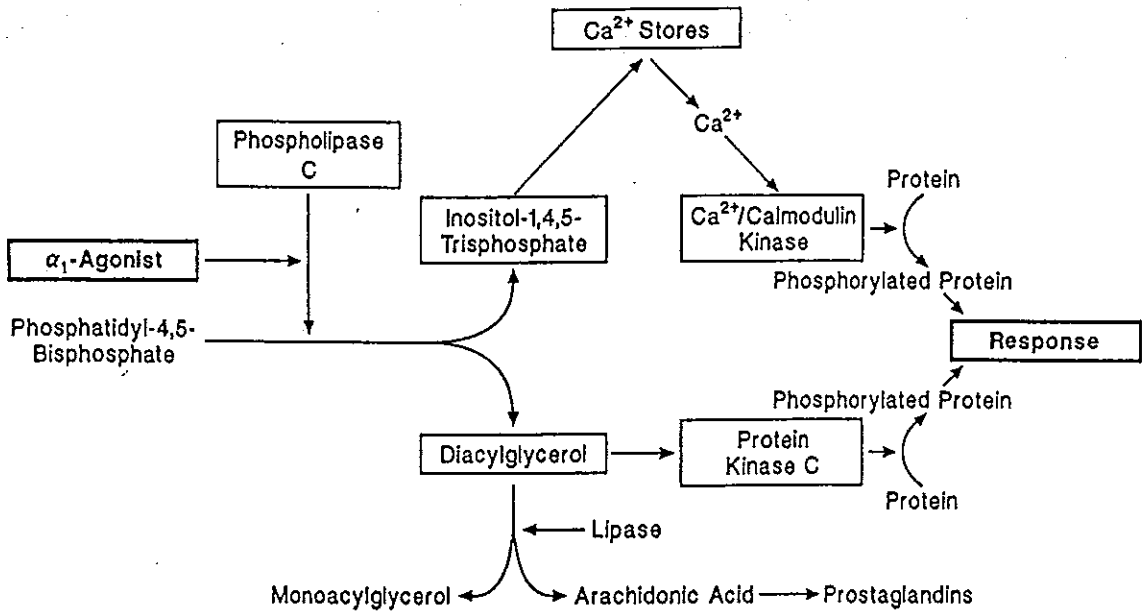


Figure 10 Pathway for hydrolysis of inositol phospholipids in activation of α_1-adrenoceptors (Source: Parkinson, 1990, pp. 19)

2. Alpha$_2$-adrenoceptors

The α_2-adrenoceptor located on the presynaptic terminals is activated by norepinephrine and similar molecules; activation diminishes further release of norepinephrine from the nerve ending. The α_2-receptor appears to be similar to other receptors that are negatively coupled to adenylate cyclase in that they lower the maximum rate of cAMP synthesis but do not affect the affinity for substrate (Parkinson, 1990). Alpha$_2$ inhibition of adenylate cyclase occurs through the mediation of an inhibitory regulatory protein (Gi), which couples

the α_2-adrenoceptor to the adenylate cyclase in an inhibitory manner. The precise mechanism by which α_2-receptor inhibits cyclase has not been elucidated. Two major possibilities were postulated. Firstly, the free beta-gamma units of Gi combine with the free alpha subunit of Gs, rendering them inactive. Secondly, the alpha subunit of Gi directly inhibits adenylate cyclase activity (Hoffman, 1998).

The beta-adrenoceptors

The β-adrenoceptors were subdivided by comparison of the relative potencies of different sympathomimetic amines in eliciting various responses in intact and isolated tissues (Lands *et al.*, 1967; Brodde, 1989; Cruichshank and Prichard, 1994). The β_1-adrenoceptors, which have been cloned, predominate in the heart, were stimulated by catecholamines with a rank order of potency; isoproterenol > epinephrine = norepinephrine. The β_2-adrenoceptors, which predominated on smooth muscle cells, isoproterenol was again more potent than epinephrine and 100 times more potent than norepinephrine (Brodde, 1989; Cruichshank and Prichard, 1994). More recently, the prejunctional β-adrenoceptors, β_2-subtype, have been described to facilitate the release of neuronal norepinephrine (see revieweed by Borkowski, 1988). The order of potency in stimulating adenylate cyclase for various β-agonists was different for the β_3-adrenoceptors when compared to β_1 and β_2-subtypes, but similar to lipolysis in rat adipocytes and relaxation of guinea-pig ileum (Cruichshank and Prichard, 1994). The rank order of potency of agonists was isoproterenol = norepinephrine > epinephrine for β_3-subtype (Lefkowitz *et al.*, 1996).

Activation of β-adrenoceptors results in an increase in cyclic adenosine monophosphate (cAMP) synthesis by stimulating adenylate cyclase. Activation of the cyclase enzyme is mediated by the stimulatory guanine nucleotide-dependent coupling protein Gs. Cyclic AMP is the major second messenger of β-adrenoceptor activation (Hoffman, 1998). The pathway of cAMP production in activation of β-adrenoceptors is illustrated in Figure 11.

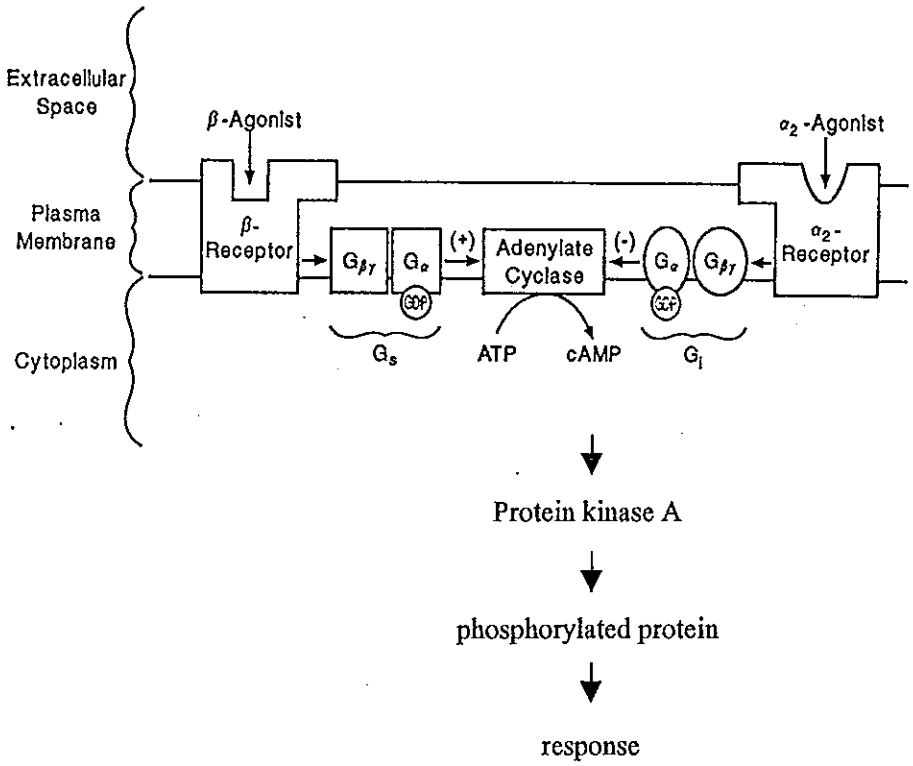


Figure 11 The pathway of β-adrenoceptors activation (Source: Parkinson, 1990, pp. 23)

Cardiac beta-adrenoceptors

The heart contains predominantly the β_1 type, which is responsible for both chronotropic and inotropic responses but there is also β_2-adrenoceptor that may be linked to the inotropic responses (Parkinson, 1990). Autoradiographic studies in man indicate that both β_1- and β_2-adrenoceptors are evenly distributed in cardiomyocytes, with a low density of β_2-adrenoceptor occurring in intramyocardial blood vessels. Ligand studies reveale that about 20 % of the receptors in the right and left ventricles and 35 % in the right atrial appendage are β_2 subtype (Cruickshank and Prichard, 1994). In guinea-pig atria, the ratio of β_1:β_2 subtypes is 75:25 (Summers and Molenarr, 1987; Brodde, 1989).

Function of cardiac beta-adrenoceptors

There is evidence that the chronotropic and inotropic effects are mediated via both β_1 and β_2 subtypes. Cruichshank and Prichard (1994) reviewed that isoproterenol-induced tachycardia was inhibited considerably more effectively by non-selective blockade than by β_1–selective blockade. In contrast, exercise-induced tachycardia is a β_1-receptor phenomenon. The peripheral β_2-vasodilation resulting in reflex vagal tachycardia occurs so as to maintain heart rate, cardiac output and blood pressure. In *vitro* studies with the electrically stimulated right atrial muscle revealed that the dose-response curves of the inotropic response to isoproterenol was shifted to the right by both β_1- and β_2-blockade, but a greater effect was seen with combined β-blockade.

During the plateau phase of the cardiac action potential, the concentration of free cytosolic calcium ions in the myocytes is rapidly increased due to the influx of extracellular calcium ions via voltage dependent calcium channels and

the release of calcium ions bound intracellularly to the sarcoplasmic reticulum. The raised cytosolic calcium leads to increased binding of calcium ions to and activation of the contractile proteins. The final common pathway of inotropic responses is the enhanced bioavailability of free cytosolic calcium during this process. Thus, catecholamines acting at the β-adrenoceptor increase not only force and rate of contraction but also the rate of myocardial relaxation and the overall shortening of the contractile events (Barnett, 1989).

Beta-adrenergic receptor of the airway smooth muscle

The airway smooth muscle is mainly regulated by three types of extrinsic autonomic nerves, excitatory parasympathetic nerves, inhibitory sympathetic nerves and non-adrenergic non-cholinergic (NANC) nerves. The distribution of types of receptors in airway smooth muscle may depend on the density of innervation and on the distance between the autonomic nerve terminals and the muscle cell membrane (see reviewed by Tomita and Bulbring, 1987). The β_1:β_2 ratio in guinea-pig trachea is 15:85 (Brodde, 1989). The regional difference of autonomic innervation is observed by the histochemical evidence. In guinea-pig airway, norepinephrine-containing fluorescent nerve fibers are distributed densely in the proximal tracheal muscle and sparsely in distal tracheal muscle but absent in bronchial muscle. Muscarinic receptors are also more pronounced in proximal than distal airway (see reviewed by Kamikawa, 1994). There is no evidence supporting direct innervation of adrenergic nerve in human airway smooth muscle (Pack and Richardson, 1983; Sheppard *et al.*, 1983; see reviewed by Kamikawa, 1994).

The mechanism of action of catecholamines to produce relaxation responses in airway smooth muscle is still unknown. However, the study in guinea-pig trachea of Kumar (1978) and Barnes (1992) showed that the relaxation of airway smooth muscle caused by relocating calcium away from the contractile protein through some process. On the other hand, Bulbring and Tomita (1987) and Ito *et al.* (1995) noted that the relaxation of guinea-pig trachea caused by isoproterenol or epinephrine was shown to be accompanied by suppression of the slow waves and membrane hyperpolarization through activation of β_2-adrenoceptor. Norepinephrine has little effects on the tracheal relaxation of mammalian airway smooth muscle. Most of the norepinephrine-containing nerve fibers are seen around peritracheo-bronchial plexus whereas norepinephrine causes a prejunctional inhibition of cholinergic neurotransmission. Norepinephrine released from adrenergic nerves preferentially acts on α_2-adrenoceptors located in cholinergic nerve cell body or terminals. In human and canine airways, the presence of prejunctional β_1- or β_2-adrenoceptors, which inhibits the release of acetylcholine was also reported (see reviewed by Kamikawa, 1994). These findings indicate that adrenergic nerves do not play a principal role in regulation of the airway but rather have modulatory function on cholinergic transmission (see reviewed by Bulbring and Tomita, 1987; Pendry and Maclagan, 1991; Barnes, 1992; see reviewed by Kamikawa, 1994).

REGULATION OF ADRENERGIC RECEPTOR FUNCTION

The radioligand binding studies of β-adrenoceptors revealed that the tissue concentration of the β-adrenoceptors is not a fixed number but rather is dynamically regulated by a variety of drugs, hormones, pathological and physiological conditions (Brodde, 1989). The regulation of signal transduction in the sympathetic nervous system of tissue containing β-adrenoceptors is firstly associated with the adenylate cyclase system. For example, exposure to agonists result in increase in the cAMP synthesis, which due to activation of the adenyl cycalse, while exposure to antagonists results in the inactivation of the adenylate cyclase system (see reviewed by Lefkowitz and Caron, 1988; Parkinson, 1990). However, it has been known that after chronic exposure to agonists, the rate of cAMP production declined and the tissue is desensitized (Brown, 1992; Chess-Wiliams, 1993). In contrast, chronic exposure to antagonists causes the hypersensitization of β-adrenoceptors (Brodde, 1989; Parkinson, 1990).

Beta-adrenoceptors desensitization

Some studies demonstrated that exposure of the tissue to catecholamine resulted in a rapid rise followed by a decrement of the intracellular levels of cAMP. Subsequent stimulation of the exposed tissues with β-agonists caused a markedly diminished in cAMP level (Brodde, 1990). Such a decrease in responsiveness to pharmacological stimuli with time is a general mechanism of cellular adaptation that is referred as desensitization, tachyphylaxis or

refractoriness. The mechanism of desensitization may involve changes in the coupling of receptor to postreceptor events. However, Arrons *et al.* (1982) proved that chronic administration of agonist such as ephedrine and terbutaline on the human lymphocyte caused a decrease in receptor density of β-adrenoceptors.

Two major mechanisms of desensitization of the adenylate cyclase-coupled β-adrenoceptors have been postulated, homologous and heterologous desensitization (Brodde, 1989; Parkinson, 1990).

1. Homologous desensitization

Chronic exposure of β-adrenoceptors to β-agonists results in the decrease in the rate of cAMP synthesis. If the response to only β-agonist is attenuated, then this is referred to as an agonist-specific or homologous desensitization. Very rapidly after occupancy of the β-adrenoceptors by a β-agonist, there is a functional uncoupling of the receptor from the adenylate cyclase activation (Brodde, 1989).

The mechanism underlying homologous desensitization (Figure 12) appears to involve phosphorylation of the receptor by β-adrenoceptor kinase. Phosphorylation begins within minutes of agonist occupation. The phosphorylation does not appear to involve cAMP or require the coupling of receptor to Gs. Rather, there is evidence for a cytosolic kinase (β-adrenoceptor kinase) that appears to phosphorylate only the agonist-occupied receptor. Presumably the conformational change subsequent to agonist occupation reveals a phosphorylation of β-receptors and uncouples them from the Gs protein, impairing their ability to stimulate cAMP synthesis. Phosphorylation

also promotes internalization of β-adrenoceptors into intracellular compartments that are not yet fully characterized. In this compartment, a phosphatase appears to dephosphorylate the receptor so it can return to the cell surface (Brodde, 1989; Parkinson, 1990).

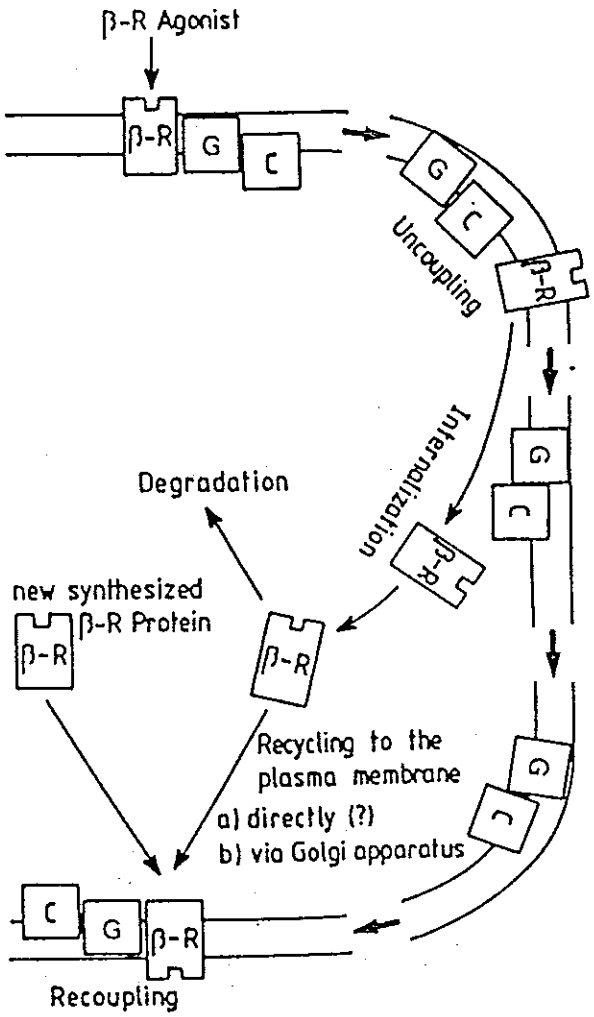


Figure 12 Model for homologous desensitization of β-adrenoceptors. β-R = β-adrenoceptor; G = G protein; C = catalytic unit of the adenylate cyclase (Source: Brodde, 1989, pp. 227)

Chang *et al.* (1982) demonstrated the effects of prolonged infusion of norepinephrine and isoproterenol on the physiological reactivity and binding properties of cardiac β-adrenoceptors. Infusion of either norepinephrine or isoproterenol significantly reduced the *in vitro* chronotropic potency of isoproterenol in isolated right atria and inotropic potency in left atria. No significant change in the density of β-adrenoceptors in either atrial or ventricular tissues was observed. The results indicated that selective desensitization of β-adrenoceptor-mediated response was unrelated to decrease binding site density.

Martin and Broadley (1994) studied the effects of chronic intravenous administration of doxepamine; a selective agonist for D_1-dopamine receptors and β_2-adrenoceptors, and isoproterenol; a nonselective β-adrenoceptor agonist, for 7 days. The β_1-adrenoceptor-mediated response of the spontaneously beating right atrium (increase in rate) and paced left atrium (increase in tension) showed significant reduction in sensitivity to isoproterenol following isoproterenol infusion. After infusion with doxepamine, however, there was no change in sensitivity of the right or left atria to the β_1-adrenoceptor stimulation by isoproterenol. The β_2-adrenoceptor-mediated relaxation response to isoproterenol of the pulmonary artery, which was preconstricted with norepinephrine, showed no significant difference in maximum response or EC_{50} in tissue from isoproterenol- or dopexamine-infused rats compared with vehicle-infused controls. Thus, there appeared to be selective down-regulation of peripheral β_1-adrenoceptors but not β_2-adrenoceptors.

2. Heterologous desensitization

In contrast to homologous desensitization, heterologous desensitization seems to be a cAMP dependent process (Brodde, 1989). This heterologous desensitization, in which many types of receptors and responses are mediated simultaneously, can occur in the absence of agonist and also involve phosphorylation of the receptor but not internalization. In part, the diminished response to agonists involves uncoupling of receptor from adenylate cyclase, and this can be correlated with cAMP-dependent receptor phosphorylation. Since the response to stimulation of other receptors and to agents that bypass receptors is also diminished, then there must be changed in the adenylate cyclase complex as well. Functional impairment of Gs and increase activity of Gi have been observed in association with heterologous desensitization of β-adrenoceptors (Parkinson, 1990).

Phosphorylation of β-receptors by cAMP-dependent kinase and protein kinase C appears to take place the same serine residues (Brodde, 1989; Parkinson, 1990). Since many different hormones and drugs can activate cAMP-dependent protein kinase and protein kinase C, this type of phosphorylation reaction may lead to cross-regulation of signaling pathways (Lefkowitz and Caron, 1988). For example, phorbol esters could induce desensitization of the β-adrenoceptors-couples adenylate cycalse. Since the common effect of β-agonists and phorbol esters is to activate protein kinase (protein kinase A via β-agonists and protein kinase C via phorbol esters) and since both classes of drugs evoke desensitization, it has been hypothesized that the common link in heterologous desensitization may be phosphorylation of the

β-adrenoceptors (Brodde, 1989). In addition, muscarinic stimulation in the heart can lead to β-adrenoceptor desensitization because some of the muscarinic receptors in heart are linked to elevate phosphatidylinositol breakdown, and this, in turn, lead to stimulation of protein kinase C. Therefore, this sequence of event illustrates how activity at one receptor (the muscarinic receptor) can modify the responsiveness of another receptor (the β-adrenoceptors) (Parkinson, 1990). The model for heterologous desensitization of β-adrenoceptors is illustrated in Figure 13.

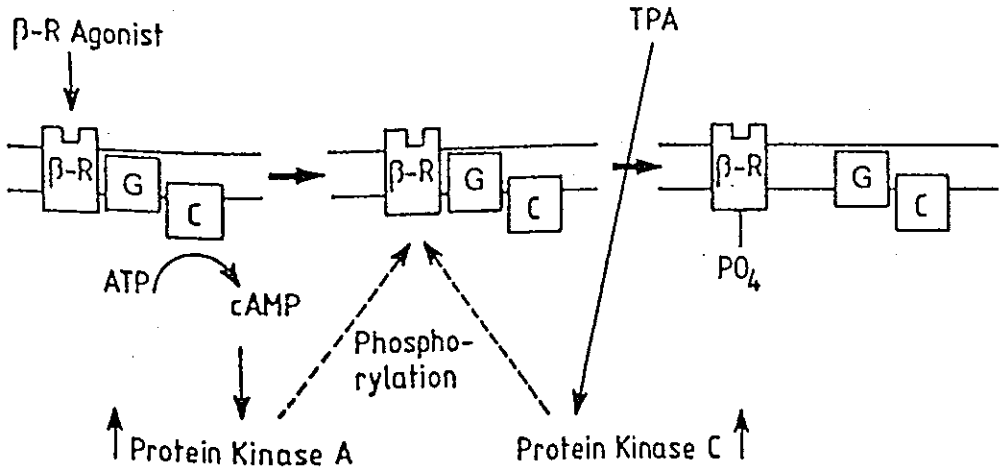


Figure 13 Model for heterologous desensitization of β-adrenoceptors. β-R = β-adrenoceptor; G = G protein; C = catalytic unit of the adenylate cyclase; TPA = 12-*O*-tetradecanoyl-phorbol-13-acetate (Source: Brodde, 1989, pp. 228)

Supersensitivity or up-regulation

In many *in vivo* studies in animals, depletion of catecholamines by treatment with 6-hydroxydopamine, reserpine and long-term treatment with adrenergic neuron blocking agent like guanethidine, are found to be associated with a supersensitivity of various tissues to the stimulation of β-agonist (Brodde, 1989). The β-adrenoceptor supersensitivity as measured by the activating effect of catecholamines on the adenylate cyclase is fairly good model of receptor supersensitivity of adenylate cyclase to catecholamine concentration. Pik and Wollemann (1977) demonstrated that in rat heart pretreated with 6-hydroxydopamine, there was a significant increase in adenylate cyclase levels after the stimulation of norepinephrine and isoproterenol. No significant difference in norepinephrine contents was observed after chemical denervation. These results seemed to emphasize the role of the receptor in short-term supersensitivity since it developed relatively early after denervation, even before the catecholamine content of the heart entirely disappeared.

In humans, supersensitivity was studied in the patients with acute myocardial ischemia. In these patients, the adenylate cyclase system became sensitized by two different and independent mechanisms. One form was linked to the β-adrenoceptors, the other was mediated by the adenylate cyclase itself. The receptor-linked sensitization was characterized by an increase in number of β-adrenoceptor and an increased capability of the β-adrenoceptor agonist to stimulate the adenylate cyclase. This receptor-specific sensitization was superimposed by a transient enzyme-coupled sensitization of the adenylate

cyclase itself. The receptor-independent elevation of adenylate cyclase activity was even retained after partial purification of the enzyme suggesting a covalent modification of the enzyme in acute myocardial ischemia. After prolonged periods of global ischemia, the persistent sensitization at the receptor level met an unresponsiveness adenylate cyclase. The mechanism of this inactivation of the enzyme activity remained to be elucidated. The significance of the persistent sensitization at the receptor level with increase of functionally coupled receptors could only be hypothesized.

Thus, β-adrenoceptors may in fact couple to different second messenger and signal transduction systems, such as ion channels. Recent evidence suggests that the activated stimulatory G protein, Gs, may couple directly to Ca^{2+} channel independently of an activation of the adenylate cyclase. By this mechanism the persistent sensitized β-adrenoceptors may additionally contribute to malignant arrhythmia in the infarcted heart even at a time when the adenylate cyclase activity is already diminished. (see reviewed by Strasser *et al.*, 1990; Cruickshank and Prichard, 1994).

Sporn *et al.* (1976) showed that 6-hydroxydopamine, an agent that destroyed adrenergic nerve terminals, caused an increase in the density of β-adrenoceptors in rat cerebral cortex without affecting their affinity for isoproterenol. The results demonstrated that the regulation of the postsynaptic response to catecholamines at central adrenergic synapse was mediated at least in part by change in the density of β-adrenoceptor.

Glaubiger *et al.* (1978) investigated that chronic withdrawal of adrenergic stimulation in rat heart, which was produced by guanethidine treatment *in vivo*

caused an increase in cardiac receptor density and responsiveness. Furthermore, in isolated, perfused heart obtained from guanethidine-treated animal, there was an increase in cAMP accumulation in response to a β-agonist, isoproterenol. These results suggested that the increased number of receptors might relate to the increased biochemical response.

