CHAPTER 2

REVIEW OF LITERATURE

SECTION I: COCAINE

1. The Origin of Cocaine

Cocaine is a naturally occurring substance derived from the leaves of coca plants (*Erythroxylon coca*) indigenous to South America, which most being cultivated in Bolivia and Peru. Coca plants are typically pruned to a desired height of 3 to 6 feet, make them easier to harvest. The leaves of the coca plant are elliptical in shape, varying from 1 to 4 inches in length and can be harvested several times a year since the plants are evergreen. Coca leaves contain a cocaine alkaloid that comprises about 0.5 to 1.0 percent of the plant's dry weight. Besides cocaine, several other alkaloids are found in coca leaves. The coca leaves have been used by South American Indians for more than 3,000 years. The use of coca leaves associated historically with religious ceremonies of the Incars and reserved specifically for nobility. (Murray, 1986; Karch, 1989; Johanson & Fischman, 1989; and Das, 1993).

Cocaine is one of 14 alkaloids extracted from the leaves of 2 species of coca, *Erythroxylum coca* (found in South America, Central America, India and Java) and *Erythroxylum novogranatense* (in South America). The leaves are steeped in alkaline, sulphuric acid, paraffin or other solvents. The mixture forms a thick brown paste, "coca paste", which contains 40 to 91

percent cocaine. Subsequently, the alkaloids are precipitated with sodium carbonate and then dissolved in dilute hydrochloric acid to produce cocaine hydrochloride containing 40 percent cocaine. Extraction of cocaine hydrochloride with ether in aqueous or alkaline solution produces "freebase" or "crack", which contains 85 to 90 percent of pure cocaine (Goldfrank, 1990).

2. General Chemistry of Cocaine

2.1. Chemical Structure of Cocaine

Cocaine's chemical name is benzoylmethylecgonine. Its molecular formula and molecular weight are $C_{17}H_{21}NO_4$ and 303.4, respectively (Reynolds, 1989). It is a weak base with a pKa = 8.6 (Murray, 1986). Figure 1 shows the chemical structure of cocaine. It contains a nitrogen base, methylecgonine and ester of benzoic acid, making its chemical known as a benzoylmethylecgonine. Cocaine contains both a hydrophilic and hydrophobic domains that are separated by an intermediate alkyl chain. The hydrophobicity of cocaine causes it to rapidly penetrate into brain tissue and to be sequestered into fat tissue. The sequestration of cocaine into lipid tissue produce a relative increase in cocaine's potency, prolong its short duration of action, and increase its potential toxicity (Ritchie & Green, 1990).

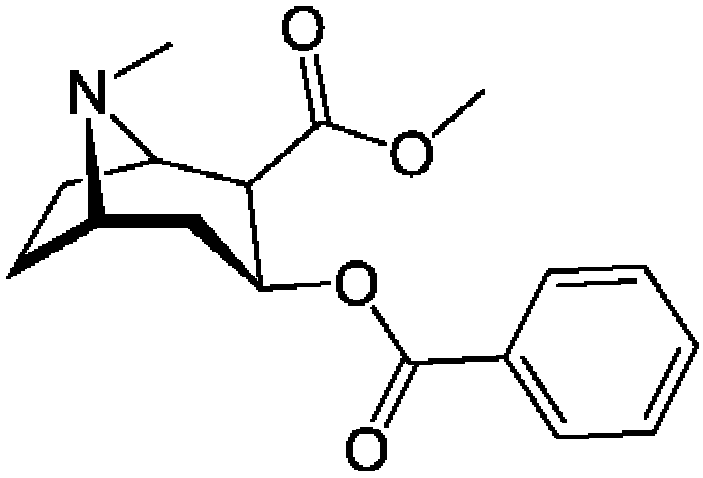

Figure 1 The chemical structure of cocaine (From Wikipedia, the free encyclopedia)

2.2 Chemical and Physical Properties of Cocaine

Cocaine usually presented in a hydrochloride salt. Cocaine hydrochloride is prepared by dissolving the cocaine alkaloid in hydrochloric acid to form a water soluble salt, as form of crystals, granules and powder. It is slightly bitter and numbs the tongue and lips. Cocaine hydrochloride decomposes on heating and melts at 195 ^{o} C. Another commercial cocaine, alkaloid or free base is the form for inhalation (smoking). 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 because cocaine base melts at 98 ^{o} C and vaporizes at higher temperature and its cocaine aqueous solution is alkaline (see reviewed by Cregler & Mark, 1986).