Korzyn *et al.* (1982) showed that the surgical thoracic sympathectomy caused a dose-dependent supersensitivity of the canine heart to norepinephrine and isoproterenol. Administration of isoproterenol to intact dogs resulted in tachycardia. Norepinephrine produced bradycardia, probably as a reflex to the hypertensive response of this catecholamine. In higher doses, arrhythmia occurred without the appearance of tachycardia. Following surgery, the heart rate at rest significantly decreased. Injection of isoproterenol now produced significantly greater heart rate, while for norepinephrine, reversal in heart rate response occurred with the appearance of tachycardia. An important mechanism of the supersensitivity, which occurred in the sympathetic systems following denervation, was disappearance of catecholamine uptake. Normally, the action of extrinsically applied catecholamine was terminated at the synaptic level to a large extent by uptake into the nerve terminals. These terminals should degenerate following ganglionectomy, thus allowing increased catecholamine concentrations to remain within the synaptic cleft for longer periods. However, isoproterenol was an inadequate substrate for the uptake process and therefore, a different mechanism might be considered for the supersensitivity to this catecholamine. One possibility of a leftward shift of the

chronotropic dose-response curve to both catecholamines could be explained by an alteration of cardiac β-adrenoceptors.

Grassby and Broadley (1986) proved that reserpine-pretreatment induced supersensitivity in atria and ileum, which are mediated by β_1-adrenoceptor but not in aorta, lung and vas deferens, which are mediated via β_2-adrenoceptor. Therefore, only responses mediated via β_1-adrenoceptor exhibited reserpine-induced supersensitivity.

DeGaris and Pennefather (1987) studied the effects of some sympathomimatic amines on the contractility of the epididymal half of the rat vas deferens denervated by vasectomy. Supersensitivity to norepinephrine was greater by day 28 after vasectomy and persisted relatively unchanged throughout the remainder of the study (183 days). The magnitude of the leftward shift in the log concentration-response curves for norepinephrine in operated epididymal segments approached that produced in nisoxetine, a neuronal uptake blocker, pretreated unoperated segments. This inhibitor of neuronal uptake did not enhance the potency of norepinephrine in operated segments. These findings indicated that postganglionic denervation of the epididymal half of the rat vas deferens by vasectomy led to a slowly developed and prolonged supersensitivity to norepinephrine, which was primarily due to the loss of the neuronal uptake facility.

Spadari *et al.* (1988) demonstrated that the swim-induced in rat reduced the norepinephrine content and induced a long-lasting increase in plasma corticosterone level. Pacemaker isolated from swim stressed rat showed supersensitivity to the chronotropic effects of isoproterenol. Sensitivity to

norepinephrine was not significantly altered. The results suggested that corticosterone mediated impairment of the extraneuronal uptake could increase the availability of endogenous epinephrine and constantly, be related to the modulation of peripheral sympathetic transmission during the acute stress response.

Mechanisms of cocaine induced supersensitivity

It has been described previously that the cardiovascular complication is the major causes related to the lethal toxicity and death among cocaine users. Cocaine increases blood pressure, heart rate and force of contraction by enhancing of the adrenergic responses to catecholamines. These sympathomimetic effects of cocaine may be attributed to the increase in cardiovascular abnormalities but the mechanisms responsible for the supersensitivity are not clear. Several hypotheses have been continually advanced to explain the mechanisms of cocaine induced supersensitivity to catecholamines, but only two are ever given much attention. Firstly, the presynaptic hypothesis suggested that supersensitivity was due to the absence of uptake-1 clearance, which depended on intact adrenergic nerves (Hammond *et al.*, 1992). The uptake and storage of catecholamines by adrenergic nerves is currently believed to play a dominant part in regulation of response to the amines. Cocaine blocks the reuptake process of catecholamines at the postganglionic sympathetic nerve ending, thus diverting the amine to the vicinity of appropriate tissue receptor and interfering amine inactivation (Kalsner and Nickerson, 1969). Because this synaptic site is the major site for

the termination of action of locally released and circulating catecholamines, thus, an increasing in the synaptic concentration of these monoamines available for binding to the adrenergic receptors might be the mechanism of enhancing the effects of endogenous or exogenously administered catecholamines (Trendelenburgs *et al.*, 1972; Surprenant and Williams, 1987). Secondly, the postsynaptic hypothesis proposes that cocaine has a direct action on the effector cells, and these actions are more obvious in either sparsely innervated or non-innervated tissues. Such postsynaptic changes may involve selective allosteric alterations of the agonist-receptor and/or non specific alteration of permeability of ion fluxes (Nakatsu and Reiffenstein, 1968; Kalsner and Nickerson, 1969; Reiffenstein and Triggle, 1974; see reviewed by Fleming *et al.*, 1990; Alburges *et al.*, 1996). However, there are still controversial that which mechanisms play a dominant part in supersensitivity induced by cocaine (see review by Fleming *et al.*, 1990; Hammond *et al.*, 1992).

1. Uptake blockade mechanism

Cocaine is one of the potent neuronal uptake blocking agents. It has been widely used to study the role of neuronal uptake in the sympathetic nerve. In 1959, Macmillian observed that the vasoconstriction effects of the sympathetic stimulation and of norepinephrine infusion were highly increased after an injection of cocaine. He suggested that cocaine could inhibit the uptake of norepinephrine, which was called uptake-1 mechanism, into the tissues and thus increasing its effective concentration at the receptor and eventually increased in the responses (Hertting *et al.*, 1961; Day, 1979). This evidence is supported

by the study of Withby *et al.* (1960) who used radioactive labeled norepinephrine to measure the uptake mechanism of various tissues of cat received an injection of cocaine. The data showed that many tissues of the cat took up norepinephrine from the blood circulation and it was greatest in those tissue, such as heart, spleen and adrenal gland, which have a rich sympathetic innervation. This experiment also showed that the uptake process was significantly prevented by cocaine. In addition, Suhara *et al.* (1996) identified the possible lethal mechanisms and the accumulation of cocaine in various organs. The effects of cocaine on [^{11}C]norepinephrine uptake in cynomolgus monkeys were measured by positron emission tomography. Cocaine (5 mg/kg) pretreatment noticeably inhibited [^{11}C]norepinephrine uptake in the heart and lung. There was a significant uptake in the liver which was decreased following cocaine pretreatment as well. The result of this study confirmed that cocaine blocked the neuronal uptake of norepinephrine in sympathetic nerve terminal in the myocardium.

Many investigators proved that the uptake blocking action related to the induced supersensitivity mechanism of cocaine. Trendelenburgs *et al.* (1972) studied the mechanism of cocaine in potentiating the effect of norepinephrine and related amines in the smooth muscles isolated from the nictitating membrane of the reserpine-pretreated cats. Cocaine significantly caused a concentration-dependent increase in the response of the isolated membrane to norepinephrine with a maximal increase of about of 115 times of normal. There was a relationship between rate of norepinephrine uptake and degree of

supersensitivity. The results indicated that the effect of cocaine on the nictitating membrane was predominantly prejunctional.

Masuda *et al.* (1980) investigated the effects of cocaine, a neuronal uptake inhibitor in comparison with metanephrine, an extra neuronal uptake blocking agent. Cocaine potentiated and prolonged the positive chronotropic and inotropic responses to norepinephrine infusion. In contrast, metanephrine neither potentiated nor prolonged the cardiac responses to norepinephrine. Data revealed that the uptake blockade mechanisms of cocaine played a pivotal role in potentiation and prolongation of the cardiac response to norepinephrine. In addition, these findings suggested that the neuronal uptake process was more important than the extraneuronal uptake process in the removal of exogenous infused norepinephrine in the heart.

Surprenant and Williams (1987) recorded the intracellular membrane potential current made from neurons of rat nucleus locus coeruleus and guinea pig submucous plexus after cocaine exposure. These neurons exhibited inhibitory postsynaptic potentials (i.p.s.p.s) which resulted from norepinephrine acting on α_2-adrenoceptors to cause an increase in potassium conductance. Cocaine (0.2-30 μM) reversibly increased the duration of the i.p.s.p. or inhibitory post synaptic current (i.p.s.c) in both locus coeruleus and submucous plexus neurons, produced a maintained hyperpolarization or outward current, and increased the amplitude and duration of spontaneous i.p.s.p.. Moreover, outward current produced by superfusion norepinephrine was greatly increased by cocaine. These results suggested that cocaine played an essential role in the neuronal uptake process of norepinephrine released from adrenergic nerve.

This study also showed that the action of cocaine in inhibiting neuronal uptake of norepinephrine released from adrenergic nerve ending played an important part in the increased sensitivity of tissue.

Abrahams *et al.* (1996) demonstrated the mechanism of cocaine involving in the sympathetic discharge. The pentobarbital anesthetized rats, which their monoamines were depleted by pretreatment with reserpine and α-methyl-meta-tyrosine, were received intraperitoneal cocaine injection (1 mg/kg). In saline-control rats, cocaine elicited marked and prolonged decrease in sympathetic nerve discharge. The magnitude and duration of these responses were significantly attenuated after 1 day of monoamine depletion. After 2 days of depletion, the sympathoinhibitory response was abolished and replaced by a small, brief increase in sympathetic nerve discharge. They concluded that a functionally intact monoaminergic system was essential for the sympathoinhibitory response to cocaine.

2. Receptor sensitizing mechanisms

Contrary to the presynaptic hypothesis, many researchers believed that the reuptake blockade alone can not portray the cocaine induced sensitizing response of adrenergic receptors. Supersensitivity causing by the absence of uptake-1 clearance can not described the supersensitivity to isoproterenol which does not depend on uptake-1 clearance (Korzyn *et al.*, 1982; Hammond *et al.*, 1992). The experiment of Summers and Tillman (1979) demonstrated that desmethylimipramine (desipramine), a more neuronal uptake blocking agent, did not increase the sensitivity to norepinephrine compared to cocaine at the

same concentration, in addition, high concentration of desipramine reduced the sensitivity and maximal response. Further supporting evidence is provided by the observation that cocaine can potentiate response to norepinephrine in isolated vas deferens of male albino rats after denervation (Nakatsu and Raffeinstein, 1968). Vasa deferentia was pretreated with phenoxybenzamine (POB), an irreversibly-adrenoceptor blocking agent. Pretreatment with POB, therefore, blocked a sufficient number of receptor so that the maximum response to the agonist was reduced. After blockade with POB and the administration of norepinephrine until the concentration of norepinephrine reached an equilibrium state, an addition of cocaine into the bathing fluid still caused an increase in the maximum response to norepinephrine. Because the portion of receptor remaining after POB blockade could not produce the maximum response of which the tissue was capable, and because the supramaximal dose of norepinephrine was unchanged indicating by the equilibrium with the additional norepinephrine, thus, the maximum response was dependent on the number of receptor remaining after POB. The maximum response of such a tissue to norepinephrine in the presence of cocaine could not be caused by the increase in the local concentration of norepinephrine, but could then only be modified by receptor or postreceptor level mechanism.

Kalsner and Nickerson (1969) investigated the mechanism of cocaine in potentiating the response to amines in isolated strip of thoracic aorta, which had been stored at 6 °C to degenerate the sympathetic innervation. Data showed that although cocaine potentiated response to norepinephrine, epinephrine and phenylephrine, and slowed their inactivation, the correlation between these

two parameters under various experimental conditions was poor. In all cases the delay in the intrinsic inactivation was inadequate to account for the observed potentiation. This effect of cocaine is similar to those of procaine, which also produced the potentiation effect but occurred without delayed amine inactivation. Procaine slowed the inactivation of phenylephrine, apparently by the same mechanism, as did cocaine. However, procaine did not potentiate response to phenylephrine in any experiment. These results could be interpreted that cocaine could act directly at the effector cells to make them hyperresponsive.

Shibata *et al.* (1971) exhibited the potentiative effects of cocaine on catecholamines in aortic strips from young and old rabbits. The histochemical study apparently demonstrated catecholamines-specific fluorescence localized in the smooth muscle layers of the media in the young rabbit aorta but not in the old rabbit. However, no significant differences were found between the mean ED_{50} values for the cocaine potentiating responses of norepinephrine in the young and old rabbit preparations. These data suggested that the mechanism of the cocaine potentiating response involved some actions on the effector cells rather than on nervous element within the tissues.

Reiffenstein and Triggle (1974) studied the supersensitivity mechanism of cocaine (3.3×10^{-7} – 3.3×10^{-5} M) in smooth muscle of human umbilical arteries, which is devoid of sympathetic nerve innervation. The concentration of cocaine, which was proved to have no effect on the uptake of norepinephrine by smooth muscle of human umbilical arteries, was found to potentiate response of the tissue to norepinephrine (3×10^{-5} M) and 5-hydroxytryptamine (3.3×10^{-4} M).

It was clear that cocaine, in this tissue, was acting via a mechanism, which was not related to blockade of norepinephrine uptake and which represented a direct action on the effector cells. This might be equivalent to the "postsynaptic" action, which was suggested to occur in innervated systems (Nakatsu and Reiffenstein, 1968; Shibata *et al.*, 1971).

Alburges and Williams (1993) evaluated the mechanism of effects of chronic cocaine exposure on the central catecholamine neurotransmitter in rats. Groups of rats were injected with cocaine (15 mg/kg, i.p., twice a day) or saline for 1, 3, 7, 14 or 21 days. Following chronic cocaine exposure, the rat dopaminergic receptors significantly increased depending on dose of cocaine. Cortical and striatal tissues were analyzed for norepinephrine, dopamine and serotonin and their metabolite concentrations using HPLC method. Chronic administration of cocaine did not change the cortical and striatal concentrations of neurotransmitters and their metabolites under this study. These results proved that changes in the dopaminergic receptor following chronic cocaine exposure were not due to change in the neurotransmitter concentration. Therefore, it was possible that the behavioral effects resulting from long term cocaine use might be more likely associated with changes in the central catecholamine receptor.

Alburges *et al.* (1996) also demonstrated that chronic cocaine administration (5, 10, 15, 20 and 25 mg/kg, i.p., twice a day for 21 days) did not significantly change the concentration of epinephrine, norepinephrine, 5-hydroxytryptamine, dopamine and their metabolites in cortical and striatal tissues of the brain. In addition, the cocaine concentrations in the brain regions

did not change with the different doses used. Accumulation of ecgonine methyl ester, a metabolite of cocaine, was the only alteration found. These observations suggested that chronic cocaine administration might cause long-term change in the brain monoamine system and the behavioral sensitization effects described during cocaine use might involve the up-regulation in the dopaminergic receptor.

The most acceptable interpretation of those data mentioned above is that cocaine may directly act at the postsynaptic sites to produce an alteration in the effector cells, thus making them capable of generally increased maximum responses (see reviewed by Fleming *et al.*, 1990 and Mouhaffel *et al.*, 1995; Alburges *et al.*, 1996).

The mechanisms of cocaine induced postsynaptic supersensitivity remains unresolved, but some explanations have been proposed to describe this responsiveness of adrenergic receptors. Cocaine acts at the receptor either to increase the number of receptor or, via an allosteric conformational change to alter the intrinsic activity or affinity of the receptor (Nakatsu and Reiffenstein, 1968; Reiffenstein and Triggle, 1974; King *et al.*, 1994; Unterwald *et al.*, 1994). Another explanation for the increase in response to catecholamine is that cocaine may change the metabolism of calcium by affecting intracellular storage site of Ca^{2+} or membrane Ca^{2+} fluxes (Shibata *et al.*, 1971; Summers and Tillman, 1979; Kalsner, 1993; He *et al.*, 1994).

Beta-adrenoceptors changes evoked by β-adrenoceptor antagonists

The β-adrenoceptor antagonists are commonly used in the therapy of angina pectoris and hypertension (Bowman and Rand, 1980; Barnett, 1989; Bowkoski, 1989). There are some reports showing that chronic treatment of β-adrenoceptor blockade may prevent progressive down-regulation of the cardiac β-adrenoceptor or induce supersensitivity and up-regulation. Such a β-adrenoceptor supersensitivity can be caused by an increase in β-adrenoceptor number as a reflection of the β-adrenoceptor blockade. In the patients who had been pretreated with propranolol and pindolol, the β-adrenoceptor density was 52% higher than non-treated subjects (Barnett, 1989; Brodde, 1989; Cruickshank and Prichard, 1994).

CHAPTER 3

MATERIALS AND METHODS

1. Experimental animals

All experiments were carried out in adult guinea-pigs. Guinea-pigs of either sex, weighing 400-600 g were supplied from the animal house, Faculty of Science, Prince of Songkla University. They were housed in an air-conditioned room (24-26 °C) with a 12 hr light/dark cycle. The animals were fed with rodent laboratory chow and water *ad libitum*.

2. Drug pretreatment

Guinea-pigs were divided into control and cocaine treated groups as follows.

2.1 Control groups

The animals in this group received 1 ml/kg of 0.9% w/v sodium chloride solution intraperitoneally twice a day for 14 days. After the last injection of the saline solution, the animals were killed by cervical dislocation and exanguinated at 1, 24 or 72 hours.

2.2 Cocaine treated groups

Guinea-pigs were chronically treated with cocaine hydrochloride as described by Darmani *et al.* (1992). They were injected with cocaine hydrochloride 1, 1.25 or 5 mg/kg intraperitoneally twice a day for 14 days, in the same way as control groups. After cocaine cessation, the animals were also killed at 1, 24 or 72 hours.

3. Experimental protocol

Part A Studies on guinea-pig isolated atria and trachea

After the cessation of saline or cocaine treatment as mentioned above, the guinea-pigs were killed by cervical dislocation and exanguinated at 1, 24 or 72 hours. The atria and trachea were then isolated for studying of their contraction and their relaxation, respectively, in responses to exogenous applied catecholamines, norepinephrine and isoproterenol.

1. Isolated atrial preparation

After guinea-pigs were killed, their hearts were quickly excised and immersed in Krebs-Henseleit solution as described by Grassby and Broadley (1986). After removal of the pericardium, ventricular tissues were separated from atria. The isolated atria were then mounted in a 25-ml organ bath containing 37 °C Krebs' solution, and aerated with 95% O_2 and 5% CO_2 (Figure 14). The atria were set up under initial resting tension of 2 g. Changes in atrial tension were recorded isometrically with a force displacement transducer (Grass Instrument CO., Quincy, Mass., U.S.A.) connected to a Grass model 79D polygraph. The change in tension signal was used to trigger a Grass tachograph so that recording of atrial tension and rate of beating were made simultaneously. Before the onset of each experiment, the atrial preparation was equilibrated for at least 45 minutes and the Krebs' solution in the organ bath was changed every 10 minutes. Each experiment was repeated in at least 5 isolated atria (Satayavivad and Kraivaphan, 1987).

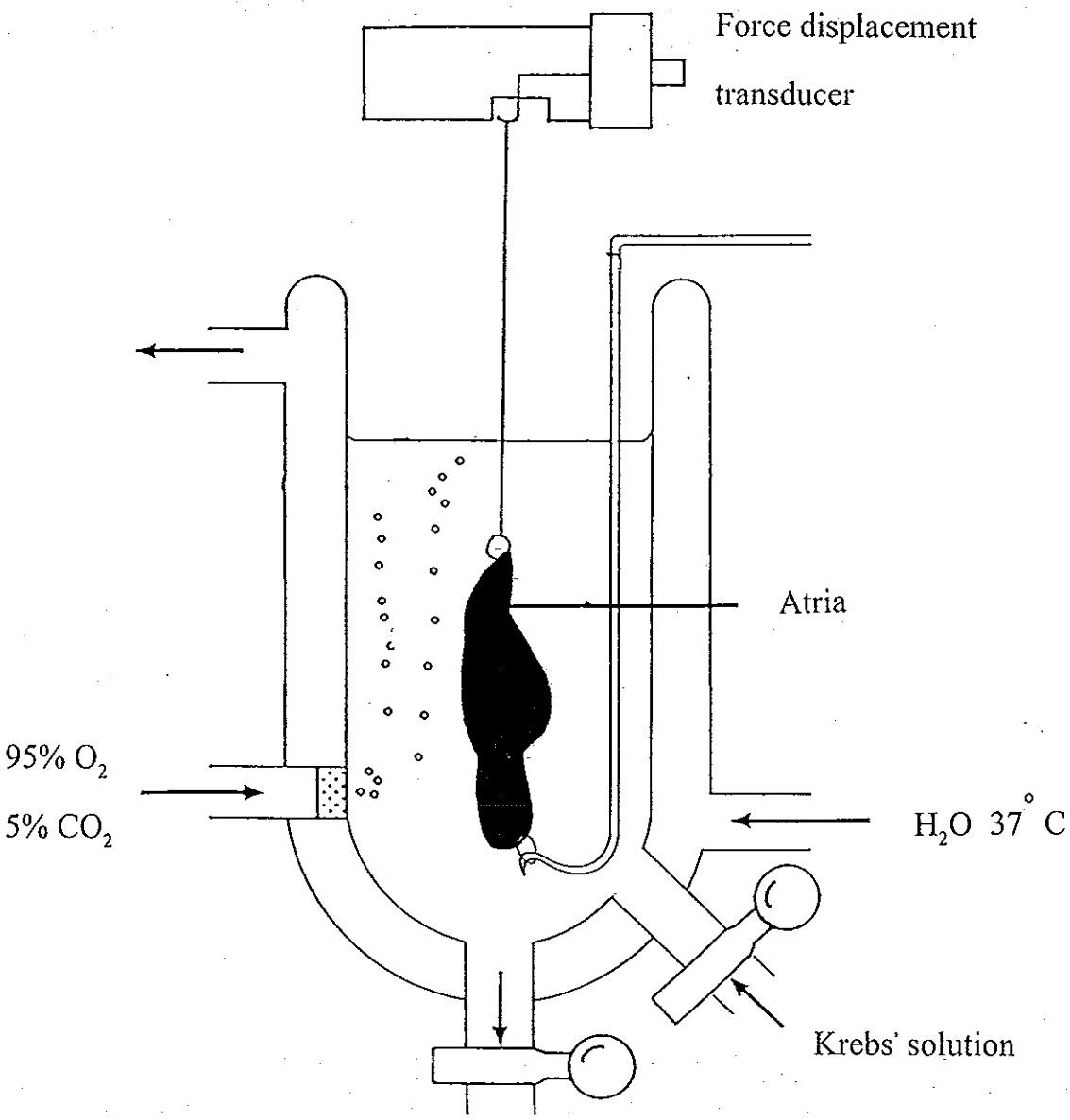


Figure 14 Set up of isolated guinea-pig atria for recording of their contraction

2. Isolated tracheal preparation

Guinea-pig tracheas from cervical to thoracic portion were dissected out. The trachea was opened by cutting longitudinally at the hyaline cartilage region which was opposite to the trachealis smooth muscle (Ito *et al.*, 1995). A segment of trachea, 2-3 cm in length, was zigzag cut at each two or three cartilaginous rings as shown in Figure 15 (Staff, Department of Pharmacology, Prince of Songkla University, 1995). The tracheal preparation was mounted in a 25-ml organ bath containing Krebs' solution maintained at 37 °C and gassed with 5% CO_2 in O_2 (Figure 16). The tissue was subjected to an imposed tension of 2 g and equilibrated for at least 1 hour before starting the experiment, during which the Krebs' solution was replaced with fresh Krebs' solution every 10 minutes. After equilibration, the tracheal preparation was induced to contract by being exposed to 1 μg/ml carbachol for 30 minutes. A stable contractile response of the trachea was observed before the beginning of each experiment. Change in tracheal tension was recorded isometrically with a force displacement transducer (Grass Instrument, Quincy, Mass., U.S.A.) connected to a Grass model 7D polygraph. Each experiment was repeated at least 5 isolated tracheas.

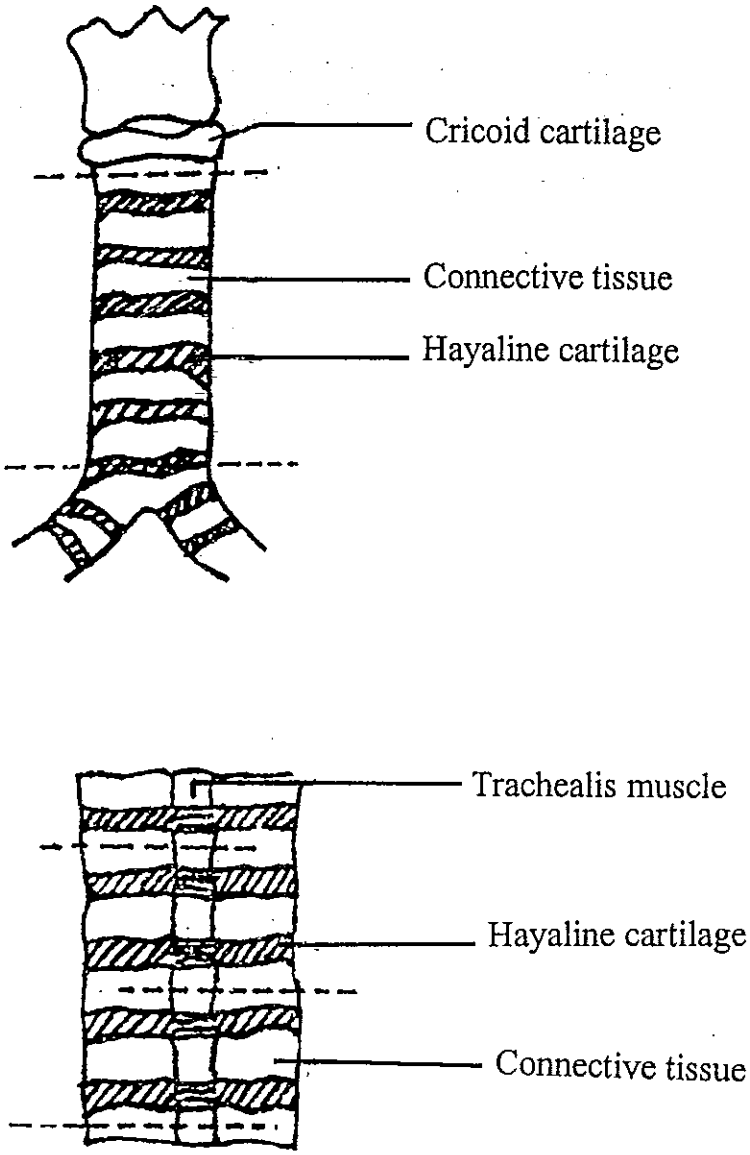


Figure 15 Diagram shows the segment of trachea dissected from guinea-pig (above) and preparation of tracheal strip by zigzag cutting (below)

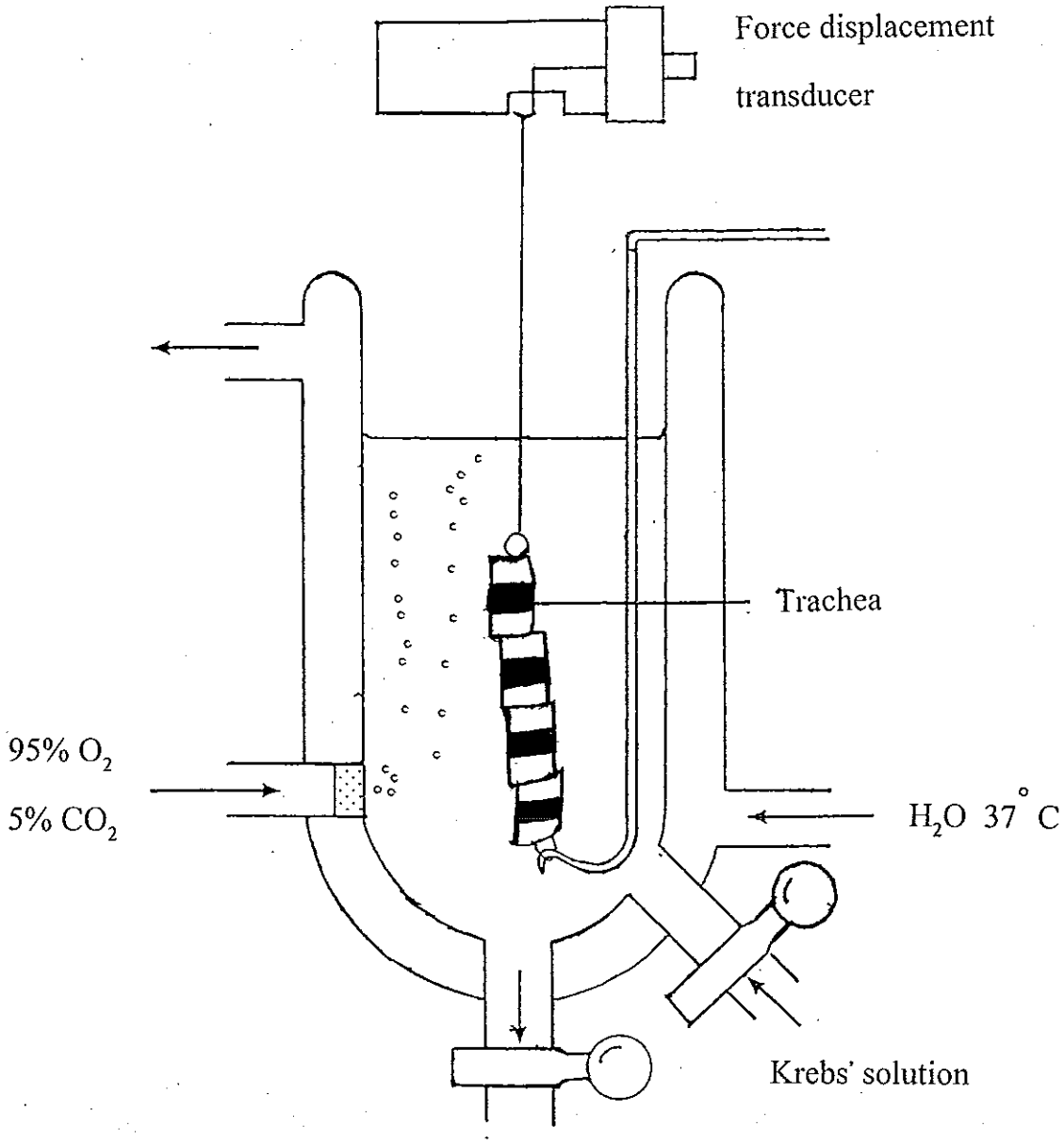


Figure 16 Set up of isolated trachea for recording of its contraction or relaxation

3. Experimental procedure

The isolated atria and trachea from cocaine-treated and control saline-treated animals were concomitantly studied in order to compare their responsiveness to catecholamines.

3.1 Determination of chronotropic and inotropic responses to catecholamines of the guinea-pig isolated atria

After the equilibration period, norepinephrine or isoproterenol was added to the bathing solution in a cumulative increase in concentration manner. The two drugs were administered in a three-fold increase in concentration. The interval between each addition was adjusted to allow the effect of each concentration to develop fully. The total volume of drug added was kept as small as possible with maximum allowance volume of 1 ml. The chronotropic and inotropic effects of each concentration of the catecholamines were recorded and the concentration-response curves were constructed.

3.2 Determination of the relaxation responses in trachea to catecholamines of the guinea-pig isolated trachea

Prior to the studies of the effects of norepinephrine or isoproterenol, the trachea was induced to contract by pretreatment with carbachol (1 μg/ml). After the maximum contraction was obtained, norepinephrine or isoproterenol was added into the bathing fluid in the cumulative increase in concentration schedule as those described for isolated atria but in a ten-fold increase in concentration. The relaxing effect of each concentration of the two catecholamines was recorded and the concentration-response curves were prepared.

4. Drugs and chemicals

Drugs used in this part of experiments were norepinephrine bitartrate (L-levaterenol), (±)-isoproterenol hydrochloride and carbamylcholine hydrochloride (carbachol). They were purchased from Sigma Chemical Company (St. Louis, U.S.A.). Cocaine hydrochloride was obtained from Diosynth (Apeldoom, Netherlands). All drugs, except cocaine hydrochloride, were prepared as stock solution (10^{-1} M for norepinephrine and isoproterenol and 1 mg/ml for carbachol) in 0.1% ascorbic acid in water. Cocaine hydrochloride was dissolved in 0.9% sodium chloride solution. All drugs were kept at -4 °C until use. For each day of experiment, working solutions were freshly diluted from the stock solutions with Krebs' solution to appropriate concentrations. The Krebs-Henseleit solution had the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.9; $NaHCO_3$, 25; $MgSO_4.7H_2O$, 1.2; KH_2PO_4, 1.2; D-glucose, 11.7 and ascorbic acid, 0.14. All chemicals were analytical grades.

5. Data analysis

Data were expressed as mean ± standard error ($\bar{X} \pm$ S.E.). For each group, the log concentration-response curves were plotted. Regression lines were fitted to the linear portion of the log concentration response curves of norepinephrine and isoproterenol by the method of least squares. $[D]_{max50}$ values (concentration required to produced 50% of their own maximum effect) were calculated. Regression lines of the responses of atria and trachea from cocaine-treated groups and those corresponding control saline-treated groups

were tested for parallelism and their $[D]_{max50}$ were compared. The pD_2 values (the negative logarithm of the $[D]_{max50}$) were also determined.

The changes in the responsiveness to norepinephrine and isoproterenol of the atria and trachea isolated from the cocaine-treated and the control guinea-pig were also determined using $[D]_{max50}$ ratio ($[D]_{max50}$ of the catecholamines of the cocaine-treated groups divided by those $[D]_{max50}$ of control).

Data were also analyzed using two way analyses of variance (ANOVA). The analyses were performed on the individual $[D]_{max50}$ values obtained from each concentration-response curves for norepinephrine and isoproterenol of the preparations from both cocaine-treated and saline-treated guinea-pigs. When a significant heterogeneity in their estimate due to treatment was observed, further comparison of individual $[D]_{max50}$ values was made by Duncan's multiple range test. Statistical significance was achieved when p value < 0.05.

Part B Analysis of cocaine comncentration in plasma and cardiac tissues

1. Animals and protocol

Guinea-pigs were chronically pretreated with either 0.9% sodium chloride solution or cocaine hydrochloride (1.25, 2.5 or 5 mg/kg) as described above. After cessation of cocaine or sodium chloride solution, animals were killed at 1, 24 or 72 hr. Five milliliters of blood was collected from heart and put into a heparinized glass tube containing 250 units of heparin sodium. The plasma was separated by centrifugation at 3,500 rpm for 15 minutes. The heart was also dissected. Atria and ventricle were separated and 2 g of each were homogenized by Grinder tissue homogenizer in 5 ml of 0.9% sodium chloride

(Robert *et al.*, 1991). One hundred milligrams of sodium fluoride was added into both plasma and homogenated cardiac tissues in order to maintain the stability of cocaine (Isenchmid *et al.*, 1989). All samples were stored at -20 °C until analysis.

2. Chemicals and reagents

Cocaine hydrochloride was purchased from Diosynth (Apeldoom, Netherlands). Acetonitrile (HPLC grade), sodium fluoride (analytical grade) and disodium hydrogen phosphate (analytical grade) were purchased from JT Baker Inc. (Philipsburg, NJ, U.S.A.). Analytical grade glacial acetic acid, sodium dihydrogen phosphate, ammonium acetate, chloroform and 2-propanol were purchased from Merck (Darmstadt, F.R. Germany). Sterile water was obtained by deionization and filtration through a Milli-Q and Nanopure system (Millipore, Molsheim, France), respectively.

3. Instrumentation and chromatographic condition

The HPLC system used consisted of a liquid chromatograph (Milton Roy, Model CM 400, Riviera Beach, Florida, U.S.A.), a manual injector (Rheodyne, Model 7125, Cotaki, CA, U.S.A.) with a 100-µl sample loop and an ultraviolet detector (Milton Roy, Model Spectromonitor, 3100). Detection was made with the variable wavelength UV detector set at 235 nm and peak area was measured with an integrator (Jasco, Model 827-IT, Tokyo, Japan). A recorder was used with attenuation set at 8 mV F.S. and chart speed at 2 mm/min.

Chromatographic separation was performed on a Novapak C_{18} column, particle size 4.5 µm, 3.9 x 150 mm I.D. (Waters Associated, Milford, U.S.A.).

A guard-pak precolumn module was used to obviate the effect of rapid column degeneration.

4. Analytical method

Cocaine concentrations in plasma, atrial and ventricular tissues were assayed by a High Performance Liquid chromatographic (HPLC) method modified from that described by Lim and Peters (1984).

4.1 Mobile phase

The mobile phase consisted of 200 ml of acetonitrlie and 700 ml of water containing 0.1 M ammonium acetate (adjusted to pH 5.15 with glacial acetic acid). The mobile phase was freshly prepared each day and was filtered through 0.45 μm filter paper (Pierce, Rock Ford, IL, U.S.A.) and degassed before use. The flow rate was 0.8 ml/min, which gave a pressure of 1,900-2,200 psi. All analyses were performed at room temperature (23-27 °C).

4.2 Stock solution

Stock solution of cocaine hydrochloride (1 mg/ml) was prepared by dissolving 10 mg of standard cocaine hydrochloride in methanol and adjusted the volume to 10 ml in volumetric flask. To assay precision and the percentage recovery, working standard solutions of cocaine (50, 100 and 500 ng/ml) were prepared by dilution of the stock standard solution with deionized water, drug-free plasma, atrial or ventricular tissue homogenates. The addition of sodium fluoride to all samples to prevent cocaine hydrolysis was necessary. The stock solution was stored at -20 °C.

4.3 Extraction procedure

Two hundred microlitters of plasma, atrial or ventricular tissue homogenates were extracted by adding 150 µl of 0.1 M Na_2HPO_4 (pH 8.9) and 5 ml of chloroform : 2-propanol (9 : 1). After vortex mixing for 2 min and centrifuging at 3,500 rpm (about 700 g) for 10 min, 4 ml of the organic phase were evaporated to dryness under air stream and the residue was reconstituted with 500 µl of 0.05 M NaH_2PO_4 (pH 5.2) and the content was transferred to a polypropylene microcentrifuge tube. After centrifugation for 10 min, 100 µl of the supernatant was injected onto the column (Tagliaro *et al.*, 1994).

4.4 Calibration procedure

Standard calibration was run on each day of analysis. Calibration samples of cocaine were obtained by adding standard cocaine hydrochloride solution to drug-free plasma, atrial or ventricular tissue homogenates and adjusting cocaine concentration to 25, 50, 200, 400 and 800 ng/ml. The spiked plasma, atrial and ventricular tissue samples were extracted and assayed as described above. The peak areas obtained from the chromatograms were plotted against cocaine concentrations. The calibration curves of cocaine in plasma, atrial or ventricular tissues were illustrated in Figure 17-19.

4.5 Detection limit

The detection limits of cocaine hydrochloride in plasma and homogenated cardiac tissues were 12.5 and 20 ng/ml, respectively.

4.6 Assay precision and variability

The within-day, between-day precision and variability were determined using plasma, atrial or ventricular tissues spiked with cocaine at low, medium

and high concentrations within a linear range of the calibration curve. Deviation of each cocaine concentration should be about ± 10% of the spiked values. The coefficient of variance (CV) at each concentration of direct injection should be less than 5% and the extraction should be less than 10% (Table 4-9).

4.7 Extraction recovery

The extraction recovery was determined by comparing the peak area obtained from the extracted samples containing a known amount of cocaine with those obtained from a direct injection of the same concentrations. The percentage recovery was calculated as follows:

$$\frac{\text{Peak area obtained from an extracted sample} \times 100}{\text{Peak area obtained from a direct injection}}$$

The extraction recovery of cocaine from plasma, atrial and ventricular tissue homogenates were demonstrated in Table 10-12.

5. Data analysis

The concentrations of cocaine in plasma, atrial and ventricular tissues were expressed as mean ± standard error ($\bar{X} \pm$ S.E.). All data were analyzed using analysis of variance (ANOVA). The differences among mean values were compared using Duncan's multiple range test. Difference was considered significant if p value < 0.05.

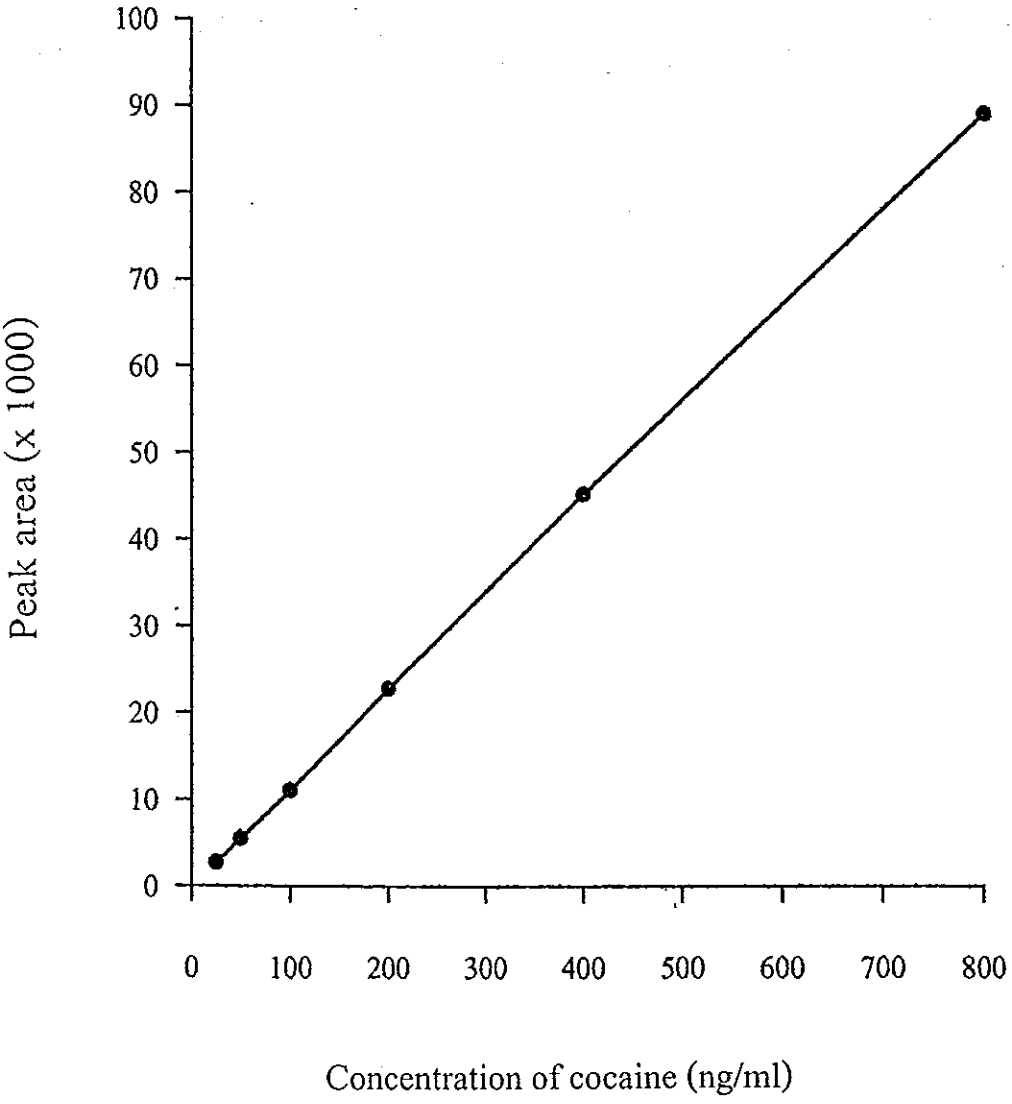


Figure 12 Calibration curve of cocaine in plasma, correlation coefficient (r) = 0.997

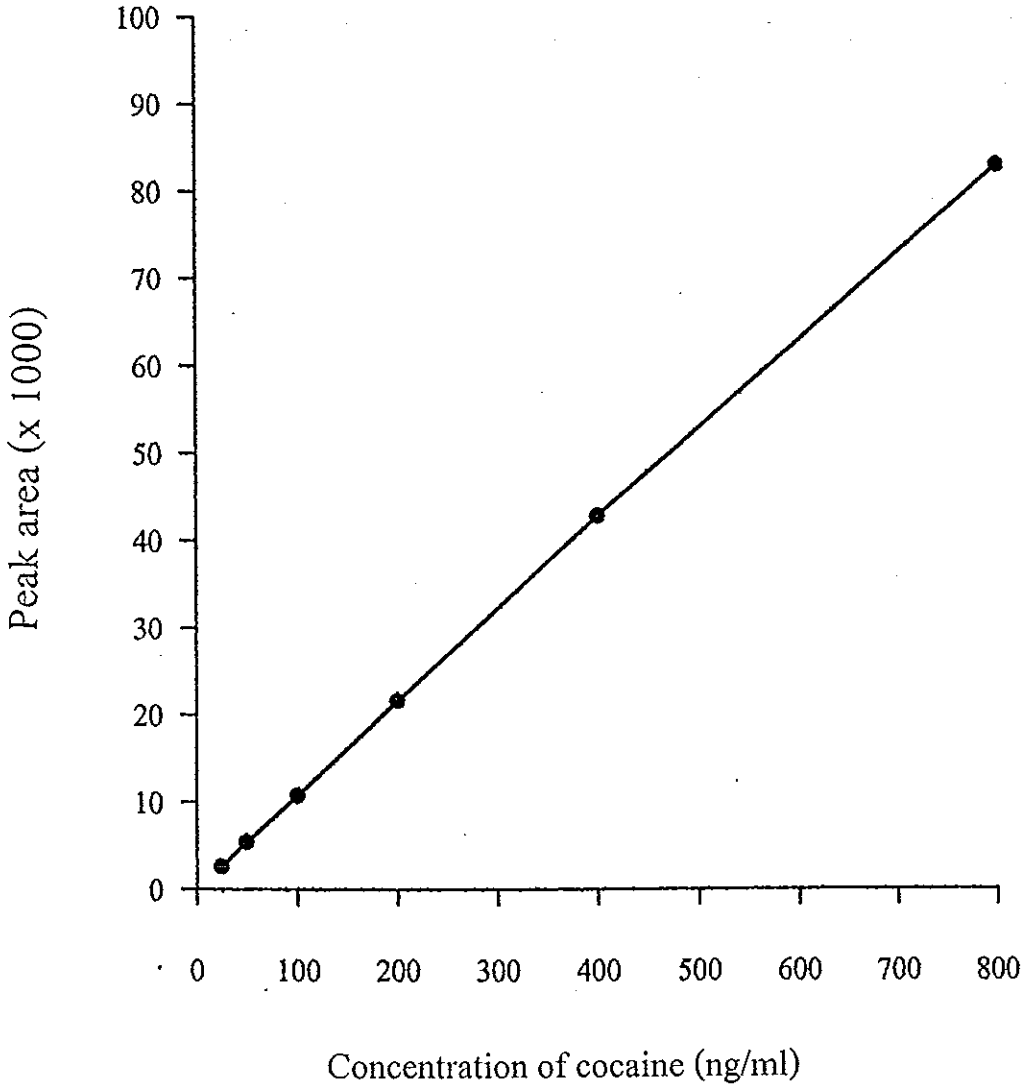


Figure 13 Concentration of cocaine in atrial tissues, correlation coefficient (r) = 0.998

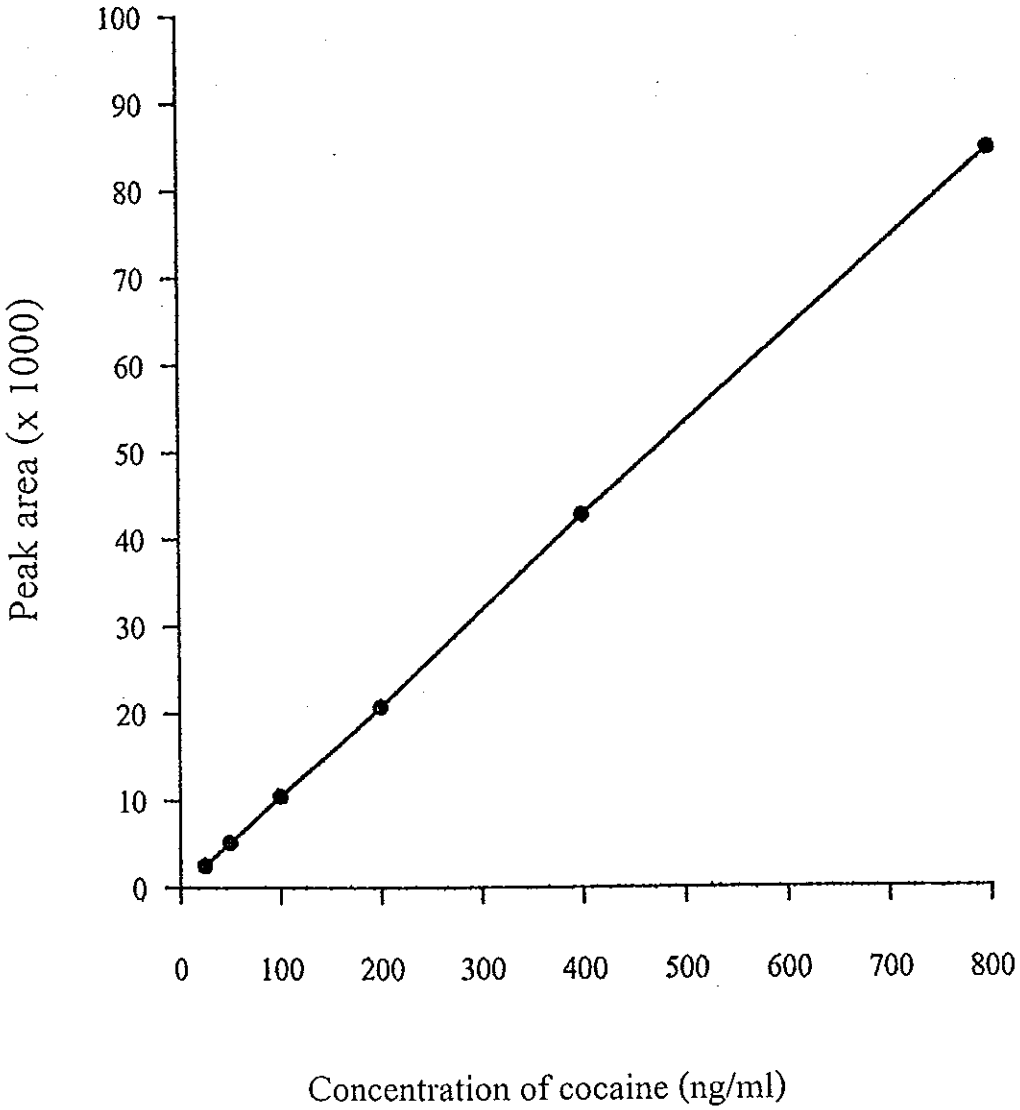


Figure 14 Calibration curve of cocaine in ventricular tissue, correlation coefficient (r) = 0.997

Table 4 The intra-assay variance of three different cocaine concentrations in (A) mobile phase and (B) plasma

(A) in mobile phase

Concentration[a] (ng/ml)	Mean peak area ± S.E. (n = 5)	CV[b] (%)
50	12792.6 ± 439.58	3.44
100	27043.8 ± 708.25	2.62
500	135507 ± 3554.75	2.62