3. History of Cocaine Use and Abuse

Coca was recognized for its ability to boost energy, relieve fatigue, and lessen hunger. Chewing the coca leaf was the predominant mode of ingestion of cocaine until 1860, when the drug was isolated by Albert Niemann. The leaves have also been steeped into teas and incorporated into beverages, such as Coca Cola (which no longer contains cocaine.) In the United States, cocaine was once an ingredient in many patented medicines. Pure cocaine was first used medicinally in the 1880s as a local anesthetic in eye, nose, and throat surgery because of its ability to provide anesthesia as well as to constrict blood vessels and limit bleeding. Many of its therapeutic applications are obsolete due to the development of safer drugs (Avois**,** et al., 2006). In the late 1880s and 1890s, cocaine use became popular and many reports of addiction began to emerge, leading to the recognition of its potentially dangerous effects. The Harrison Narcotic Act of 1914, which was subsequently modified in 1922, prohibited the importation of cocaine and coca leaves, except for pharmaceutical purposes. The Controlled Substances Act of 1970 prohibits the manufacture, distribution, and possession of cocaine, except for limited medical purposes. The use of cocaine in the United States of America tapered in the 1920s, when amphetamines replaced cocaine as the most prevalent stimulant. In the 1970s and 1980s, the development of "freebase" and "crack" cocaine revolutionized the use of the drug by providing a form that could be smoked (Warner, 1993).

4. Pharmacology of Cocaine

4.1. Forms of Cocaine and Methods of Uses

Cocaine can be absorbed through any mucous membrane, smoked, or injected intravenously. Data from the National Household Drug Survey show that 90 percent of cocaine users have snorted cocaine, making intranasal insufflations the most common route of use. About one third of users have smoked the drug, and fewer than 10 percent of users have injected it (Jeffcoat, et al., 1989).

4.1.1 Coca Leaves

The leaves can be chewed directly. Due to differing environmental factors, the cocaine content of the coca leaf ranges between 0.1 percent and 0.8 percent. Coca plants grown at higher altitudes contain a higher percentage of the cocaine alkaloid than those grown at lower altitudes and are consequently more potent. Coca leaves typically are chewed but can be rolled into cigarettes or cigars and smoked or infused in liquid and consumed like tea (Hawks, 1977 and Jaffe, 1990).

4.1.2. Coca Paste

The paste is the middle step between the leaf and the powder cocaine. It cannot be injected or snorted, therefore the only use of the coca paste is to burn the substance and inhale. Coca paste is much more popular in South American countries then in

the United States of America (Jeffcoat, et al., 1989 and Warner, 1993).

4.1.3. Powder Cocaine

Powder cocaine is a white, powdery substance derived from dissolving coca paste with hydrochloric acid and water. It is the most widely used form of cocaine. Usually cocaine is not necessarily pure. Normally the cocaine is mixed with different additives such as sugars, local anesthetics, or other drugs. Powder cocaine is soluble in water and therefore can be snorted, injected, or ingested. Because of, its high decompose at the high temperature, the powder base cocaine cannot be smoked, unlike crack cocaine. Powder cocaine loses its potency when the drug is heated above 198 ^{o} C/388 ^{o} F. The cocaine alkaloid decomposes at these temperatures and in return does not produce the physiological or psychotropic effects (Jeffcoat, et al., 1989 and Warner, 1993).

4.1.4. Cocaine Base

Cocaine base is a product of powder cocaine. It is created when the cocaine alkaloid is freed from the salt substrate and thus similarly resembles cocaine paste. At this form the cocaine is much more volatile at low temperatures. Where as the powder cocaine would lose potency at 198 ^{o} C, the cocaine base loses its mind altering affects at 98 ^{o} C. Cocaine base is not easily absorbed throughout the body and thus does not carry the potency level of powder cocaine (Jeffcoat, et al., 1989 and Smart, 1991).

4.1.4.1. Freebase Cocaine

Freebase cocaine is derived from powder cocaine that has been dissolved in water and a strong alkaloid solution, typically ammonia. Ether or another organic solvent is added, and a solid substance separates from the solution. This solid substance is the cocaine base. Because freebase cocaine is significantly purer than coca paste or powder cocaine, many users believed that it was a healthier form of the drug. Even though an estimated 10 to 20 percent of the cocaine-abusing population was using freebase cocaine during the 1970s, many resisted the freebasing process because of its complexity and potential danger. Ether, a highly volatile and flammable solvent, will ignite or explode if the freebase cocaine is smoked before the ether has evaporated entirely (Jeffcoat, et al., 1989 and Smart, 1991).

4.1.4.2. Crack Cocaine

Crack cocaine, another form of cocaine base, also is derived from powder cocaine (or solid form of powder cocaine). The purpose of crack cocaine is to allow the user to smoke the cocaine instead of injection or insufflations. It is lipid-soluble and resists thermal degradation, so that it can be smoked (reviewed by Baldwin, et al., 2002). Unlike the processing of freebase cocaine, converting powder cocaine into crack cocaine does not involve any flammable solvents. The powder cocaine is simply dissolved in a solution of sodium bicarbonate and water. The solution is boiled and a solid substance separates from the boiling mixture. This solid substance, crack cocaine, is removed and allowed to dry. The crack cocaine is broken or cut into "rocks," each typically weighing from one-tenth to one-half a gram. One gram of pure powder cocaine

will convert to approximately 0.89 grams of crack cocaine. The name crack comes from the popping sound the drug makes when it heated (Brown, et al. 1992). Today, this alkalinized form of cocaine is widely smoked throughout the world (Cook & Jeffcoat, 1990 and Kuczkowski, 2004).

4.2 Routes of Administration of Cocaine

The method of cocaine use affects its pharmacokinetics (Jatlaw, 1987). While cocaine in any forms such as paste, powder, freebase, or crack produces the same type of physiological and psychotropic effects, the onset, intensity, and duration of its effects are related directly to the method of use (Johanson, & Fischman, 1989).

4.2.1 Ingestion (Oral route)

Users who ingest cocaine typically chew the coca leaves in their mouths much like chewing tobacco. Coca leaves typically are mixed with an alkaline substance (such as lime) and chewed into a wad that is retained in the mouth between gum and cheek and sucked of its juices. The juices are absorbed slowly by the mucous membrane of the inner cheek and by the gastrointestinal tract when swallowed. Alternatively, coca leaves can be infused in liquid and consumed like tea. Ingesting coca leaves generally is an inefficient means of administering cocaine. Because cocaine is hydrolyzed (rendered inactive) in the acidic stomach, it is not readily absorbed. Only when mixed with a highly alkaline substance (such as lime), it can be absorbed into the bloodstream through the stomach. Absorption of orally administered cocaine is

limited by two additional factors. First, the drug is partly metabolized in the liver. Second, capillaries in the mouth and esophagus constrict after contact with the drug, reducing the surface area over which the drug can be absorbed. Orally administered cocaine takes approximately 30 minutes to enter the bloodstream. Typically, only 30 percent of an oral dose is absorbed, although absorption has been shown to reach 60 percent in controlled settings. Given the slow rate of absorption, maximum physiological and psychotropic effects are attained approximately 60 minutes after cocaine is administered by ingestion. While the onset of these effects is slow, the effects are sustained for approximately 60 minutes after their peak is attained (Smart, 1991).

4.2.2 Nasal Insufflations or Snorting (Nasal route)

One of the most common non-medical methods for the administration of cocaine is nasal insufflations (snorting) or inhalation as the white powder. Users who insufflate cocaine "snort" the drug into their nasal passages. The powder cocaine typically is apportioned into "lines," each representing between 10 and 35 mg of cocaine. The powder is drawn into each nostril through a thin straw and absorbed into the bloodstream through the capillaries of the mucous membranes of the nasal cavity. The conversion of cocaine to the un-ionized freebase at an alkaline pH facilitates its absorption across nasal mucous membrane. Administration of cocaine by nasal inhalation may lead to irritation of the mucosa, sinusitis and perforated septum. However, nasal insufflations are not the most efficient route of cocaine administration. Cocaine constricts the capillaries in the nasal membranes, thus reducing the surface area and making absorption slow and incomplete. Absorption following snorting cocaine is

dose-dependent, with larger doses more completely absorbed than smaller doses. One study found that only 28 percent of a 64 mg intranasal dose of cocaine was absorbed compared to almost 69 percent of a 96 mg dose. Cocaine snorted through the nasal passages appears in the blood three to five minutes after administration, significantly faster than the 30 minutes required for it to reach the bloodstream through ingestion. However, both ingestion and insufflations result in approximately the same proportion of the drug being absorbed: 30 to 60 percent. Compare to ingestion, the faster absorption of insufflated cocaine results in quicker attainment of maximum drug effects. Snorting cocaine produces maximum physiological effects within 40 minutes and maximum psychotropic effects within 20 minutes. Similar to ingestion of cocaine, physiological and psychotropic effects from nasally insufflated cocaine are sustained for approximately 60 minutes after the peak effects are attained (Fischman, 1984 and Johanson & Fischman, 1989).