(B) in plasma

Concentration[c] (ng/ml)	Mean peak area ± S.E. (n = 5)	CV[b] (%)
50	13078.6 ± 509.11	3.89
100	26482 ± 859.12	3.24
500	135208 ± 4470.66	3.31

[a] Various concentrations of standard cocaine were directly injected into HPLC system

[b] Standard deviation divided by mean, expressed in percent

[c] Various concentrations of standard cocaine were added to drug-free plasma sample prior to extraction

Table 5 The inter-assay variance of three different cocaine concentrations in (A) mobile phase and (B) plasma

(A) in mobile phase

Concentration[a] (ng/ml)	Mean peak area ± S.E. (n = 5)	CV[b] (%)
50	13356.22 ± 509.11	4.47
100	27296.46 ± 890.67	3.26
500	132648.9 ± 3647.49	2.75

(B) in plasma

Concentration[c] (ng/ml)	Mean peak area ± S.E. (n = 5)	CV[b] (%)
50	13451.6 ± 648.89	4.82
100	26988.35 ± 1165.17	4.32
500	131509.9 ± 3705.95	3.17

[a] Various concentrations of standard cocaine were directly injected into HPLC system

[b] Standard deviation divided by mean, expressed in percent

[c] Various concentrations of standard cocaine were added to drug-free plasma sample prior to extraction

Table 6 The intra-assay variance of three different cocaine concentrations in (A) mobile phase and (B) atrial tissue homogenate

(A) in mobile phase

Concentration[a] (ng/ml)	Mean peak area ± S.E. (n = 3)	CV[b] (%)
50	13933.33 + 272.96	1.96
100	28638.67 + 291.93	1.03
500	138859.3 + 1798.55	1.3

(B) in atrial tissue homogenate

Concentration[c] (ng/ml)	Mean peak area ± S.E. (n = 3)	CV[b] (%)
50	13160 ± 548.48	4.17
100	26756.67 ± 651.44	2.43
500	131818.3 ± 3607.74	2.74

[a] Various concentrations of standard cocaine were directly injected into HPLC system

[b] Standard deviation divided by mean, expressed in percent

[c] Various concentrations of standard cocaine were added to drug-free plasma sample prior to extraction

Table 7 The inter-assay variance of three different cocaine concentrations in (A) mobile phase and (B) atrial tissue homogenate

(A) in mobile phase

Concentration[a] (ng/ml)	Mean peak area ± S.E. (n = 3)	CV[b] (%)
50	13539.11 ± 474.13	3.5
100	27585.39 ± 1040.82	2.75
500	138122.3 ± 2961.94	2.1

(B) in atrial tissue homogenate

Concentration[c] (ng/ml)	Mean peak area ± S.E. (n = 3)	CV[b] (%)
50	12765.92 ± 466.05	3.65
100	26020.06 ± 842.78	3.23
500	130526.1 ± 3808.14	2.92

[a] Various concentrations of standard cocaine were directly injected into HPLC system

[b] Standard deviation divided by mean, expressed in percent

[c] Various concentrations of standard cocaine were added to drug-free plasma sample prior to extraction

Table 8 The intra-assay variance of three different cocaine concentrations in (A) mobile phase and (B) ventricular tissue homogenate

(A) in mobile phase

Concentration[a] (ng/ml)	Mean peak area ± S.E. (n = 5)	CV[b] (%)
50	13815.4 ± 463.43	3.35
100	28303.2 ± 789.48	2.79
500	140830 ± 3623.42	2.57

(B) in ventricular tissue homogenate

Concentration[c] (ng/ml)	Mean peak area ± S.E. (n = 5)	CV[b] (%)
50	13019 ± 517	4.38
100	27015.5 ± 980.63	3.63
500	134892 ± 4723	3.5

[a] Various concentrations of standard cocaine were directly injected into HPLC system

[b] Standard deviation divided by mean, expressed in percent

[c] Various concentrations of standard cocaine were added to drug-free plasma sample prior to extraction

Table 9 The inter-assay variance of three different cocaine concentrations in (A) mobile phase and (B) ventricular tissue homogenate

(A) in mobile phase

Concentration[a] (ng/ml)	Mean peak area ± S.E. (n = 5)	CV[b] (%)
50	13998.08 ± 504.07	3.6
100	28535.74 ± 886.53	3.1
500	138577.7 ± 4136.56	2.99

(B) in ventricular tissue homogenate

Concentration[c] (ng/ml)	Mean peak area ± S.E. (n = 5)	CV[b] (%)
50	13303.8 ± 741.29	4.76
100	26836.55 ± 1114.05	4.15
500	129957.8 ± 4601.41	3.54

[a] Various concentrations of standard cocaine were directly injected into HPLC system

[b] Standard deviation divided by mean, expressed in percent

[c] Various concentrations of standard cocaine were added to drug-free plasma sample prior to extraction

Table 10 The percentage recovery of standard cocaine in plasma

Concentration (ng/ml)	Peak area in mobile phase (Mean ± S.E.) (n = 5)	Peak area in plasma (Mean ± S.E.) (n = 5)	% recovery (Mean ± S.E.)
50	13356.22 ± 597.04	13451.6 ± 648.89	100.71 ± 1.42
100	27296.46 ± 890.67	26988.35 ± 1165.17	98.85 ± 1.32
500	132648.9 ± 3647.49	131509.9 ± 3705.95	98.83 ± 0.77

Table 11 The percentage recovery of standard cocaine in atrial tissue homogenate

Concentration (ng/ml)	Peak area in mobile phase (Mean ± S.E.) (n = 5)	Peak area in atrial tissue homogenate (Mean ± S.E.) (n = 5)	% recovery (Mean ± S.E.)
50	13539.11 ± 474.13	12765.92 ± 466.05	94.31 ± 0.13
100	27585.39 ± 1040.82	26020.06 ± 842.78	94.34 ± 0.83
500	138122.3 ± 2916.94	130526.1 ± 3808.14	93.05 ± 0.69

Table 12 The percentage recovery of standard cocaine in ventricular tissue homogenate

Concentration (ng/ml)	Peak area in mobile phase (Mean ± S.E.) (n = 5)	Peak area in ventricular tissue homogenate (Mean ± S.E.) (n = 5)	% recovery (Mean ± S.E.)
50	13998.08 ± 504.07	13303.8 ± 741.29	93.61 ± 1.92
100	28535.74 ± 886.53	26836.55 ± 1114.05	92.99 ± 1.44
500	138577.7 ± 4136.56	129957.8 ± 4601.41	92.33 ± 1.33

CHAPTER 4

RESULTS

The results expressed in this study were divided into 2 main sections as follows:

Section 1 Effects of chronic cocaine treatment on the responsiveness of guinea-pig isolated atria and trachea to exogenous catecholamines

Part A The effects of chronic cocaine treatment on the responsiveness of guinea-pig isolated atria to catecholamines

1. The responsiveness to norepinephrine

Norepinephrine had the same general pattern of effects on isolated atria of saline-control and all cocaine-treated guinea-pigs, the representative tracing was shown in Figure 20. But quantitatively, the atria of all cocaine-treated groups were more sensitive to norepinephrine than those of saline-treated groups. As shown in Figure 24 and 25, cocaine (1.25-5 mg/kg) significantly caused dose-dependent parallel shift to the left of the log concentration-response curves of chronotropic and inotropic effects of norepinephrine, respectively, without changing the maximum response. The shift was also depended on the times after cocaine cessation as shown in Figure 26 and 27. The pD_2 values of both effects of norepinephrine in atria of all cocaine-treated groups were significant

higher than those of corresponding control groups (Table 13 & 14). $[D]_{max50}$ ratio of norepinephrine in all cocaine-treated groups increased in a cocaine dose-dependent manner, and was highest at 1 hr after cocaine cessation and subsequently decreased with time.

2. The responsiveness to isoproterenol

Similar to norepinephrine, isoproterenol also had the same general pattern of effects on both atria from saline-control and cocaine-treated groups, in which the representative tracing was shown in Figure 21. Chronic cocaine (1.25-5 mg/kg) treatment also produced dose-dependent parallel leftward shift of the concentration-response curves to isoproterenol without changing the maximum response (Figure 28 & 29). The magnitude of the shift was highest at 1 hr after cocaine cessation and then decreased when the times after cessation were longer (Figure 30 & 31). The pD_2 values of all cocaine-treated animals (Table 15 & 16) significantly increased compared to their controls. $[D]_{max50}$ ratio of isoproterenol in all cocaine-treated groups also increased in the cocaine dose-dependent manner. This ratio was highest at 1 hr after cocaine cessation and then diminished with time.

Part B The effects of chronic cocaine treatment on the responsiveness of guinea-pig isolated trachea to catecholamines

1. The responsiveness to norepinephrine

The representative tracing of the relaxing effects of norepinephrine on carbachol (1 μg/ml)-induced contraction in the isolated trachea of cocaine-treated groups was illustrated in Figure 22. The cumulative increase in

concentration of norepinephrine produced the same general pattern of effects in trachea obtained from both saline and cocaine-treated groups. The concentration-response curves to norepinephrine of cocaine-treated groups were demonstrated in Figure 32 and 33. Cocaine significantly induced the leftward shift of the concentration-response curves, which were depended on doses of cocaine and time after cocaine cessation. Low dose of cocaine (1.25 mg/kg) could not induced the supersensitivity to norepinephrine (Figure 33), while at higher doses (2.5-5 mg/kg), the increases in responsiveness were observed as shown by higher pD_2 values compared to the control groups. The hyperresponsiveness persisted till 72 hr after cocaine cessation. $[D]_{max50}$ ratio, as shown in Table 17, was highest at 1 hr after cocaine cessation and then decreased with time (except at a cocaine dose of 1.25 mg/kg).

2. The responsiveness to isoproterenol

As of norepinephrine, isoproterenol had the same general pattern of the relaxing effect on carbachol-induced contraction of isolated trachea from both saline-control and all cocaine-treated groups. Figure 23 illustrated the typical tracing of relaxing effects to isoproterenol of cocaine-treated groups. The concentration-response curves obtained from cocaine-treated groups significantly shifted to the left compared to their corresponding control groups (Figure 34 & 35). The leftward shifts depended on doses and times after cocaine cessation. The pD_2 values of cocaine-treated groups were significant higher than those of control only at the high doses (2.5-5 mg/kg) and still persisted up to 72 hr after cocaine cessation (Table 18). The orders of $[D]_{max50}$ ratio were at 1 hr > 24 hr > 72 hr after cocaine cessation.

Part C Comparison between the degree of supersensitivity in isolated guinea-pig atria and trachea

Table 19 demonstrated the comparison of $[D]_{max50}$ ratio of the positive chronotropic and positive inotropic effects in atria and relaxing effect in trachea of norepinephrine and isoproterenol obtained from cocaine (1.25, 3.5 and 5 mg/kg)-treated groups and their corresponding control saline-treated groups. The $[D]_{max50}$ ratio of the chronotropic and inotropic effects in atria of both norepinephrine and isoproterenol revealed that the degree of supersensitivity induced by cocaine directly correlated with doses of cocaine administration and times after cocaine cessation. In trachea, the degree of supersensitivity to both norepinephrine and isoproterenol was less than that observed in the atria. The degree of supersensitivity of relaxing effect to norepinephrine in trachea was lower than the degree of supersensitivity of chronotropic and inotropic effects in atria by about 1.6-6 and 1.6-8 times, respectively, at the cocaine dose of 1.25 mg/kg; 8-210 and 7-320 times, respectively, at 2.5 mg/kg; and 75,000-190,000 and 42000-155,000, respectively, at 5 mg/kg cocaine. The degree of supersensitivity of relaxing effect to isoproterenol, similar to norepinephrine, was lower than the degree of supersensitivity of chronotropic and inotropic effects by about 2-4 and 1.3-4, respectively, at the cocaine dose of 1.25 mg/kg; 4-117 and 6.5-130 times, respectively, at 2.5 mg/kg; and 53,000-154,000 and 45,000-76,000 times, respectively, at 5 mg/kg.

In atria, the degree of supersensitivity to norepinephrine was significantly higher than those of isoproterenol at 1 and 24 hr after cocaine cessation in most

of cocaine-treated groups, but this significant difference was not observed in trachea.

Section 2 Analysis of cocaine concentrations in plasma and cardiac tissues of guinea-pigs

Part A Determination of cocaine concentration in plasma, atrial and ventricular tissues of guinea-pigs at various times after cocaine cessation

The representative chromatograms of cocaine in plasma, atrial and ventricular tissues were exhibited in Figure 36, 37 and 38, respectively.

1. At 1 hr after cocaine cessation

The concentrations of cocaine in plasma, atrial and ventricular tissues after 1 hr cessation of cocaine (1.25, 2.5 and 5 mg/kg) were shown in Table 20. The concentrations of cocaine in plasma, atrial and ventricular tissues following cocaine administration of a 1.25 mg/kg dose were 40.91 ± 4.03 ng/ml, 127.4 ± 15.6 ng/g and 121.56 ± 8.54 ng/g, respectively; after the administration of a 2.5 mg/kg dose were 70.73 ± 5.65 ng/ml, 245.48 ± 12.48 ng/g and 221 ± 18.29 ng/g, respectively; and after a 5 mg/kg dose were 285.64 ± 17.06 ng/ml, 629.37 ± 32.77 ng/g and 613.14 ± 47.64 ng/g, respectively. The concentrations of cocaine in plasma, atrial and ventricular tissues directly correlated with doses of cocaine administration.

Part B Comparison between the $[D]_{max50}$ ratio of the chronotropic, inotropic and the relaxing effects of norepinephrine and isoproterenol with the cocaine concentration in plasma, atrial and ventricular tissues of various doses of cocaine-treated guinea-pigs.

1. At 1 hr after cocaine cessation

The $[D]_{max50}$ ratio of the chronotropic, inotropic effects in isolated atria, the relaxing effects in isolated trachea of norepinephrine and isoproterenol and the concentrations of cocaine in plasma, atrial and ventricular tissues taken from guinea-pig at 1 hr after cocaine cessation were shown in Table 23. The $[D]_{max50}$ ratio values indicated the degree of supersensitivity of atria and trachea to norepinephrine and isoproterenol. The results demonstrated that the supersensitivity to both catecholamines were observed in atria and trachea of all cocaine-treated groups. The degree of supersensitivity was correlated with the concentration of cocaine in plasma, atrial and ventricular tissues.

2. At 24 hr after cocaine cessation

The $[D]_{max50}$ ratio of the chronotropic, inotropic effects in isolated atria, the relaxing effects in isolated trachea of norepinephrine and isoproterenol and the concentrations of cocaine in plasma, atrial and ventricular tissues taken from guinea-pig at 24 hr after cocaine cessation were shown in Table 24. It is likely that there was no correlation between the degree of supersensitivity and the concentration of cocaine in plasma, atrial and ventricular tissues because cocaine could not be detected in any tissue when low dose (1.25 mg/kg) of cocaine was administered while the supersensitivity still existed. Even at higher dose (2.5 mg/kg), cocaine were detected only in plasma and atria of

some guinea-pigs, whereas at the highest dose (5 mg/kg), that cocaine was detected in all atria and ventricular tissues.

3. At 72 hr after cocaine cessation

The supersensitivity responses to the two catecholamines in both atria and trachea were still presented even at 72 hr after the cocaine cessation although the cocaine in plasma, atrial and ventricular tissues could not be detected (Table 25)

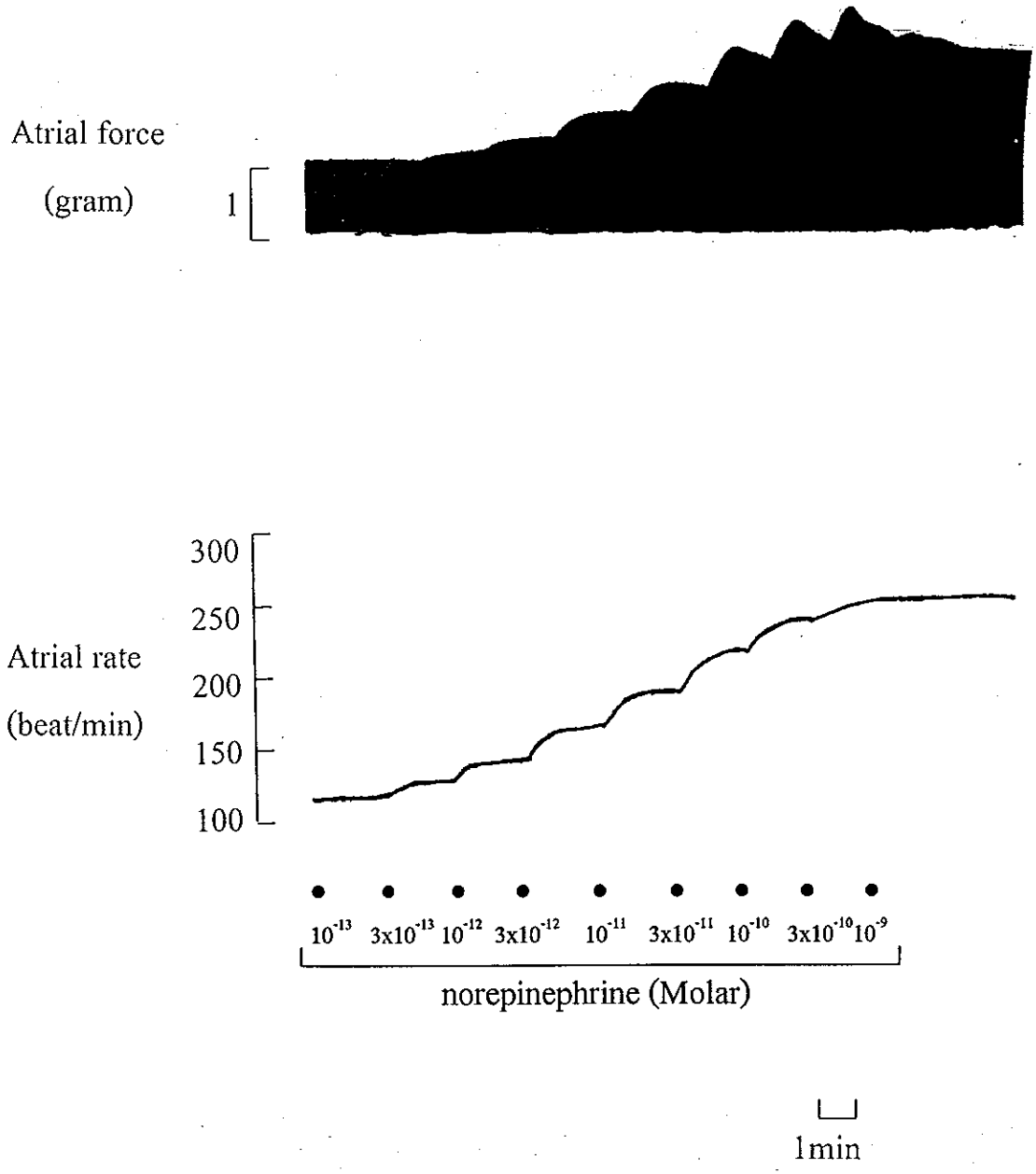


Figure 20 The representative tracing of inotropic and chronotropiceffects of the cumulative increase in concentrations of norepinephrine in isolated atria of cocaine-treated (2.5 mg/kg) guinea-pig

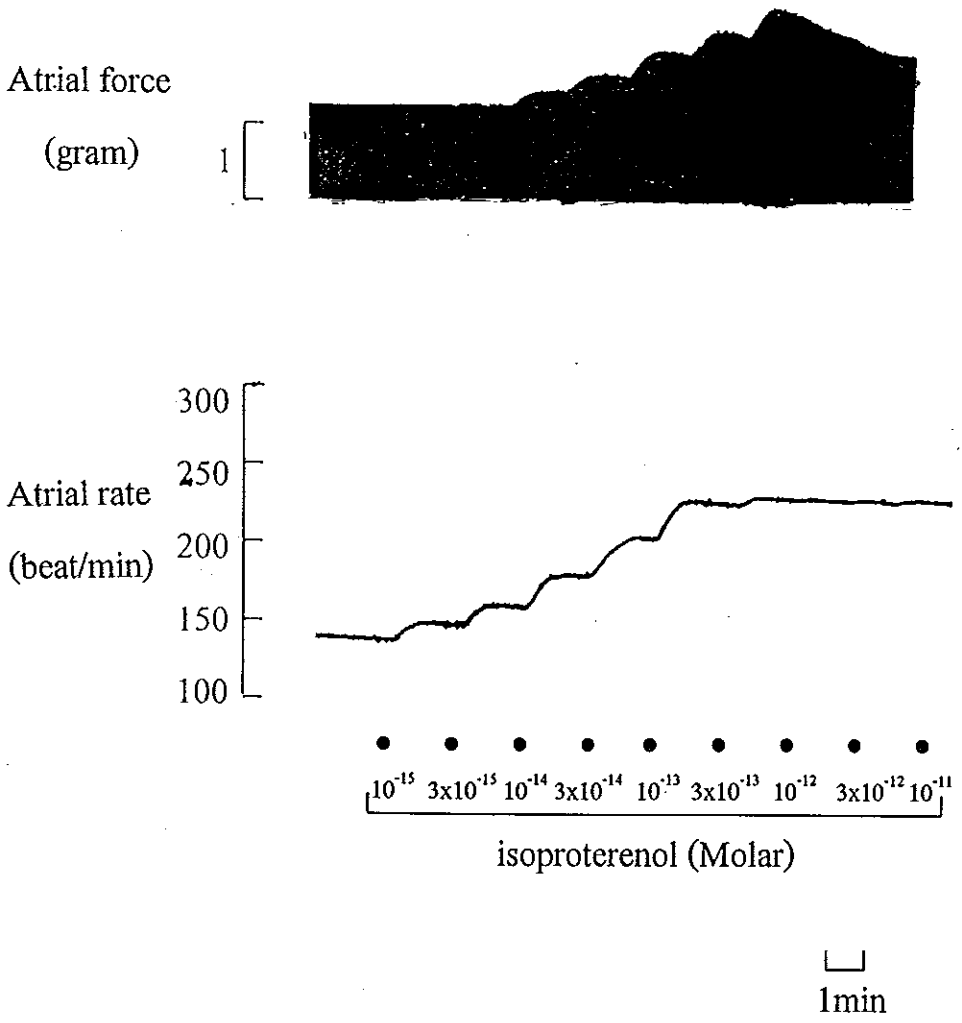


Figure 21 The representative tracing of inotropic and chronotropic effects of the cumulative increase in concentrations of isoproterenol in isolated atria of cocaine-treated (2.5 mg/kg) guinea-pig

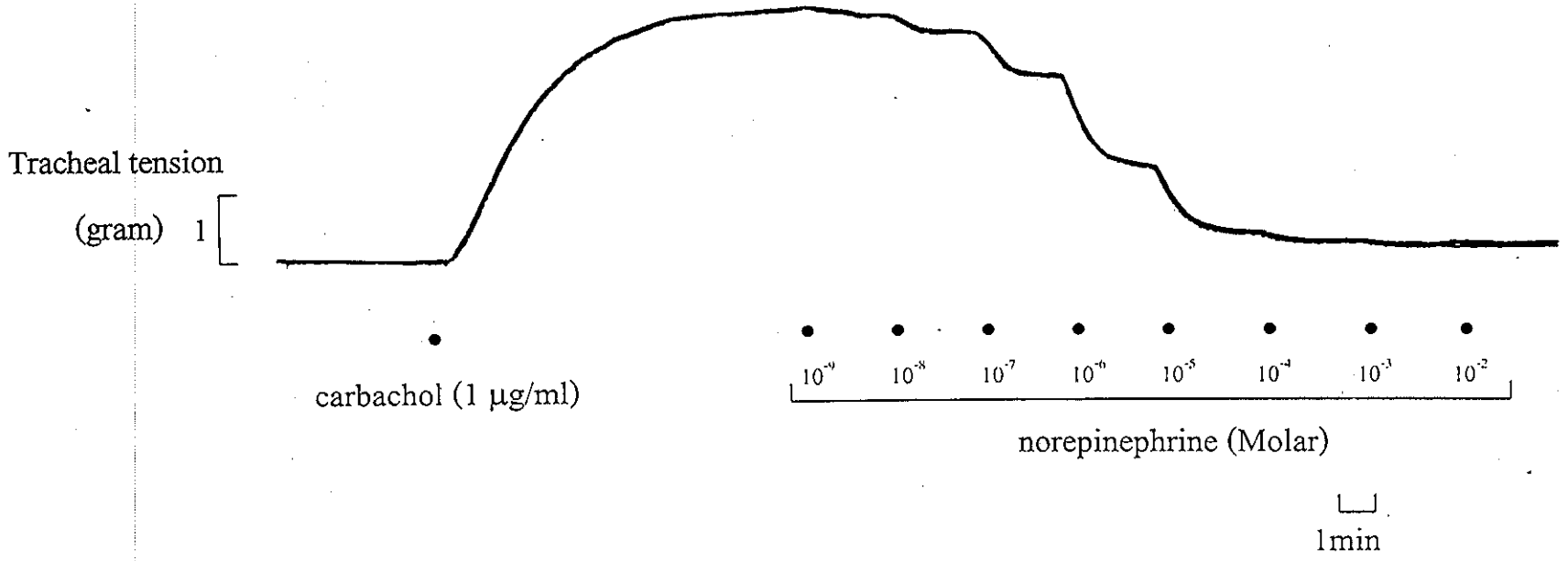


Figure 22 The representative tracing of relaxing effect of the cumulative increase in concentrations of norepinephrine on carbachol (1 μg/ml)-pretreated isolated trachea of chronic cocaine-treated (2.5 mg/kg) guinea-pig