4.2.3. Injection (Intravenous route)

Cocaine injectors dissolve powder cocaine in water and inject the mixture into a vein, typically in the arm, using a hypodermic syringe. While injection is an effective method of delivering a drug dose, it is potentially problematic. Because the drug is injected directly into the bloodstream, natural safeguards are bypassed. Given the unknown purity of street doses, intravenous drug users are less able to monitor and correct dosages, and therefore are subject to unexpected drug reactions or overdoses. Further, safe intravenous administration requires sterile conditions - conditions typically not associated with illicit drug use. Consequently, illicit drug users who inject drugs are generally at a greater risk of health problems than illicit drug users who use

drugs in other fashions. Intravenously administered cocaine is directly entered into the bloodstream and requiring only 1 minute reaches to the brain. The time interval to attain maximum physiological and psychotropic effects is much shorter than the interval following either ingestion or intranasal administration. Maximum physiological effects occur in 10 minutes; maximum psychotropic effects in 4 minutes. These effects are sustained for approximately 30 minutes. 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 (Fischman, 1984 and Johanson & Fischman, 1989).

4.2.4. Inhalation or Smoking (pulmonary route)

Smoking cocaine may be the second most effective route of drug administration because it causes rapid and efficient pulmonary absorption. Cocaine administered by this route produces a very intense euphoric "rush" within minutes of administration and the user may have a craving to repeat their drug use within 10 to 30 minute following their initial use. Cocaine administered by this route is rapidly delivered to brain tissue, and concentrations in brain tissue far exceed the concentrations found in plasma. Cocaine base typically is smoked in pipes constructed of glass bowls fitted with one or more fine mesh screens that support the drug. The user heats the side of the bowl (usually with a lighter), and the heat causes the cocaine base to vaporize. The user inhales the cocaine-laden fumes through the pipe. Alternatively, crack cocaine can be sprinkled in cigarettes and smoked. Smoking cocaine combines the efficiency of intravenous administration with the relative ease of consumption of ingestion and insufflations. Facilitated by the large surface area of the lungs' air sacs, cocaine

administered by inhalation is absorbed almost immediately into the bloodstream, taking only 19 seconds to reach the brain. However, only 30 to 60 percent of the available dose is absorbed due to incomplete inhalation of the cocaine-laden fumes and variations in the heating temperature. Cocaine smokers achieve maximum physiological effects approximately two minutes after inhalation. Maximum psychotropic effects are attained approximately one minute after inhalation. Similar to intravenous administration, the physiological and psychotropic effects of inhaled cocaine are sustained for approximately 30 minutes after the peak effects are attained. 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 (Foltin & Fischman, 1992 and Kleerup, et al. 2002).

4.2.5 Intra-Arterial Administration

Cocaine can also be injected directly into the arterial supply (e.g., the carotid artery). Intra-arterial administration of a drug via the carotid artery is the most efficient route of drug delivery. This route of administration assures virtually 100 percent drug delivery to the brain capillaries. There is no first-pass elimination (i.e., hepatic metabolism) or pulmonary excretion of the drug with an intra-arterial injection. The only factor limiting drug distribution is its diffusion through the capillary walls to brain tissue. However, the high fatality rate associated with intra-arterial administration has caused most users to find alternative routes for drug delivery (Dodd, et al., 1994). In general, limb ischemia and subsequent tissue necrosis in drug abusers has been widely reported after intra-arterial injection (Coughlin & Mavor, 2006).