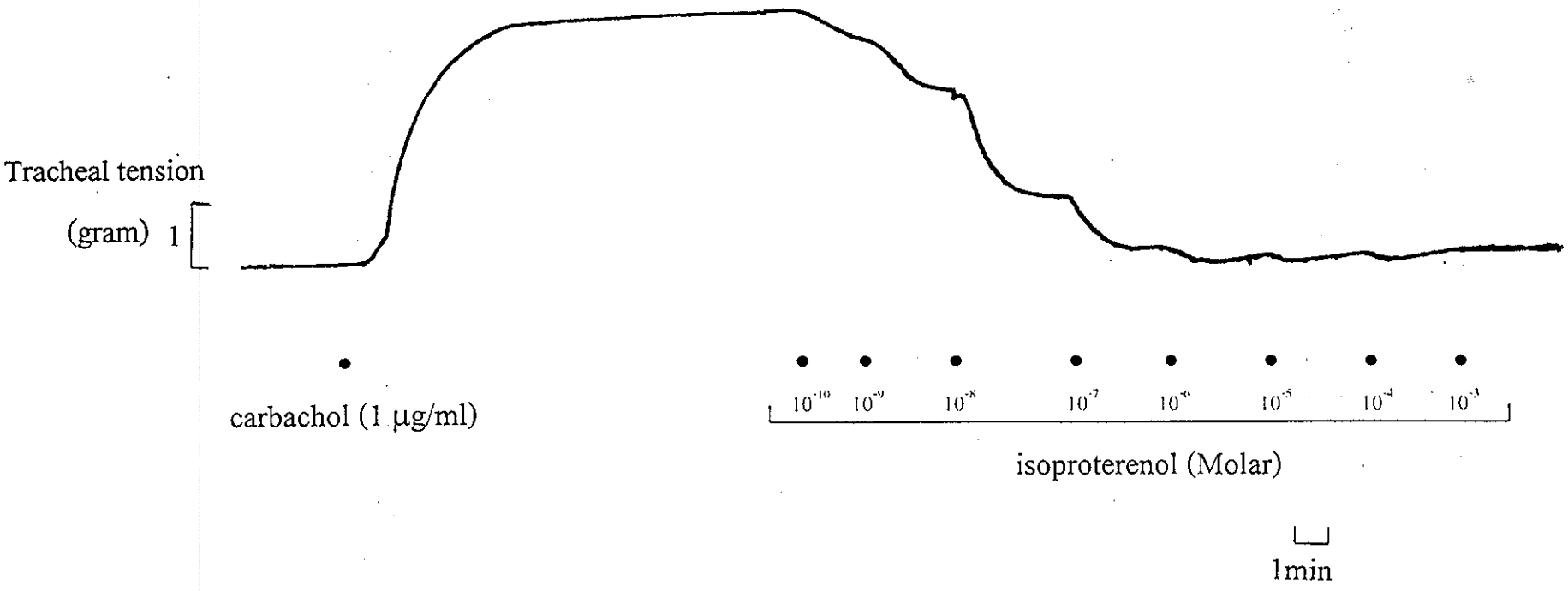
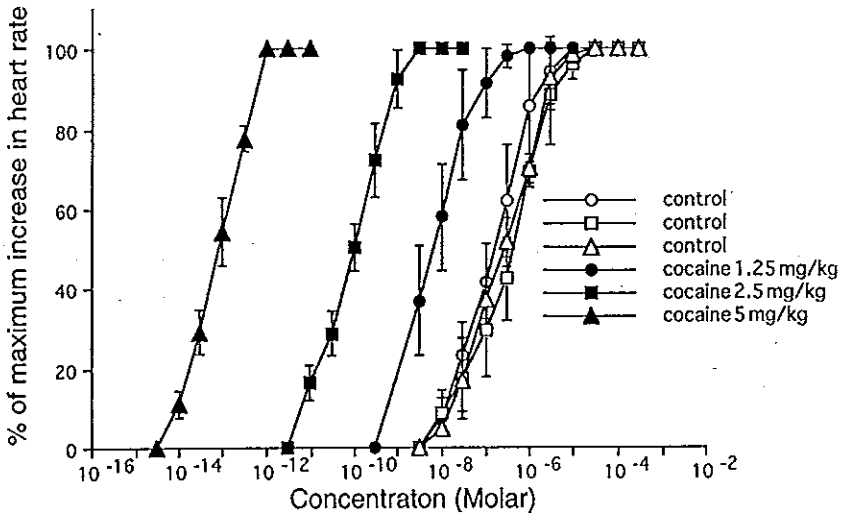
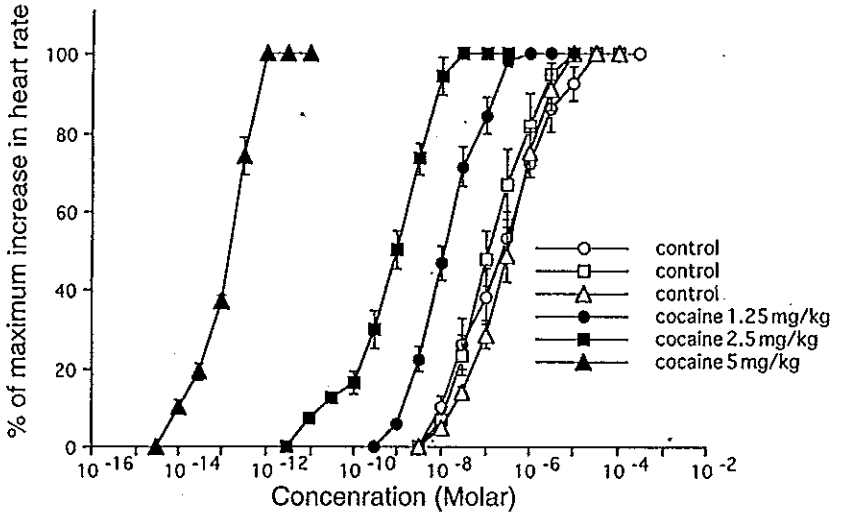


Figure 23 The representative tracing of relaxing effect of the cumulative increase in concentrations of isoproterenol on carbachol (1 μg/ml)-pretreated isolated trachea of chronic cocaine-treated (2.5 mg/kg) guinea-pig

(A) Cocaine cessation at 1 hr



(B) Cocaine cessation at 24 hr



(C) Cocaine cessation at 72 hr

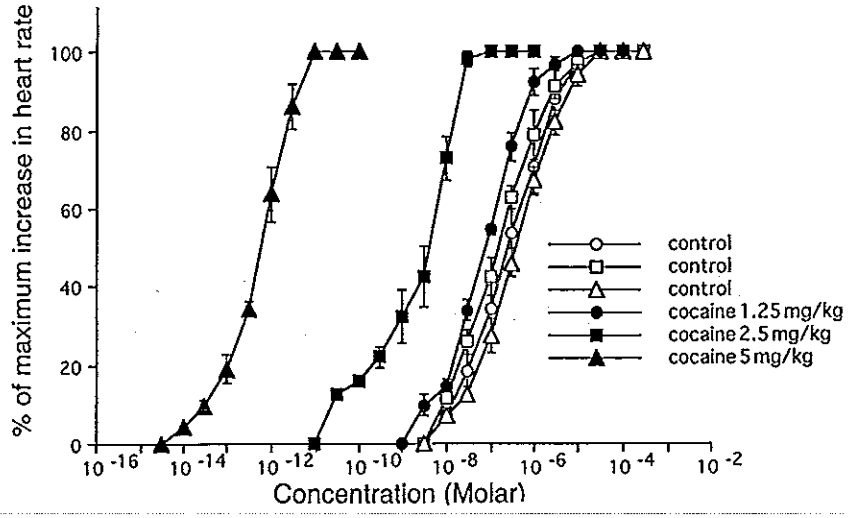
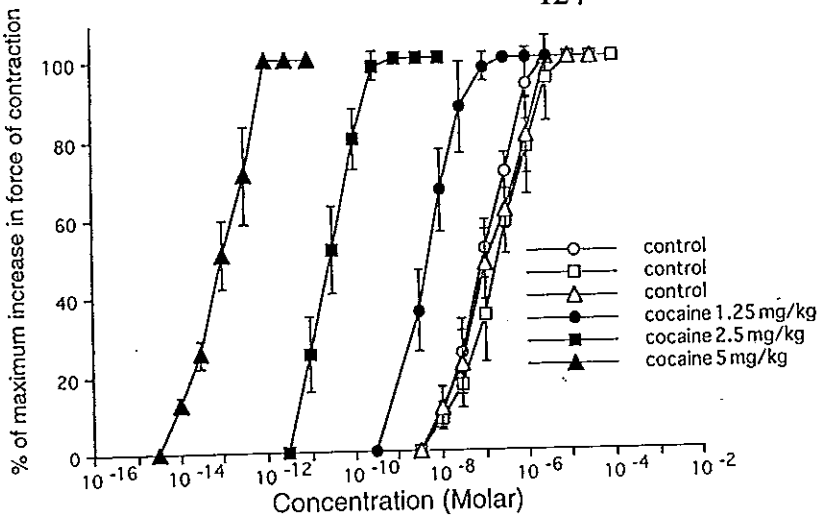
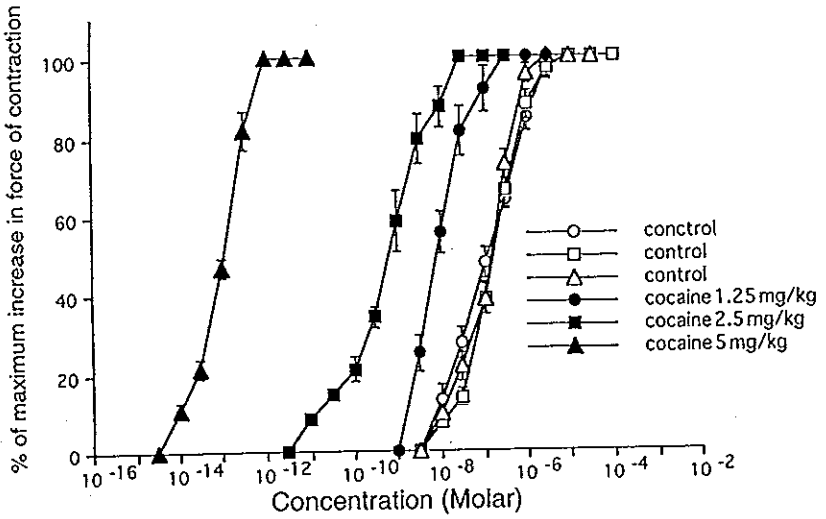


Figure 24 Comparison of the effects of various doses of chronic cocaine treatment (1.25, 2.5 and 5 mg/kg, i.p.) on the concentration-response curves of the ***chronotropic effect*** of NE on the isolated atria, at various times (1, 24 and 72 hr) after cocaine cessation. Symbols represent means and vertical bars represent standard error of means (n = 5)

(A) Cocaine cessation at 1 hr



(B) Cocaine cessation at 24 hr



(C) Cocaine cessation at 72 hr

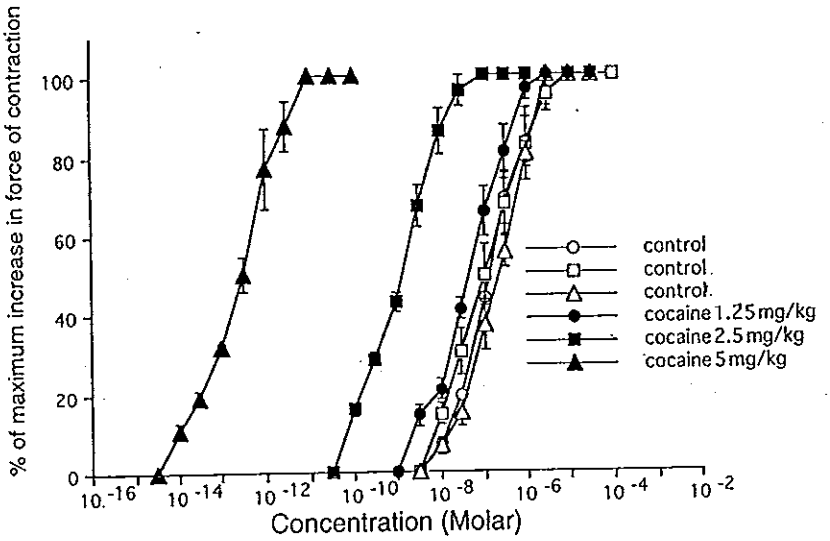
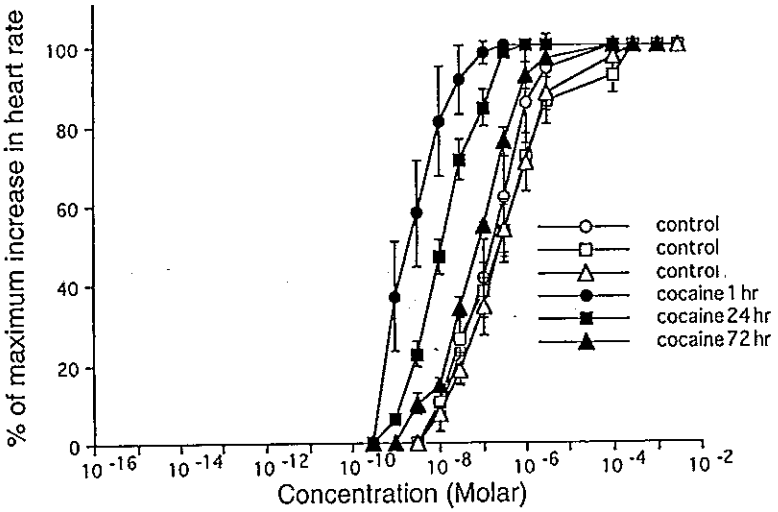
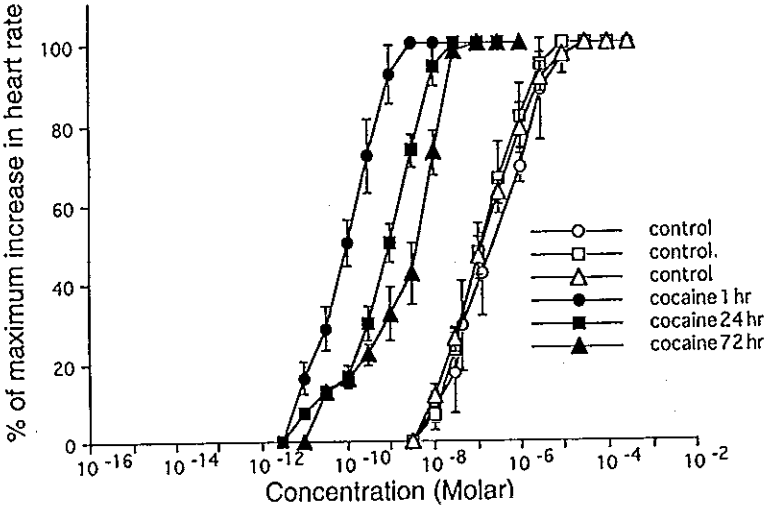


Figure 25 Comparison of the effects of various doses of chronic cocaine treatment (1.25, 2.5 and 5 mg/kg, i.p.) on the concentration-response curves of the ***inotropic effect*** of NE on the isolated atria, at various times (1, 24 and 72 hr) after cocaine cessation. Symbols represent means and vertical bars represent standard error of means (n = 5)

(A) Cocaine 1.25 mg/kg



(B) Cocaine 2.5 mg/kg



(C) Cocaine 5 mg/kg

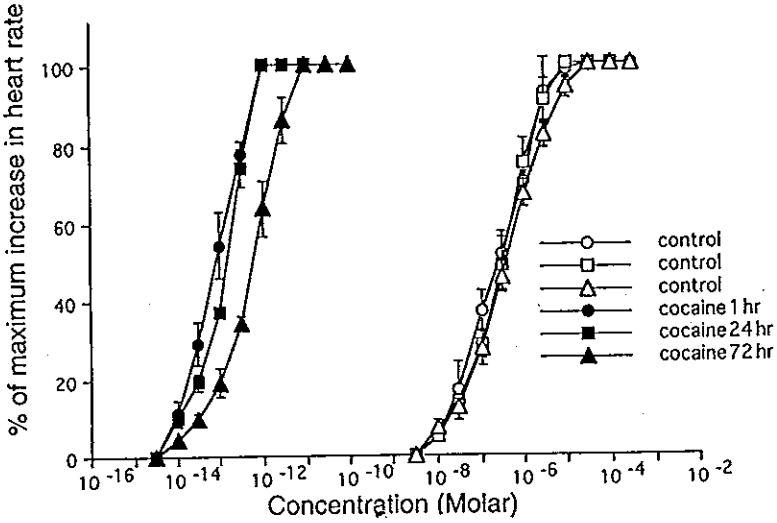
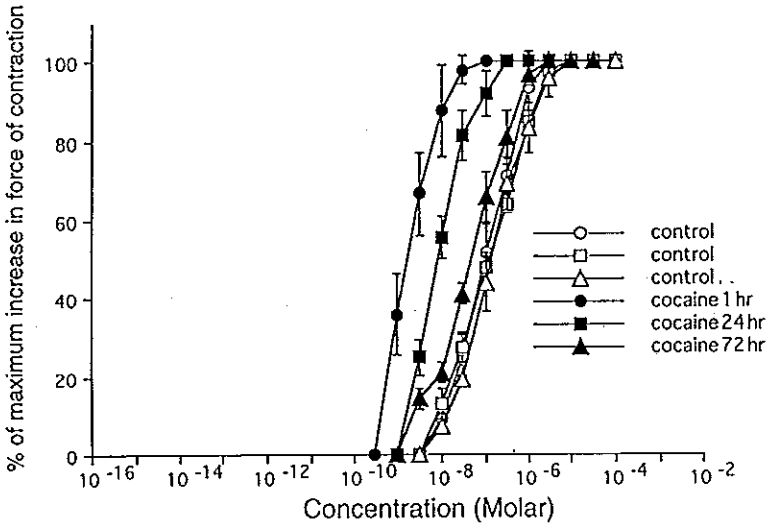
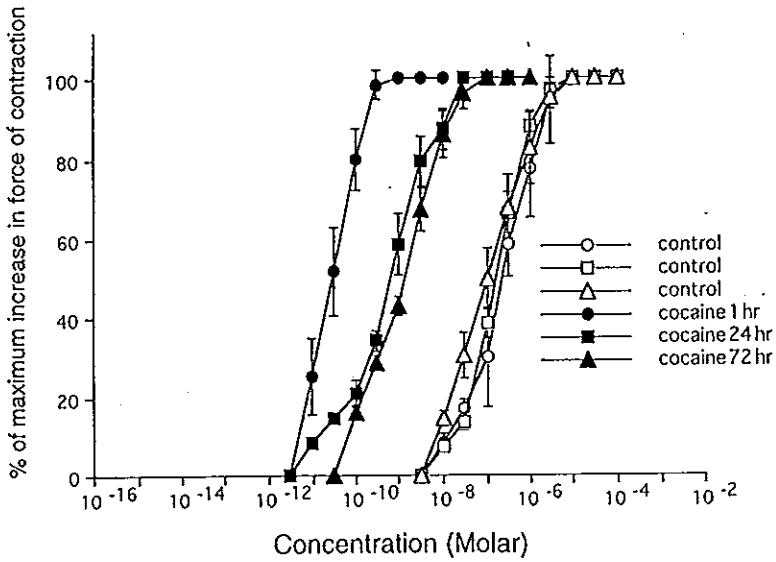


Figure 26 Comparison between various times (1, 24 and 72 hr) after cocaine cessation on the concentration-response curves of the ***chronotropic effect*** of NE on the atria isolated from chronic cocaine-treated (1.25, 2.5 and 5 mg/kg, i.p., b.i.d. for 14 days) guinea-pig. Symbols represent means and vertical bars represent standard error of means (n = 5)

(A) Cocaine 1.25 mg/kg



(B) Cocaine 2.5 mg/kg



(C) Cocaine 5 mg/kg

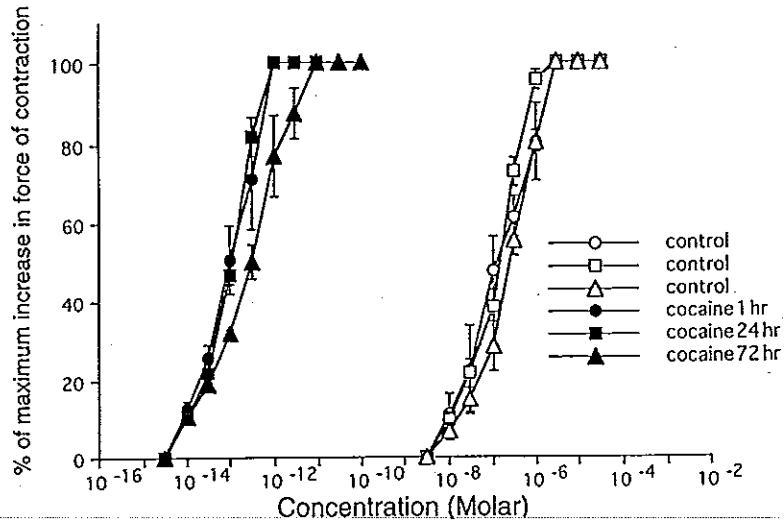
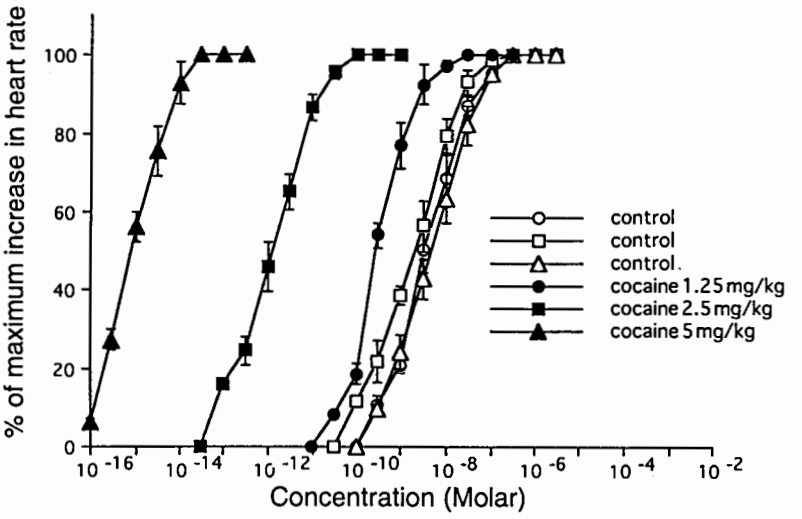
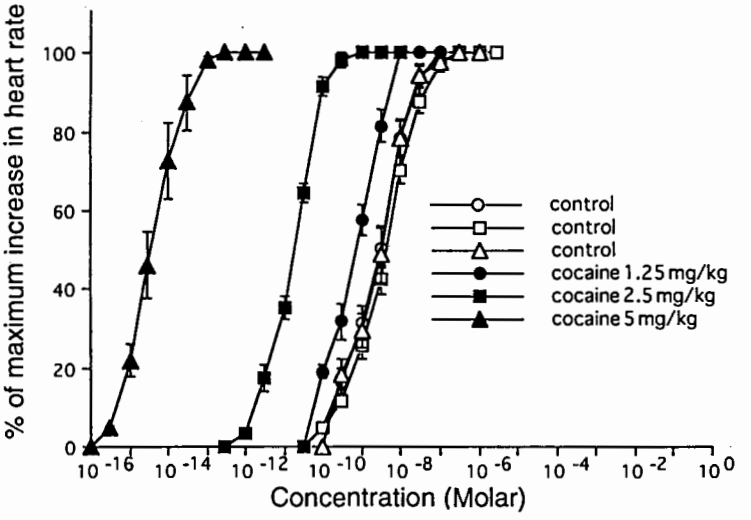


Figure 27 Comparison between various times (1, 24 and 72 hr) after cocaine cessation on the concentration-response curves of the ***inotropic effect*** of NE on the atria isolated from chronic cocaine-treated (1.25, 2.5 and 5 mg/kg, i.p., b.i.d. for 14 days) guinea-pig. Symbols represent means and vertical bars represent standard error of means (n = 5)