4.3. Pharmacokinetic of Cocaine

The pharmacokinetic of cocaine has been extensively characterized in humans (Inaba, 1989; Jeffcoat et al., 1989; Ambre et al., 1991) and in animals (Nayak et al., 1976; Benuck et al., 1987; Booze et al., 1997; Mets et al., 2000) after various routes of administration. As mentioned above, cocaine can be administered into the body by many routes. It is absorbed from all body mucous membrane, including nose, lung and gastrointestinal tract. Onset, duration of action, level of drug, and half life range from a few second till many hours depending on the route of administration and dose of drug taken (see reviewed by Mouhaffel et al., 1995). The onset of effects, peak effects, duration, and plasma half-life for different routes of administration are shown in Table 1

Table 1 Pharmacokinetic of cocaine according to the route of administration

Route	Onset of action	Peak effects	Duration of action	Half-life
Inhalation (smoking)	3-5 sec	1-3 min	5-15 min	40-60 min
Intravenous	10-60 sec	3-5 min	20-60 min	40-60 min
Intranasal or mucosal	1-5 min	15-20 min	60-90 min	60-90 min
Oral (gastrointestinal)	10-20 min	60-90 min	60-180 min	60-90 min

(Johanson & Fiscman, 1989 and Egred & Davis, 2007)

4.3.1. Absorption

Cocaine is absorbed by all routes of administration (Goldfrank & Hoffman, 1991) but the proportion absorbed depends on the route of administration (Haddad & Winchester, 1990). They directly affect the rate at which the drug will be absorbed into the bloodstream and transported to the central nervous system where it produces physiological and psychotropic effects. Absorption of a drug into the bloodstream is regulated by two primary factors: the amount of blood flowing to the site of ultimate consumption (e.g., the stomach or small intestine); and the surface area over which the drug is absorbed. Following nasal insufflations (snorting), for example, the surface area is limited to the nasal mucosa in the nasal cavity. In contrast, following cocaine inhalation (smoking), the drug is absorbed by the air sacs of the lungs which have a surface area the size of a football field. In some properties of cocaine, it is a weak base (with a pKa of 8.6), orally consumed tend to be ionized in the digestive system resulted in slowing the rate of absorption (reviewed by Carrera, et al, 2004).

The impact of a drug is additionally governed by the proportion of the drug distributed to various parts of the body. Of ultimate importance is the proportion of the drug reaching the central nervous system, particularly the brain- the primary site of action for drug of abuse. For example, when a drug is injected intravenously, 100 percent of the drug is distributed to the body. Other routes of administration result in smaller proportions of the administered dose being available for distribution to the central nervous system. This phenomenon is attributable both to the smaller fraction of the drug being absorbed into the bloodstream

and to natural safeguards in the body (e.g., metabolism) (Fleming et al., 1990).
The faster a drug reaches the bloodstream, the faster it is distributed throughout the body and the faster the user feels the desired physiological and psychotropic effects. The level of effect and the length of time until maximum effect differ according to the method of administration. Intoxication begins soon after drug use and is perceived as more intense when use is through injection or smoking. The psychotropic feelings, described as "stimulated" or "high," are correlated to the rate of increased concentration of cocaine in the blood, particularly blood flowing to the brain. The faster cocaine reaches the brain, the greater the intensity of the psychotropic effects. However, these intense psychotropic responses also dissipate more quickly. Consequently, routes of cocaine administration with the more immediate and intense psychotropic responses (specifically, injection of powder cocaine or smoking cocaine vapors) maintain the intensity for shorter periods of time than slower routes of administration (Johanson, & Fiscman, 1989).

4.3.2. Distribution

Cocaine is distributed within all body tissues, and crosses the blood brain barrier (Ellenhorn & Barceloux, 1988). In large, repeated doses, it is probably accumulated in the central nervous system and in adipose tissue, as a result of its lipid solubility (Cone & Weddington, 1989). The volume of distribution is, according to different authors, between 1 and 3 L/kg (Clarke, 1986; Ellenhorn & Barceloux, 1988; Baselt, 1989). Cocaine

crosses the placenta by simple diffusion, and accumulates in the fetus after repeated use (Finster Pedersen, 1991). After administration, cocaine is rapidly redistributed from the plasma, reaching high concentration in vessel-rich body compartments (e.g., brain) (White & Lambe, 2003). The highest concentrations of cocaine appear in the brain, spleen, kidney, and lung. Although cocaine is rapidly cleared from plasma, it is more slowly cleared from other tissues, and can be detected in the brain, ocular fluid, and liver for 8 or more hours after initial use (reviewed by Boghdadi et al, 1997). The disposition of drugs in the body includes incorporation into growing hair, thereby making hair a useful matrix for monitoring drug exposure across a wide time frame. Several mechanisms for incorporation of drugs into hair have been proposed. Drugs diffuse from arterial capillaries near the root into hair matrix cells at the base of hair follicles (Nakahara, 1999); however, drugs also distribute into sweat and sebum, other matrices that contact hair and may play roles in drug incorporation (Joseph et al., 1998; Huestis et al., 1999; Liberty et al., 2004).