(A) Cocaine cessation at 1 hr



(B) Cocaine cessation at 24 hr



(C) Cocaine cessation at 72 hr

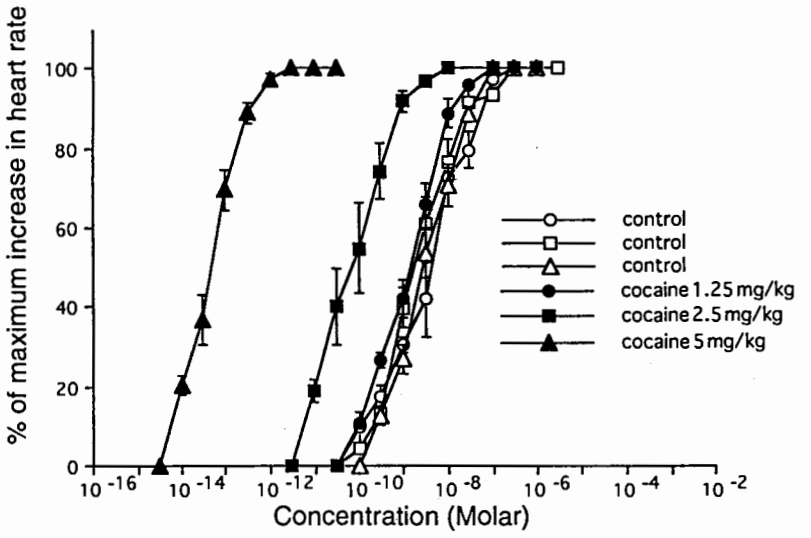
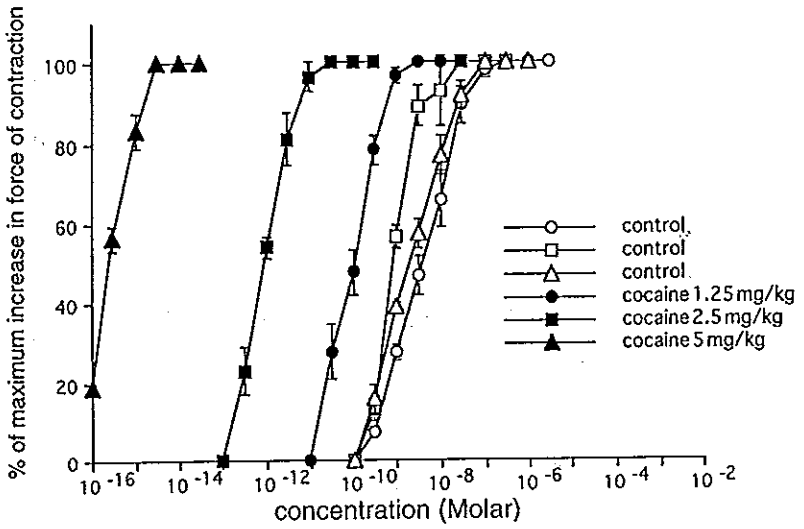
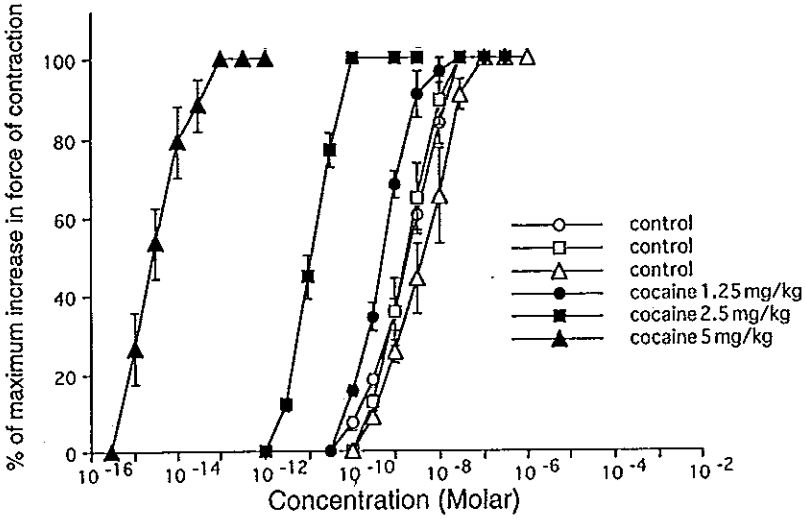


Figure 28 Comparison of the effects of various doses of chronic cocaine treatment (1.25, 2.5 and 5 mg/kg, i.p.) on the concentration-response curves of the ***chronotropic effect*** of **ISO** on the isolated atria, at various times (1, 24 and 72 hr) after cocaine cessation. Symbols represent means and vertical bars represent standard error of means (n = 5)

(A) Cocaine cessation at 1 hr



(B) Cocaine cessation at 24 hr



(C) Cocaine cessation at 72 hr

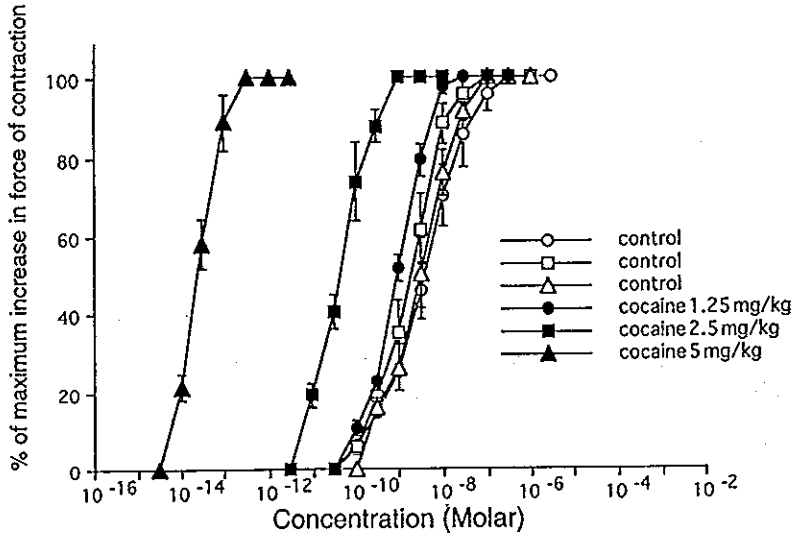


Figure 29 Comparison of the effects of various doses of chronic cocaine treatment (1.25, 2.5 and 5 mg/kg, i.p.) on the concentration-response curves of the ***inotropic effect*** of ISO on the isolated atria, at various times (1, 24 and 72 hr) after cocaine cessation. Symbols represent means and vertical bars represent standard error of means (n = 5)

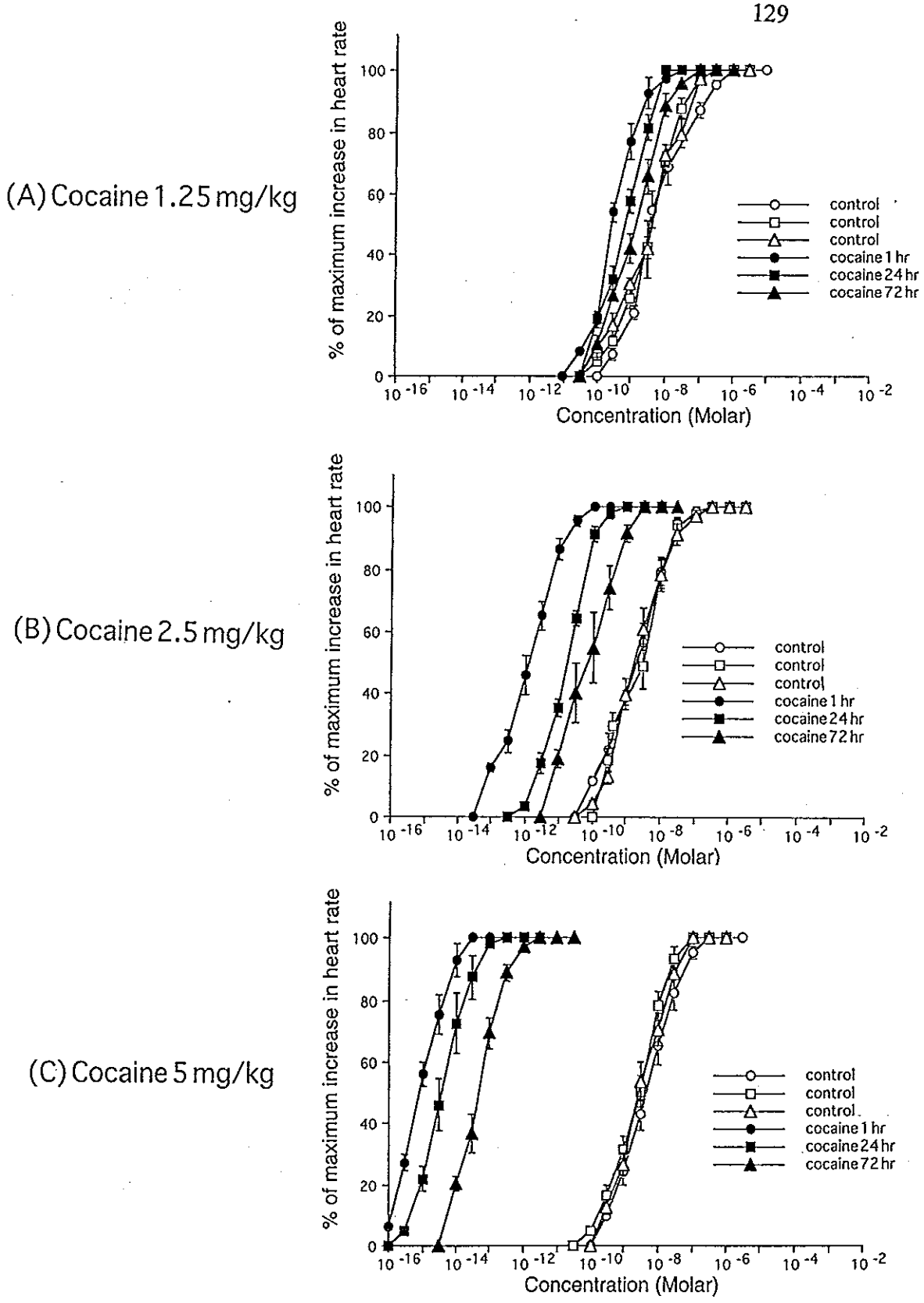
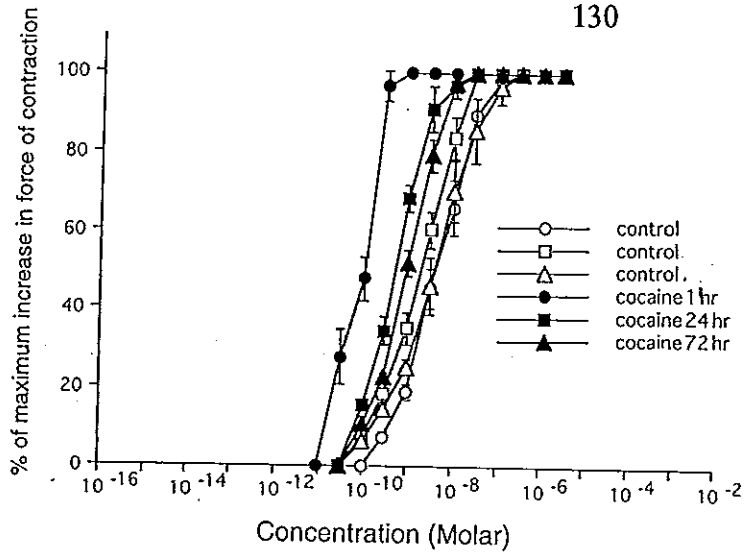
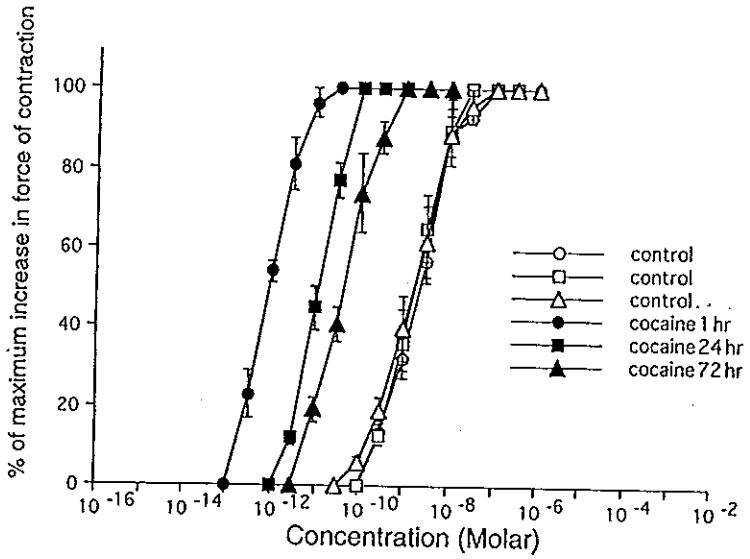


Figure 30 Comparison between various times (1, 24 and 72 hr) after cocaine cessation on the concentration-response curves of the ***chronotropic effect*** of ISO on the atria isolated from chronic cocaine-treated (1.25, 2.5 and 5 mg/kg, i.p., b.i.d. for 14 days) guinea-pig. Symbols represent means and vertical bars represent standard error of means (n = 5)

(A) Cocaine 1.25 mg/kg



(B) Cocaine 2.5 mg/kg



(C) Cocaine 5 mg/kg

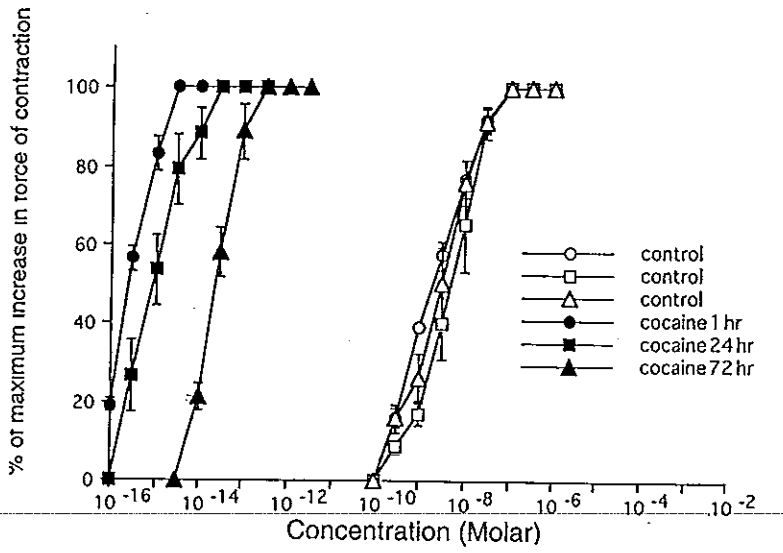
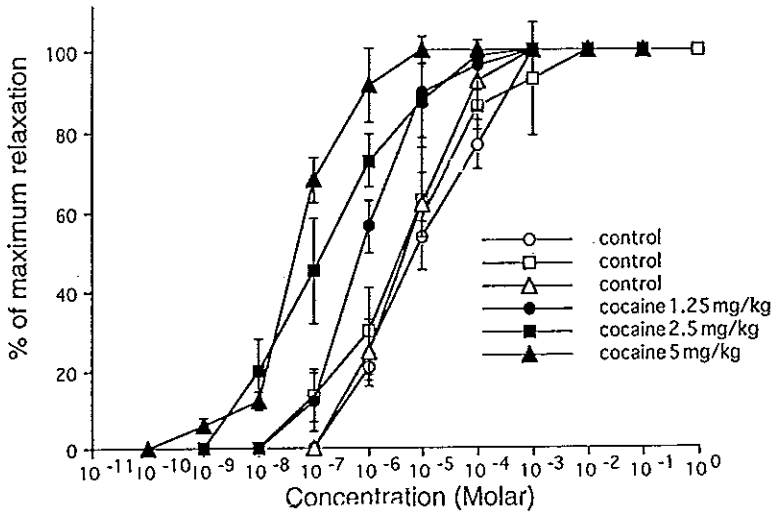
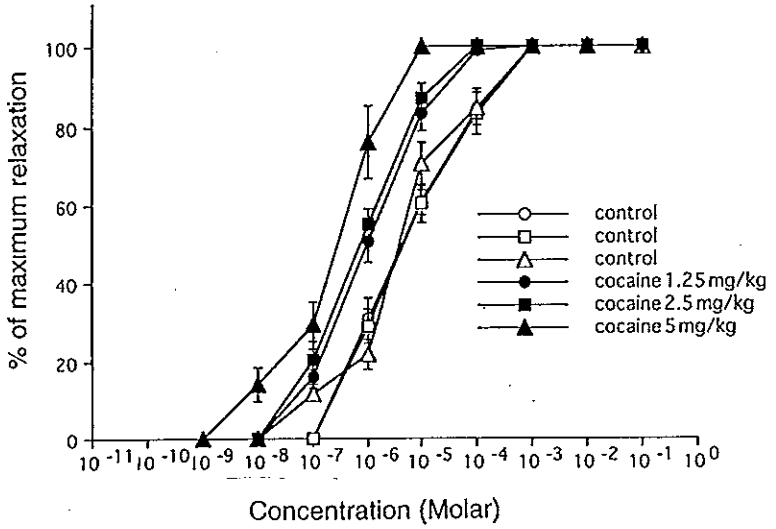


Figure 31 Comparison between various times (1, 24 and 72 hr) after cocaine cessation on the concentration-response curves of the ***inotropic effect*** of ISO on the atria isolated from chronic cocaine-treated (1.25, 2.5 and 5 mg/kg, i.p., b.i.d. for 14 days) guinea-pig. Symbols represent means and vertical bars represent standard error of means (n = 5)

(A) Cocaine cessation at 1 hr



(B) Cocaine cessation at 24 hr



(C) Cocaine cessation at 72 hr

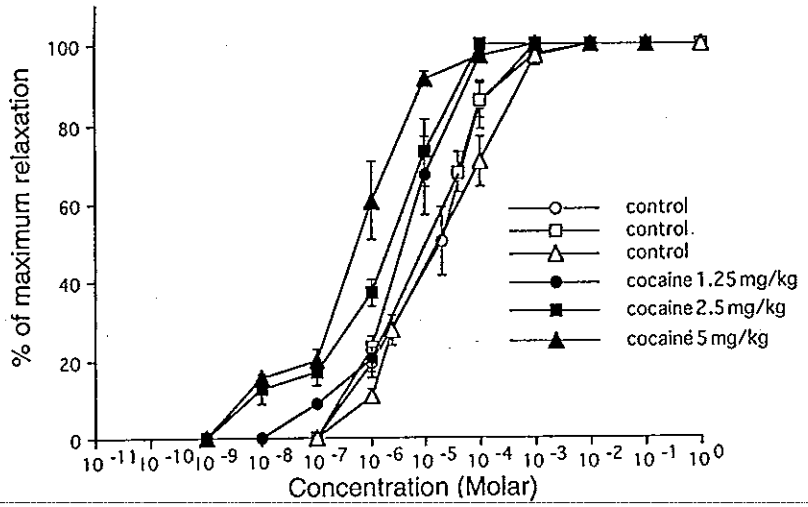
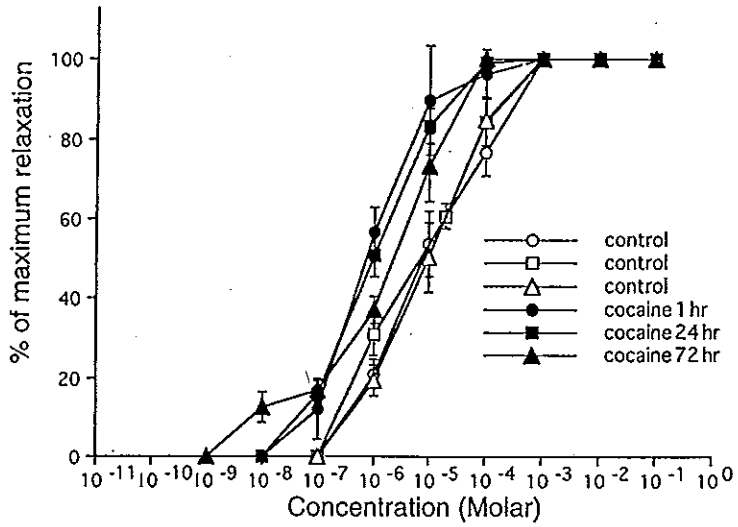
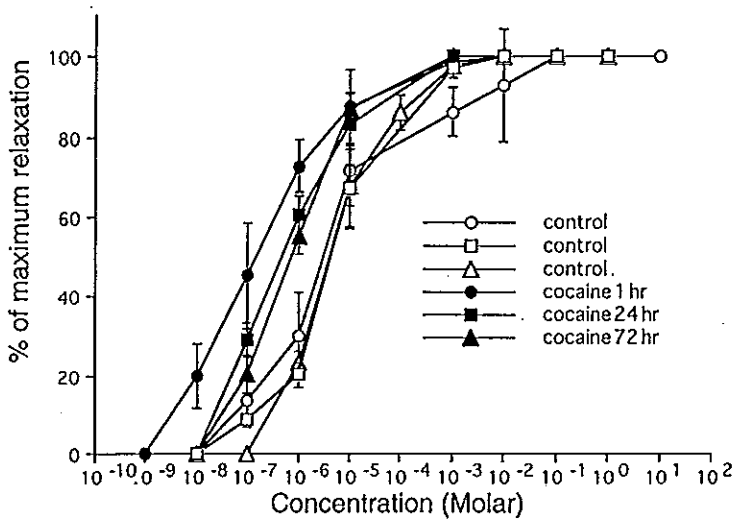


Figure 32 Comparison of the effects of various doses of chronic cocaine treatment (1.25, 2.5 and 5 mg/kg, i.p.) on the concentration-response curves of the ***relaxing effect*** of NE on the isolated trachea, at various times (1, 24 and 72 hr) after cocaine cessation. Symbols represent means and vertical bars represent standard error of means (n = 5)

(A) Cocaine 1.25 mg/kg



(B) Cocaine 2.5 mg/kg



(C) Cocaine 5 mg/kg

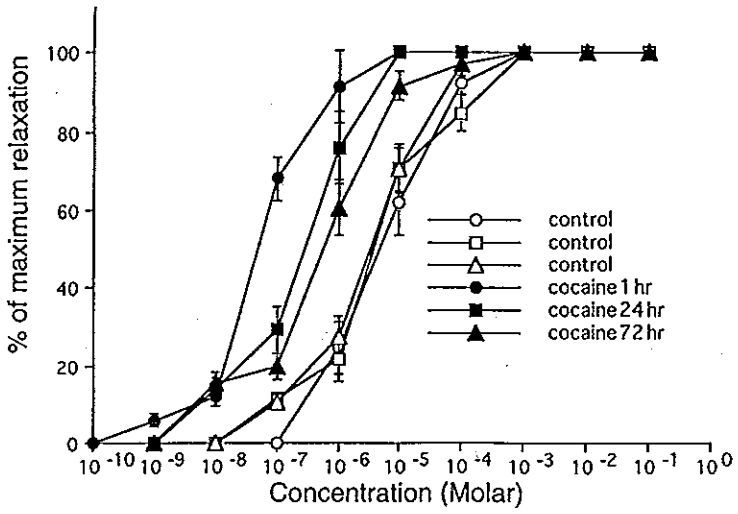
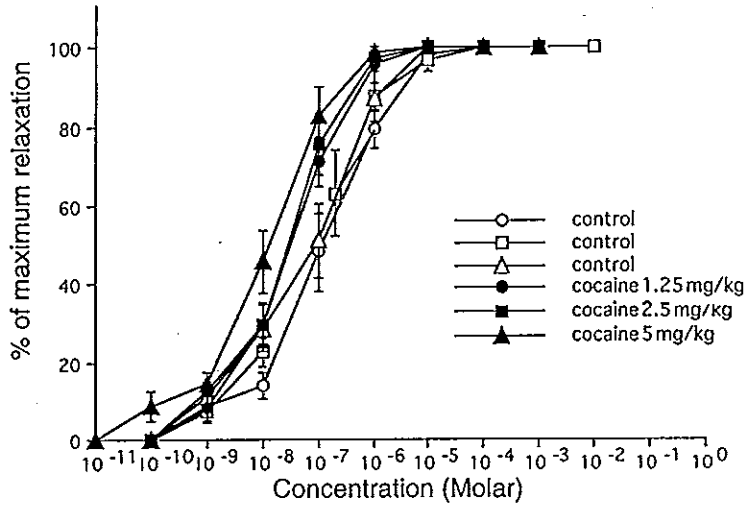
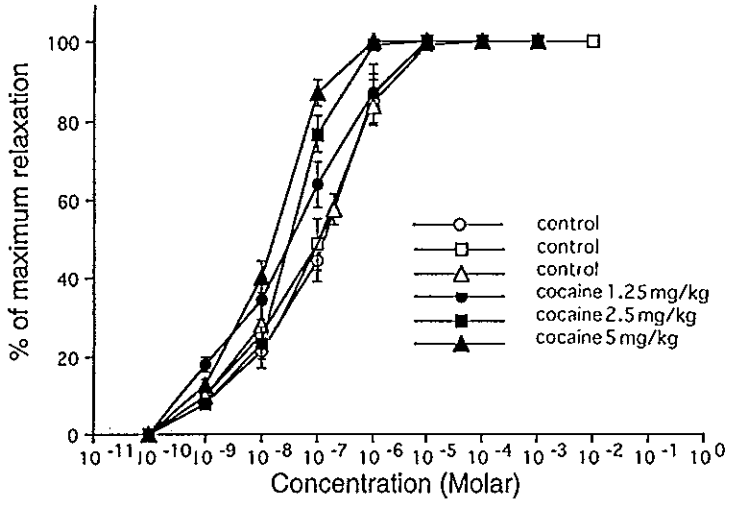


Figure 33 Comparison between various times (1, 24 and 72 hr) after cocaine cessation on the concentration-response curves of the ***relaxing effect*** of NE on the trachea isolated from chronic cocaine-treated (1.25, 2.5 and 5 mg/kg, i.p., b.i.d. for 14 days) guinea-pig. Symbols represent means and vertical bars represent standard error of means (n = 5)

(A) Cocaine cessation at 1 hr



(B) Cocaine cessation at 24 hr



(C) Cocaine cessation at 72 hr

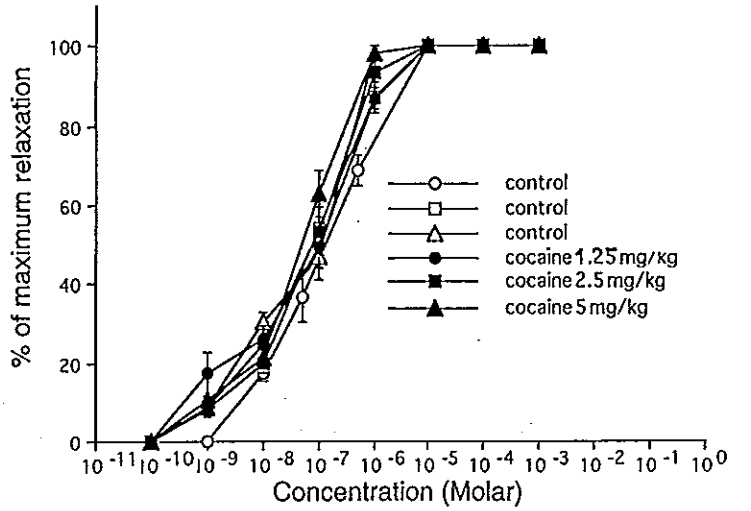
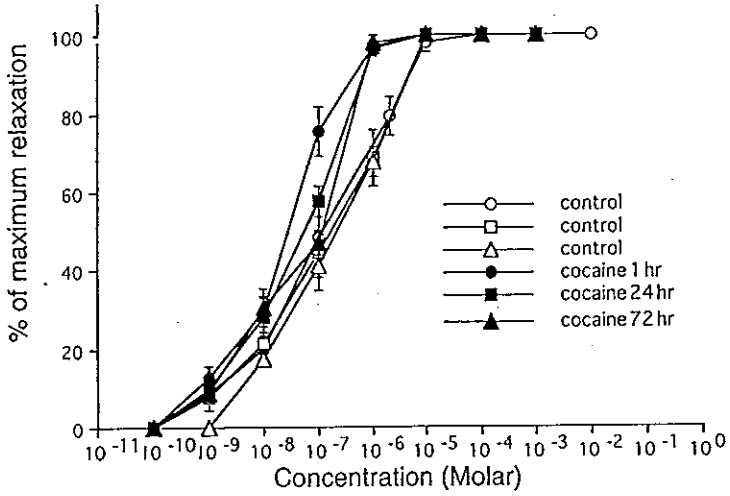
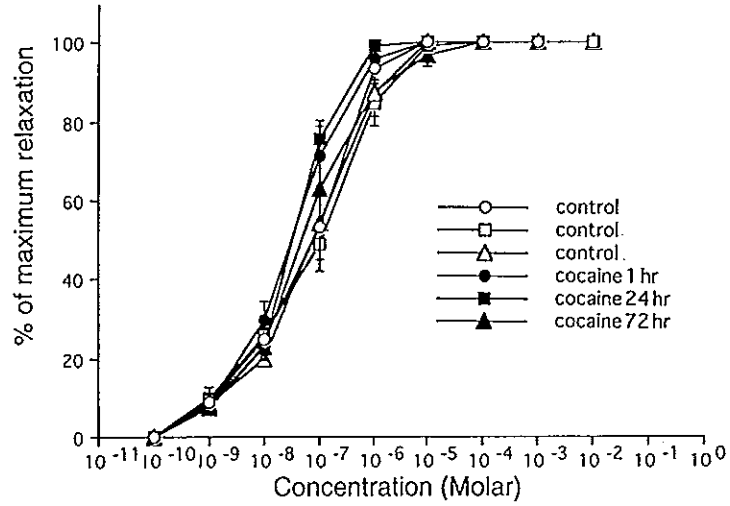


Figure 34 Comparison of the effects of various doses of chronic cocaine treatment (1.25, 2.5 and 5 mg/kg, i.p.) on the concentration-response curves of the ***relaxing effect*** of ISO on the isolated trachea, at various times (1, 24 and 72 hr) after cocaine cessation. Symbols represent means and vertical bars represent standard error of means (n = 5)

(A) Cocaine 1.25 mg/kg



(B) Cocaine 2.5 mg/kg



(C) Cocaine 5 mg/kg

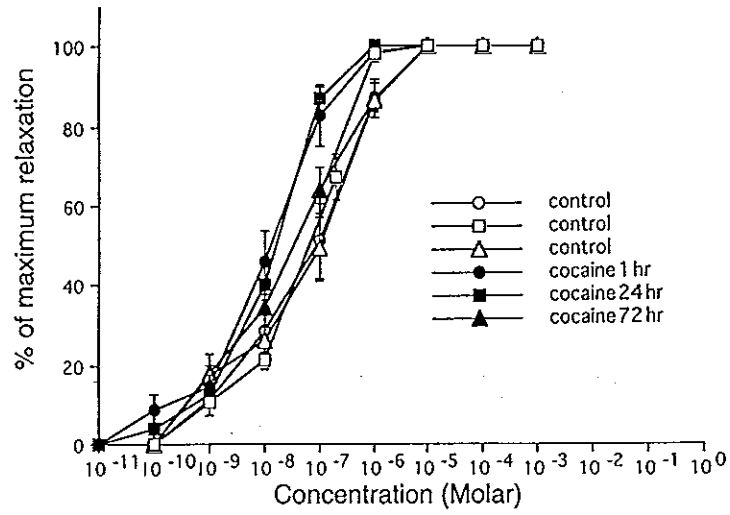


Figure 35 Comparison between various times (1, 24 and 72 hr) after cocaine cessation on the concentration-response curves of the ***relaxing effect*** of ISO on the trachea isolated from chronic cocaine-treated (1.25, 2.5 and 5 mg/kg, i.p., b.i.d. for 14 days) guinea-pig. Symbols represent means and vertical bars represent standard error of means (n = 5)

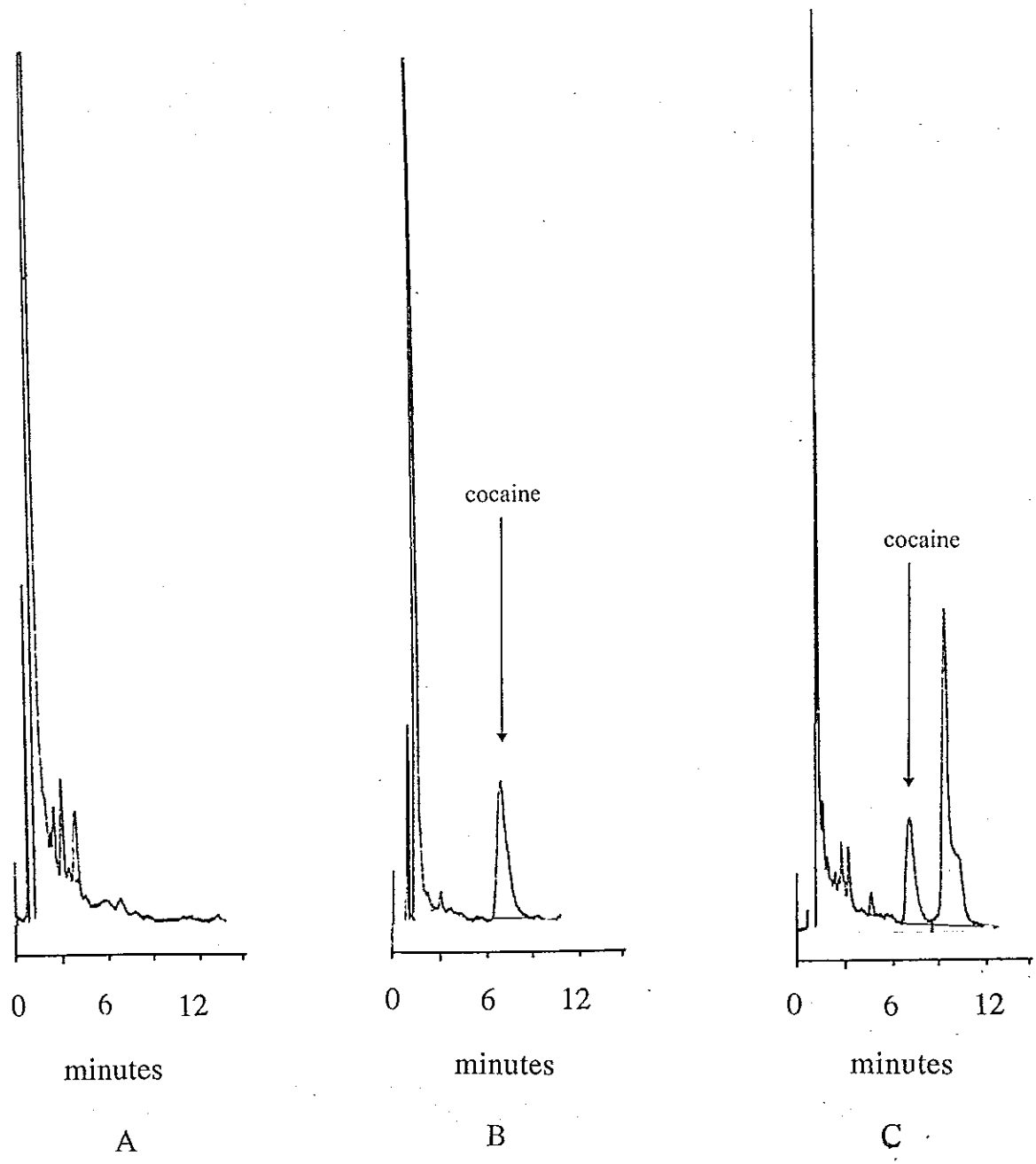


Figure 36 The representative chromatograms of (A) blank plasma; (B) plasma spiked with standard cocaine HCL, 500 ng/ml; and (C) plasma of guinea-pig at 1 hr after cocaine cessation (5 mg/kg). Chart speed 2 mm/min. Attenuation was 8 mV F.S.

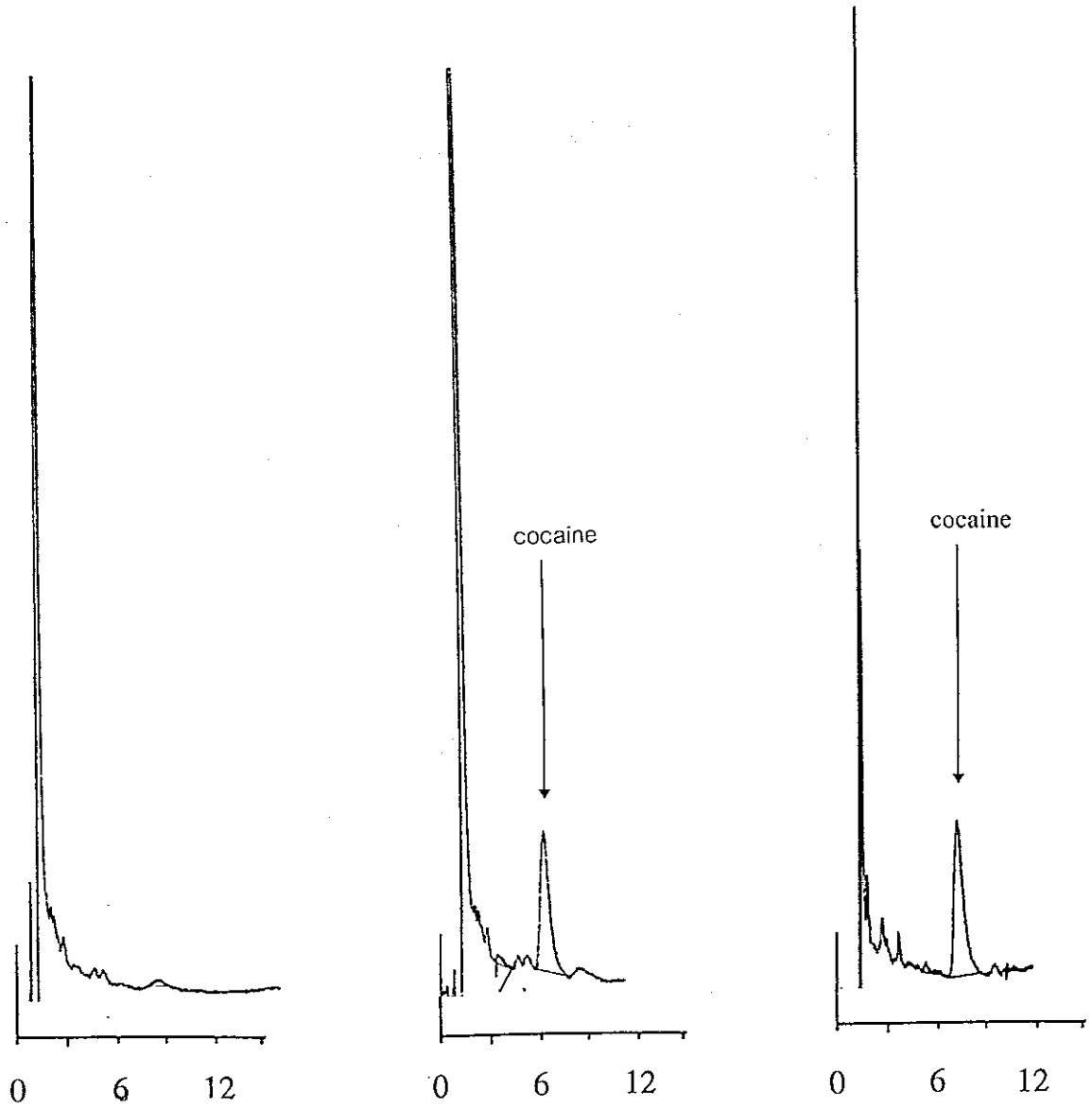


Figure 37 The representative chromatograms of (A) blank atiral tissues; (B) atrial tissues spiked with standard cocaine HCL, 500 ng/ml; and (C) atrial tissues of guinea-pig at 1 hr after cocaine cessation (5 mg/kg). Chart speed 2 mm/min. Attenuation was 8 mV F.S.

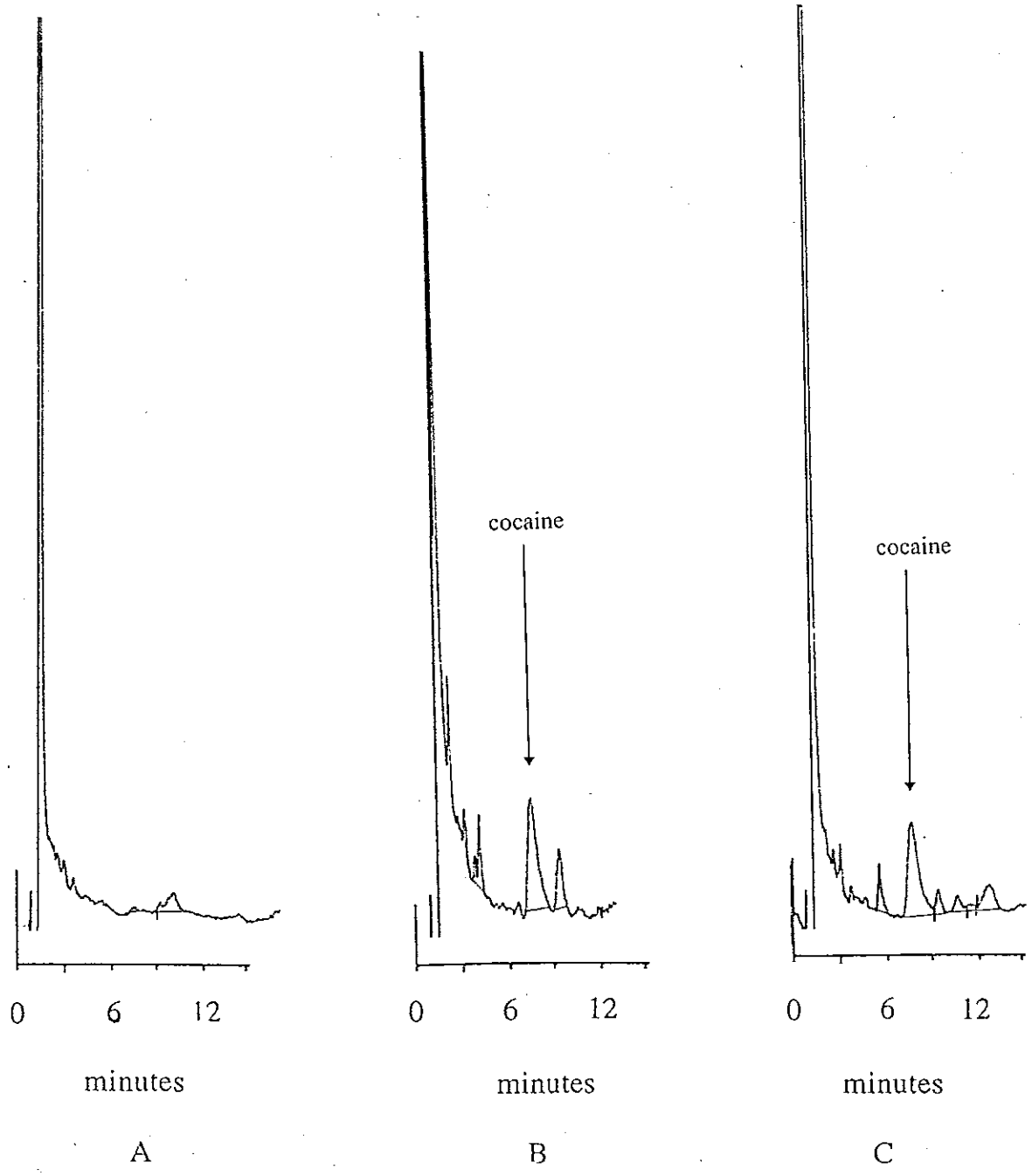


Figure 38 The representative chromatograms of (A) blank ventricular tissues; (B) ventricular tissues spiked with standard cocaine HCL, 500 ng/ml; and (C) ventricular tissues of guinea-pig treated at 1 hr after cocaine cessation (5 mg/kg). Chart speed 2 mm/min. Attenuation was 8 mV F.S.

Table 13 The pD_2 and $[D]_{max50}$ ratio values of the chronotropic effect of norepinephrine in isolated guinea-pig atria.

Experimental groups (n = 5)	pD_2 values (Mean ± S.E.) (n = 5)			$[D]_{max50}$ ratio (Mean ± S.E.) (n = 5)		
	At 1 hr	At 24 hr	At 72 hr	At 1 hr	At 24 hr	At 72 hr
Control	6.8±0.007	6.61±0.009	6.51±0.004			
Cocaine 1.25 mg/kg	8.7±0.008 [a]	8.26±0.27 [ac]	7.1±0.003 [ac]	62.09±4.7	30.07±1.96 [c]	7.74±0.72 [c]
Control	6.4±0.009	6.92±0.12	6.89±0.009			
Cocaine 2.5 mg/kg	10±0.003 [ab]	9.±0.009 [abc]	8.52±0.07 [abc]	3749.63±287.66 [b]	123.09±3.76 [bc]	48.87±3.97 [bc]
Control	6.57±0.09	6.52±0.006	6.4±0.005			
Cocaine 5 mg/kg	13.1±0.03 [ab]	12.9±0.004 [abc]	12.3±0.005 [abc]	$5.5\times10^{6}\pm3.4\times10^{5}$ [b]	$3.02\times10^{6}\pm2\times10^{5}$ [bc]	$6.9\times10^{5}\pm5.7\times10^{4}$ [bc]

pD_2 values : (negative log $[D]_{max50}$); the statistical significant differences were determined from $[D]_{max50}$ values

$[D]_{max50}$ ratio: $[D]_{max50}$ of NE or ISO of the cocaine-treated groups divided by those $[D]_{max50}$ of control

Statistical significant differences ($p < 0.05$) in relation to: [a] the control groups, [b] previous dose of cocaine and [c] previous time, respectively

Table 14 The pD_2 and $[D]_{max50}$ ratio values of the inotropic effect of norepinephrine in isolated guinea-pig atria.

Experimental groups (n = 5)	pD_2 values (Mean ± S.E.) (n = 5)			$[D]_{max50}$ ratio (Mean ± S.E.) (n = 5)		
	At 1 hr	At 24 hr	At 72 hr	At 1 hr	At 24 hr	At 72 hr
Control	7.03±0.14	6.92±0.007	6.93±0.006			
Cocaine 1.25 mg/kg	8.69±0.007[a]	8.±0.005[ac]	7.4±0.005[ac]	89.08±7.9	18.39±0.52[c]	7.44±0.72[c]
Control	6.68±0.01	6.79±0.003	6.88±0.1			
Cocaine 2.5 mg/kg	10.57±0.8[ab]	9.2±0.006[abc]	8.9±0.005[abc]	6722.46±250.9[b]	137.14±6.94[bc]	51.1±3.04[bc]
Control	6.82±0.008	6.84±0.05	6.63±0.004			
Cocaine 5 mg/kg	13±0.004[ab]	12.9±0.003[abc]	12.61±0.07[abc]	$3.1\times10^{6}\pm3.5\times10^{5}$[b]	$2.46\times10^{6}\pm6.3\times10^{5}$[bc]	$9.\times10^{5}\pm2.6\times10^{4}$[bc]

pD_2 values : (negative log $[D]_{max50}$); the statistical significant differences were determined from $[D]_{max50}$ values

$[D]_{max50}$ ratio: $[D]_{max50}$ of NE or ISO of the cocaine-treated groups divided by those $[D]_{max50}$ of control

Statistical significant differences ($p < 0.05$) in relation to: [a] the control groups, [b] previous dose of cocaine and [c] previous time, respectively

Table 15 The pD_2 and $[D]_{max50}$ ratio values of the chronotropic effect of isoproterenol in isolated guinea-pig atria.

Experimental groups (n = 5)	pD_2 values (Mean ± S.E.) (n = 5)			$[D]_{max50}$ ratio (Mean ± S.E.) (n = 5)		
	At 1 hr	At 24 hr	At 72 hr	At 1 hr	At 24 hr	At 72 hr
Control	8.65±0.12	8.74±0.005	8.68±0.004			
Cocaine 1.25 mg/kg	9.5±0.004 [a]	9.2±0.005 [ac]	8.85±0.007 [c]	44.81±1.62	18.16±0.76 [c]	7.37±0.67 [c]
Control	8.7±0.69	8.54±0.004	8.73±0.09			
Cocaine 2.5 mg/kg	11.9±0.009 [ab]	10.7±0.003 [abc]	10.19±0.14 [abc]	2278.69±159.61 [b]	151.06±8.02 [bc]	27.06±1.73 [bc]
Control	8.63±0.007	8.47±0.007	8.75±0.007			
Cocaine 5 mg/kg	15.1±0.007 [ab]	14.5±0.008 [abc]	13.29±0.06 [abc]	$4.33\times10^{6}\pm3.5\times10^{5}$ [b]	$2.8\times10^{5}\pm6.5\times10^{4}$ [bc]	$6.22\times10^{5}\pm3.0\times10^{4}$ [bc]

pD_2 values : (negative log $[D]_{max50}$); the statistical significant differences were determined from $[D]_{max50}$ values

$[D]_{max50}$ ratio: $[D]_{max50}$ of NE or ISO of the cocaine-treated groups divided by those $[D]_{max50}$ of control

Statistical significant differences ($p < 0.05$) in relation to: [a] the control groups, [b] previous dose of cocaine and [c] previous time, respectively

Table 16 The pD_2 and $[D]_{max50}$ ratio values of the inotropic effect of isoproterenol in isolated guinea-pig atria.

Experimental groups (n = 5)	pD_2 values (Mean ± S.E.) (n = 5)			$[D]_{max50}$ ratio (Mean ± S.E.) (n = 5)		
	At 1 hr	At 24 hr	At 72 hr	At 1 hr	At 24 hr	At 72 hr
Control	8.51±0.11	8.73±0.003	8.83±0.006			
Cocaine 1.25 mg/kg	9.98±0.005 [a]	9.09±0.005 [ac]	9.01±0.007 [ac]	47.97±3.07	10.99±0.87 [c]	4.47±0.18 [c]
Control	8.66±0.03	8.75±0.01	8.5±0.007			
Cocaine 2.5 mg/kg	12±0.003 [ab]	10.9±0.007 [abc]	10.4±0.009 [abc]	2528.87±105.91 [b]	156.69±7.81 [bc]	42.86±2.24 [bc]
Control	8.7±0.007	8.41±0.009	8.93±0.004			
Cocaine 5 mg/kg	15.6±0.003 [ab]	14.6±0.009 [abc]	13.6±0.005 [abc]	$6.19\times10^{6}\pm3.86\times10^{5}$ [b]	$8\times10^{5}\pm4.9\times10^{4}$ [bc]	$5.03\times10^{5}\pm2.7\times10^{4}$ [bc]

pD_2 values : (negative log $[D]_{max50}$); the statistical significant differences were determined from $[D]_{max50}$ values

$[D]_{max50}$ ratio: $[D]_{max50}$ of NE or ISO of the cocaine-treated groups divided by those $[D]_{max50}$ of control

Statistical significant differences ($p < 0.05$) in relation to: [a] the control groups, [b] previous dose of cocaine and [c] previous time, respectively

Table 17 The pD_2 and $[D]_{max50}$ ratio values of relaxing effect of norepinephrine on carbachol (1 μg/ml)-induced contraction of isolated guinea-pig trachea

Experimental groups (n = 5)	pD_2 values (Mean ± S.E.) (n = 5)			$[D]_{max50}$ ratio (Mean ± S.E.) (n = 5)		
	At 1 hr	At 24 hr	At 72 hr	At 1 hr	At 24 hr	At 72 hr
Control	5.55±0.26	5.65±0.25	5.57±0.21			
Cocaine 1.25 mg/kg	6.44±0.009 [a]	6.05±0.17 [a]	5.71±0.13 [c]	10.77±0.43	9.2±0.9	4.75±0.18 [c]
Control	5.6±0.12	5.47±0.11	5.35+0.38			
Cocaine 2.5 mg/kg	6.8±0.13 [ab]	6.12±0.007 [ac]	5.83+0.007 [ac]	17.85±0.76 [b]	9.89±0.76 [c]	6.85±0.41 [bc]
Control	5.48±0.01	5.53±0.23	5.51±0.007			
Cocaine 5 mg/kg	7.11±0.009 [ab]	6.34±0.14 [abc]	6.32±0.009 [ab]	73.25±2.24 [b]	15.88±1.4 [bc]	10.47±0.36 [bc]

pD_2 values : (negative log $[D]_{max50}$); the statistical significant differences were determined from $[D]_{max50}$ values

$[D]_{max50}$ ratio: $[D]_{max50}$ of NE or ISO of the cocaine-treated groups divided by those $[D]_{max50}$ of control

Statistical significant differences ($p < 0.05$) in relation to: [a] the control groups, [b] previous dose of cocaine and [c] previous time, respectively

Table 18 The pD_2 values and $[D]_{max50}$ ratio of relaxing effect of isoproterenol on carbachol (1 μg/ml)-induced contraction of isolated guinea-pig trachea

Experimental groups (n = 5)	pD_2 values (Mean ± S.E.) (n = 5)			$[D]_{max50}$ ratio (Mean ± S.E.) (n = 5)		
	At 1 hr	At 24 hr	At 72 hr	At 1 hr	At 24 hr	At 72 hr
Control	6.93±0.11	6.99±0.13	6.59±0.009			
Cocaine 1.25 mg/kg	7.62±0.009 [a]	7.44±0.007 [ac]	7.17±0.009 [c]	14.14±1.06	6.58±0.47 [c]	3.47±0.2 [c]
Control	7.01±0.18	7.1±0.008	6.92±0.007			
Cocaine 2.5 mg/kg	7.55±0.11 [a]	7.43±0.008 [a]	7.21±0.11 [bc]	19.49±2.19 [b]	10.88±1.29 [bc]	6.57±0.5 [c]
Control	7.1±0.13	7.08±0.01	7.02±0.12			
Cocaine 5 mg/kg	7.92±0.11 [ab]	7.8±0.007 [ab]	7.53±0.006 [abc]	81.72±2.58 [b]	17.87±0.7 [bc]	9.82±0.69 [bc]

pD_2 values : (negative log $[D]_{max50}$); the statistical significant differences were determined from $[D]_{max50}$ values

$[D]_{max50}$ ratio: $[D]_{max50}$ of NE or ISO of the cocaine-treated groups divided by those $[D]_{max50}$ of control

Statistical significant differences ($p < 0.05$) in relation to: [a] the control groups, [b] previous dose of cocaine and [c] previous time, respectively

Table 19 Comparison of the $[D]_{max50}$ ratio values of norepinephrine and isoproterenol in atria and trachea isolated from various doses of cocaine-treated guinea-pigs.

Tissues	Drugs	Cocaine 1.25 mg/kg			Cocaine 2.5 mg/kg			Cocaine 5 mg/kg		
		At 1 hr	At 24 hr	At 72 hr	At 1 hr	At 24 hr	At 72 hr	At 1 hr	At 24 hr	At 72 hr
Atria	NE	62.09	30.1	7.74	3750	123	48.87	5.5×10^{6}	3.02×10^{6}	6.9×10^{5}
(rate)	ISO	44.81	18.16	7.37	2278.69	151.06	27.06	4.33×10^{6}	2.76×10^{6}	6.22×10^{5}
Atria	NE	89.08	18.39	7.44	6722.46	137.14	51.1	3.1×10^{6}	2.46×10^{6}	9.01×10^{5}
(force)	ISO	47.97	10.99	4.47	2528.87	156.69	42.86	6.19×10^{6}	8×10^{5}	5.03×10^{5}
Trachea	NE	10.77	9.2	4.75	17.85	9.89	6.85	73.25	15.88	10.47
	ISO	14.14	6.58	3.47	19.49	10.88	6.57	81.72	17.87	9.82

$[D]_{max50}$ ratio: $[D]_{max50}$ of NE or ISO of the cocaine-treated groups divided by those $[D]_{max50}$ of control

Table 20 The concentrations of cocaine in plasma, atrial and ventricular tissues after 1 hr cessation of cocaine (1.25, 2.5 and 5 mg/kg)

Concentration of cocaine in	Dose of cocaine (n = 5)		
	1.25 mg/kg	2.5 mg/kg	5 mg/kg
Plasma (ng/ml)	40.9 ± 4.03	70.7 ± 5.65 [a]	285.6 ± 17.1 [a]
Atrial tissue (ng/g)	127.4 ± 15.76	245.4 ± 12.48 [a]	629.4 ± 32.8 [a]
Ventricular tissue (ng/g)	121.56 ± 8.54	221 ± 18.29 [a]	613 ± 47.6 [a]

Data expressed as mean ± S.E.

[a] Significant difference compared with previous concentration of cocaine ($p < 0.05$)

Table 21 The concentrations of cocaine in plasma, atrial and ventricular tissues after 24 hr cessation of cocaine (1.25, 2.5 and 5 mg/kg)

Concentration of cocaine in	Dose of cocaine (n = 5)		
	1.25 mg/kg	2.5 mg/kg	5 mg/kg
Plasma (ng/ml)	ND	27.63 (N=1) ND (n=4)	36.48 (n=2) ND (n=3)
Atrial tissue (ng/g)	ND	38.8 (n=2) ND(n=3)	69.57 ± 10.67
Ventricular tissue (ng/g)	ND	ND	62.58 ± 8.38

Data expressed as mean ± S.E.

ND: not detectable; the detection limit of cocaine in plasma and cardiac tissues = 12.5 and 20 ng/ml, respectively

Table 22 The concentrations of cocaine in plasma, atrial and ventricular tissues after 72 hr cessation of cocaine (1.25, 2.5 and 5 mg/kg)

Concentration of cocaine in	Dose of cocaine (n = 5)		
	1.25 mg/kg	2.5 mg/kg	5 mg/kg
Plasma (ng/ml)	ND	ND	ND
Atrial tissue (ng/g)	ND	ND	ND
Ventricular tissue (ng/g)	ND	ND	ND

Data expressed as mean ± S.E.

ND: not detectable; the detection limit of cocaine in plasma and cardiac tissues = 12.5 and 20 ng/ml, respectively

Table 23 Comparison between the $[D]_{max50}$ ratio and cocaine concentrations in plasma, atrial and ventricular tissues following 1.25, 2.5 and 5 mg/kg cocaine administration **at 1 hr after cessation**

Experimental groups (n = 5)	$[D]_{max50}$ ratio						Cocaine concentration (Mean ± S.E.)		
	Atrial rate		Atrial force		Tracheal relaxation		Plasma (ng/ml)	Atria (ng/g)	Ventricle (ng/g)
	NE	ISO	NE	ISO	NE	ISO			
Cocaine 1.25 mg/kg	62.09	44.81	89.09	49.97	10.77	14.14	40.9±4.3	127.4±15.76	121.56±8.54
Cocaine 2.5 mg/kg	3749.63	2278.69	6722.46	2528.87	17.85	19.49	70.7±5.65	245.5±12.48	221±18.29
Cocaine 5 mg/kg	$5.5x10^6$	$4.3x10^6$	$3.1x10^6$	$6.2x10^6$	73.25	81.72	285.6±17.1	629.4±32.8	613.14±47.6

$[D]_{max50}$ ratio: $[D]_{max50}$ of NE or ISO of the cocaine-treated groups divided by those $[D]_{max50}$ of control

Table 24 Comparison between the $[D]_{max50}$ ratio and cocaine concentrations in plasma, atrial and ventricular tissues following 1.25, 2.5 and 5 mg/kg cocaine administration **at 24 hr after cessation**

Experimental groups (n = 5)	$[D]_{max50}$ ratio						Cocaine concentration (Mean ± S.E.)		
	Atrial rate		Atrial force		Tracheal relaxation		Plasma (ng/ml)	Atria (ng/g)	Ventricle (ng/g)
	NE	ISO	NE	ISO	NE	ISO			
Cocaine 1.25 mg/kg	30.07	18.16	18.39	10.99	9.2	6.58	ND	ND	ND
Cocaine 2.5 mg/kg	123.09	151.06	137.14	156.69	9.89	10.88	26.73 (n=1) ND(n=4)	38.08 (n=2) ND (n=3)	ND
Cocaine 5 mg/kg	$3.02x10^6$	$9.76x10^5$	$2.46x10^6$	$8x10^5$	15.88	17.87	36.84 (n=2) ND (n=3)	69.57±10.67	62.58±8.38

$[D]_{max50}$ ratio: $[D]_{max50}$ of NE or ISO of the cocaine-treated groups divided by those $[D]_{max50}$ of control

ND: not detectable, the detection limit of cocaine in plasma and cardiac tissues = 12.5 and 20 ng/ml, respectively

Table 25 Comparison between the $[D]_{max50}$ ratio and cocaine concentrations in plasma, atrial and ventricular tissues following 1.25, 2.5 and 5 mg/kg cocaine administration **at 72 hr after cessation**

Experimental groups (n = 5)	$[D]_{max50}$ ratio						Cocaine concentration (Mean ± S.E.)		
	Atrial rate		Atrial force		Tracheal relaxation		Plasma (ng/ml)	Atria (ng/g)	Ventricle (ng/g)
	NE	ISO	NE	ISO	NE	ISO			
Cocaine 1.25 mg/kg	7.74	7.37	7.44	4.47	4.75	3.47	ND	ND	ND
Cocaine 2.5 mg/kg	29.87	27.06	51.1	42.86	6.15	6.57	ND	ND	ND
Cocaine 5 mg/kg	$6.91x10^5$	$6.22x10^5$	$9.01x10^5$	$5.03x10^5$	10.47	9.82	ND	ND	ND

$[D]_{max50}$ ratio: $[D]_{max50}$ of NE or ISO of the cocaine-treated groups divided by those $[D]_{max50}$ of control

ND = not detectable; the detection limit of cocaine in plasma and cardiac tissues = 12.5 and 20 ng/ml, respectively

CHAPTER 5

DISSCUSSION

In the present study, the pD_2 values, which indicated the responsiveness of atria and trachea to exogenously administered norepinephrine and isoproterenol, of cocaine-treated groups were significantly higher than those of control groups. These findings demonstrated that low doses of chronic cocaine treatment induced supersensitivity of β-adrenoceptor-mediated responses. The supersensitivity occurred in both atria (mainly β_1-adrenoceptors) and trachea (mainly β_2-adrenoceptors) and still persisted at 72 hr after cocaine cessation. Isoproterenol was more potent than norepinephrine in producing responses in both atria and trachea. The D_{max50} ratio, which indicated the degree of supersensitivity, showed that the degree of supersensitivity to norepinephrine and isoproterenol was similar in trachea but in atria, the supersensitivity to norepinephrine was higher than to isoproterenol. The present results also demonstrated that cocaine potentiated the chronotropic and inotropic responses in atria more than the relaxation responses in trachea. The degree of supersensitivity in β_1-adrenoceptors depended on doses and times after cocaine cessation while the degree of supersensitivity in β_2-adrenoceptors was saturated in nature and was much less depended on doses of cocaine or times after cocaine cessation. Therefore, it appeared that the supersensitivity of different subtypes of β-adrenergic receptors might be produced by different mechanisms.

The concentrations of cocaine in plasma, atrial and ventricular tissues depended on doses and times after cocaine cessation as well and they could be detected in all cocaine-treated groups at 1 hr after cocaine cessation, but after 24 hr of the cessation, the cocaine concentrations were not observed in some animals. At 72 hr after cocaine cessation, the cocaine concentrations could not be detected either in plasma, atrial or ventricular tissues. The concentration of cocaine in plasma, atrial and ventricular tissues directly correlated with the degree of supersensitivity at 1 hr after cocaine cessation but the correlation was not clearly observed at 24 hr after cocaine cessation. There was no such correlation between the degree of supersensitivity and the concentrations of cocaine in plasma, atrial and ventricular tissues at 72 hr of the cessation at any doses given.

It has been known that cocaine can potentiate the responses to catecholamines (Kalsner and Nickerson, 1969; Levy and Blattberg, 1978; Summers and Tillman, 1979; Unterwald *et al.*, 1994). The sympathomimetic effects of cocaine induce a dose-dependent increase in heart rate, force of contraction and systemic arterial pressure which may result in cardiovascular complications, but the mechanisms responsible for these cardiotoxic effects of cocaine are still controversial. Several hypotheses have been continually advanced to describe the mechanism of action of cocaine-induced supersensitivity of cardiac responses to catecholamines, but only two are ever given much attention. It has been suggested that uptake and storage of norepinephrine by adrenergic nerves play a dominant part in regulation of responses to the amine. Cocaine blocks the reuptake process of catecholamines

at the postganglionic sympathetic nerve ending, the major mechanism for the termination of action of locally released and circulating catecholamines. The uptake blocking action of cocaine diverts amine to the vicinity of appropriate tissue receptors and interferes amine inactivation, thus increasing the synaptic concentrations of these monoamines available for binding to the adrenergic receptors and enhancing the effects of exogenously administered norepinephrine at the region of the adrenoceptor sites (Withby *et al.*, 1960; Trendelenburgs *et al.*, 1972; Day, 1979; Abrahams, *et al.*, 1996; Suhara, *et al.*, 1996). However, this hypothesis can not explain the effects of cocaine in some conditions. If the mechanism of cocaine in potentiation of the responses to catecholamines is the inhibition of uptake process, it is necessary that the concentration of cocaine must be high enough to block the reuptake, moreover, the sympathetic nerve terminals should be intact, and the amine should be taken up into the nerve terminals under normal physiological condition (Reiffenstein and Triggle, 1974). On the other hand, they have some experiments that do not support this hypothesis. Reiffenstein and Triggle (1974) proved that the concentrations of cocaine (3.3×10^{-7} - 3.3×10^{-5} M), which could not block the uptake process of catecholamine, induced supersensitivity responses in isolated human umbilical artery which is devoid of adrenergic innervation. In addition, cocaine also produced the leftward shifts of the dose-response curves to oxymetazoline, which is not a substrate for neuronal uptake, in isolated splenic capsular strip of the cat. Comparison of the potentiative effects between cocaine and desmetylimipramine, a more potent catecholamine uptake blocker than cocaine, showed that desmetylimipramine failed to produce potentiation

responses to oxymetazoline (Summers and Tillman, 1979). Cocaine also induced supersensitivity in isolated aortic strip of the rabbit that had been stored at 6 °C for 10 days to permit degeneration of the sympathetic nerves (Kalsner and Nickerson, 1969; Shibata *et al*, 1971).

Besides the β-adrenoceptors, cocaine still induced supersensitivity to α_1-adrenoceptors. Nakatsu and Reiffenstein (1968) showed that cocaine potentiated norepinephrine induced contraction responses in isolated vas deferen which is blocked a sufficient number of α_1-adrenoceptors by phenoxybenzamine, an irreversibly α_1-receptor blocking agent, thus the maximum response is dependent on the number of receptor remaining and not on the mechanical limitation of the tissue. After blockade with phenoxybenzamine, norepinephrine was administered in organ bath to produce the maximum response. Cocaine, which introduced during response to norepinephrine also caused the maximum response, enhanced an increase in the maximum response to norepinephrine. So, the increase in response induced by cocaine was not related to the local concentration of norepinephrine because the portion of receptor remaining after blockade could not produce the maximum response of which tissue is capable and because the equilibrium were not changed in the addition of norepinephrine. Therefore, cocaine-induced supersensitivity could then only be modified by the postsynaptic site mechanism. All these studies did not support the concept that the uptake blocking action of cocaine plays an important role in cocaine-induced supersensitivity.

In the present study, low doses of chronic cocaine treatment potentiated chronotropic and inotropic responses in atria and relaxation responses in trachea to norepinephrine and isoproterenol, which persisted up to 72 hr after cocaine cessation. These results corresponded with the study of Kalsner and Nickerson (1969) in the rabbit isolated aortic strip that clearly proved that cocaine enhanced the contractile responses to norepinephrine for at least 28 hr at 37 °C.

The concentrations of cocaine in plasma, atrial and ventricular tissues were also analyzed in order to determine the relationships between the cocaine concentrations and the degree of supersensitivity. The concentrations of cocaine directly correlated with the degree of supersensitivity at 1 hr after cocaine cessation. However, it is unlikely that the correlation at 1 hr after cocaine cessation would be due to the neuronal uptake blocking action of cocaine, especially at cocaine doses of 1.25 and 2.5 mg/kg, because according to the study in cat isolated nictitating membrane of Trendelenburges *et al.* (1972) and in human isolated umbilical artery of Reiffenstein and Triggle (1974) showed that the concentrations of cocaine lower than 340 ng/ml could not inhibit the neuronal uptake process of norepinephrine. Nevertheless, from this study, the cocaine concentrations of most cocaine-treated groups, except at 1 hr after cocaine cessation, were much less than this level or even undetectable in some animals while the supersensitivity still remained. At 1 hr after cocaine cessation, the cocaine concentrations in atrial (629 ng/g) and ventricular tissues (613 ng/g) were quite high, therefore, the neuronal uptake inhibition of cocaine could not be excluded.

The present study also exhibited that cocaine could induce supersensitivity responses of β-adrenoceptors to isoproterenol, which is much less taken up by the nerve ending (Day, 1979; Hammond *et al.*, 1992), although the degree of supersensitivity was lesser than that of norepinephrine.

The present study in trachea, which is almost entirely not innervated by the adrenergic nerves (Barnes, 1992; Kamikawa, 1994) showed that chronic cocaine treatment produced supersensitivity responses to exogenous catecholamines. This study provided additional evidence that the major action of cocaine in potentiation responses to sympathetic amines is unrelated to the inhibition of uptake process.

In this study, the tissue concentration of norepinephrine was not determined, so there was no evidence to prove whether the concentration of norepinephrine was high or low at the synaptic site, but Levy and Blattberg (1978) demonstrated that in open-chest, anesthetized dogs, the coronary sinus norepinephrine concentration was not increase by cocaine infusion. Sunbhanich (1980) determined the K_D values of norepinephrine under non-equilibrium condition compared to $[D]_{max50}$ obtained under equilibrium condition in guinea-pig isolated right atria. The K_D value obtained under non-equilibrium condition was assumed to represent the concentration of catecholamine in the biophase and $[D]_{max50}$ represented the concentration in the organ bath. In control experiment, the ratio of $Kd/[D]_{max50}$ was more than unity, suggested that the concentration of norepinephrine in the biophase was higher than that in the bathing solution. In contrast, the ratio of $Kd/[D]_{max50}$ decreased toward unity in the presence of cocaine, suggesting that the concentration of norepinephrine in

the biophase was decreased. Therefore, potentiation of norepinephrine in guinea-pig isolated atria was likely due to the receptor sensitizing action of cocaine rather than its neuronal uptake blocking action. Knuepfer *et al.* (1993) determined whether there is a relationship between the different effects of cocaine administration on cardiovascular responses and atrial norepinephrine concentration. This study showed that atrial norepinephrine concentrations were not affected by cocaine. Alburges and Wamsley (1993) and Alburges *et al.* (1996) demonstrated that chronic cocaine treatment (5, 10, 15, 20 and 25 mg/kg, intraperitoneally, twice a day for 14 days) did not change the concentrations of norepinephrine and its metabolites at certain areas of the brain. Only ecgonine methyl ester, not cocaine and its other metabolites was found to be accumulated in the brain. Consequently, low doses of chronic cocaine treatment in this study might not involve with the inhibition of uptake process but possibly occurred at the postsynaptic levels.

There are some other mechanisms could describe cocaine-induced supersensitivity at postsynaptic sites. Firstly, cocaine may alter either the intrinsic activity or affinity of the adrenoceptors (Nakatsu and Reiffenstein, 1968; Reiffenstein and Triggle, 1974; King *et al.*, 1994), but this mechanisms has not been proved yet. Secondly, cocaine may increase the densities of adrenoceptors. In chronic cocaine treatment, it was found that the concentrations of dopaminergic receptors in certain areas of the brain were increased (Unterwald *et al.*, 1994) but there is no evidence to support that cocaine could increase the densities of β-adrenoceptors. In contrast, Avakian *et al.* (1987) demonstrated that chronic cocaine treatment (5 mg/kg,

subcutaneously, twice a day for 14 days and 20 mg/kg, subcutaneously, twice a day for 21 days) did not change either the number or the affinity of β-adrenoceptors in cardiac tissues. On the other hand, some studies showed that cocaine has a direct effect on calcium influx across the cell membrane during response to β-adrenoceptors agonists (Kalsner, 1992; Tomita *et al.*, 1993; He *et al.*, 1994). However, the relationship of supersensitivity induced by cocaine and the conformational changes still has not been studied. It has been known that stimulation of β-adrenoceptors activated the adenylate cyclase system via a second messenger, Gs protein (Rang and Dale, 1991; Cruickshank and Prichard, 1994; Katzung, 1998) but it has no evidence to prove that whether cocaine may produce the conformational changes which result in changes in sensitivity of β-adrenoceptors.

The results of this present study suggested that chronic cocaine treatment induced supersensitivity in β-adrenoceptors might not due to neuronal uptake blockade action of cocaine. Therefore, other mechanisms e.g. the conformational changes or increase in the number of β-adrenoceptors or an alteration of the adenylate cyclase systems might involve in the supersensitivity induced by cocaine. Further studies should undergo to clarify the mechanism of cocaine-induced postsynaptic supersensitivity. These findings may lead to understand its cardiotoxic mechanism, the most important complication of cocaine users, and to prevent its lethal cardiac toxicity.

The different responsiveness between β_1- and β_2-adrenoceptors might introduced some mechanisms of cocaine-induced supersensitivity at postsynaptic site. Intracellular calcium was known to involve in the action of

catecholamines. For the contraction of the excitatory cardiac muscle, catecholamines may increase calcium influx and/or release calcium from intracellular stores. The relaxation of smooth muscle may also be caused either by suppression of calcium influx, sequestration of calcium into the cellular store or interference with the contractile machinery (see reviewed by Bulbring and Tomita, 1987). Summers and Tillman (1979) and Kalsner (1993) demonstrated that cocaine induced increases in the calcium influx in cat spleen strip and coronary artery, respectively, which could inhibit by the calcium channel blocker. Tomita *et al.* (1993) noted that in skinned rat heart muscle loaded the sarcoplasmic reticulum with 10^{-6} M calcium, when cocaine was applied during the calcium loading periods, the amount of calcium accumulated by the sarcoplasmic reticulum significantly increased. It seemed that cocaine could directly alter the ability of the sarcoplasmic reticulum to release and accumulate calcium. However, in this present study, the increased intracellular calcium could not describe the characteristic of supersensitivity in β_2-adrenoceptor because the concentration of calcium, the composition of Krebs' solution, was stable but the responsiveness between cocaine-treated and control groups was different. In fact, Kumar (1978) showed that in the dog tracheal muscle, which induced contraction by 120 mM potassium (14.5 mM sodium), the relaxation caused by isoproterenol depended on the external calcium concentration. In the presence of 10 mM calcium, the relaxation by 10 mM isoproterenol was only 10%, while in 0.1 mM calcium, the relaxation reached 70%. It appeared that excessive loading of intracellular calcium store could reduce the relaxant effect of isoproterenol. The study of Adelstein *et al.* (1978) which suggested that the

increase in intracellular cAMP that inhibited the tension development in smooth muscle, was decreased by a rise in calcium, thus this study provided the supporting evidence that the supersensitivity induced by cocaine might not be due to a direct action on calcium channel alone. On the other hand, it has been known that β-adrenoceptors interact with a stimulatory guanine nucleotide binding protein, G_s, which activates the adenylate cyclase system. The increased intracellular cAMP controls cardiac muscle contractile function and rate of heart beating by activating a protein kinase and it may regulate the intracellular calcium distribution (Cruickshank and Prichard, 1994). The relaxation of smooth muscle caused by β-agonists is thought to involve an increase in cAMP formation by stimulating adenylate cyclase via G_s protein (Range and Dale, 1991; Katzung 1998) because the relaxation is correlated with a concomitant increase in cAMP content and cAMP mimics the effects of β-agonists (see reviewed by Bulbring and Tomita, 1987). Therefore, increase in the amount of G protein or cAMP might involve in the supersensitivity induced by cocaine. If chronic cocaine treatment increased G protein activity or amount and then enhanced the amount of cAMP, it could describe that the unsaturated, concentration-dependent supersensitivity in β_1-adrenoceptors occurred because, in β_1-adrenoceptors, increased cAMP produced increase in intracellular calcium level and then resulted in increased automaticity and contraction in atria. This increased cAMP also could explain the saturated, concentration-independent supersensitivity in β_2-adrenoceptors. The increased cAMP caused the increase in rate and/or amount of relocation of calcium away from the contractile protein and/or suppression of calcium sequestration from its store. The limitation of

calcium amount in the contractile protein or calcium store might be the reason that why the supersensitivity in β_2-adrenoceptors was saturated and only slightly depended on the concentration of cocaine. Bulbring and Tomita (1987) also noted that in the cat tracheal muscle, the action of isoproterenol occurred only when calcium was released from the intracellular store (in the presence of muscarinic agonists) and this calcium store could nearly saturated.

Cocaine induced supersensitivity in α_1-adrenoceptor has been noted. In this study, which the α_1-adrenoceptors were unblocked, the supersensitivity in α_1-adrenoceptor might actually occur in atria that consist of both α_1 and β_1-adrenoceptor. Therefore, the degree of supersensitivity in β_1-adrenoceptor to norepinephrine, which was higher than to isoproterenol, might be caused by the synergistic effects of α_1 and β_1-adrenoceptor, in response to norepinephrine, which has more selectivity to α_1-adrenoceptor than isoproterenol.

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