Plasma levels of cocaine have been determined after oral, intranasal, intravenous, or smoked cocaine (Lange, et al., 1989; Evans et al., 1996; Booze, et al., 1997). Such as, the study effects of intranasal cocaine (10 percent cocaine hydrochloride; 2 mg per kilogram of body weight) on the blood flow in and dimensions of the coronary arteries and on myocardial oxygen demand in 45 patients, peak plasma levels of cocaine after intranasal administration are typically in the range of 100-500 μg/L, occurring 20-40 min after application. These concentrations are rarely associated with apparent signs of toxicity, but they are capable of eliciting euphoria. While experimental studies in humans on the effects of cocaine typically use low dose to avoid toxicity, intravenous or smoked cocaine naturally results in a more rapid onset of hemodynamic responses due to more rapid

increases in plasma level often exceeding 1-2 mg/L (Evans et al., 1996). It is more difficult to obtain reliable values for peak plasma level after illicit use of cocaine. In addition, the toxicity is often delayed, so plasma level may not reflect concentrations at the time of the perception of toxicity when cardiac ischemia may occur. Nonetheless, the evidence from a number of studies of patients presenting with apparent myocardial ischemia after cocaine use suggests that during angina pectoris, the concentration of cocaine in the venous blood is not very high. Plasma levels are typically 0.4-6 mg/L (Knuepfer, 2003).

4.3.3 Metabolism

Cocaine is metabolized by enzymes called esterases that are present throughout all the body. High levels of esterase activity are present in the liver and serum, while moderate esterase activity has been identified in other organs including the brain (Foldes, 1978; Jones, 1984). Cholinesterase activity can vary greatly between individuals and between species (Jones, 1984), and it may therefore be one factor that predicts how quickly cocaine is metabolized. The variation in responses to cocaine exhibited by different subjects may be attributed in part to dissimilar rates of cocaine metabolism. The primary metabolites of cocaine include norcocaine, benzoylecgonine, benzoylnorecgonine, and ecgonine. Esterases do not metabolize all cocaine present in the body, and a small amount of cocaine is usually excreted unchanged via the urine. Cocaine metabolism takes place mainly in the liver, within 2 hours of administration. The rate of metabolism varies according to plasma concentration (Baselt, 1989; Haddad & Winchester, 1990).

There are 3 routes of bio-transformation (Fig. 2) the major route is hydrolysis of cocaine by hepatic and plasma

esterases, with loss of a benzoyl group to give ecgonine methyl ester. Esterase activity varies substantially from one subject to another (Fleming et al., 1990). The secondary route is spontaneous hydrolysis, probably non-enzymatic, which leads to benzoylecgonine by demethylation (Fleming et al., 1990). The final degradation of cocaine, which is a sequel to both the principle and secondary routes of metabolism, benzoylecgonine and ecgonine methyl ester are further hydrolyzed to ecgonine (Jatlow, 1987, Burnat & Le Brumant-Payen, 1992). N-demethylation (by cytochrome P450 enzyme systems) of cocaine is a minor route leading to an active metabolite, norcocaine. One to five percent of cocaine is not metabolized but is cleared in urine, where it can be detected for 3-6 hr after cocaine use (Fleming et al., 1990 and reviewed by Pozner et al, 2005). The principle metabolites are therefore benzoylecgonine and ecgonine methyl ester, which are not very active and are often used for forensic purposes with a half-life of 7.5 hours (Branch & Knuepfer, 1994). In contrast, norcocaine is pharmacologically active, eliciting similar cardiovascular effects (Baselt, 1989; Burnat & Le Brumant-Payen, 1992). The small amount of norcocaine produced and it roughly equipotent effects suggests that it could contribute to hemodynamic responses, but is not likely to be a major determinant of toxicity (Knuepfer, 2003). The concomitant use of cocaine and alcohol has a dangerous and multiplicative cardiovascular risk. They are metabolised in the liver to cocaethylene, which has been associated with a 40-fold increase in risk for acute cardiac events and 25-fold increase in sudden death (Egred & Davis, 2005).

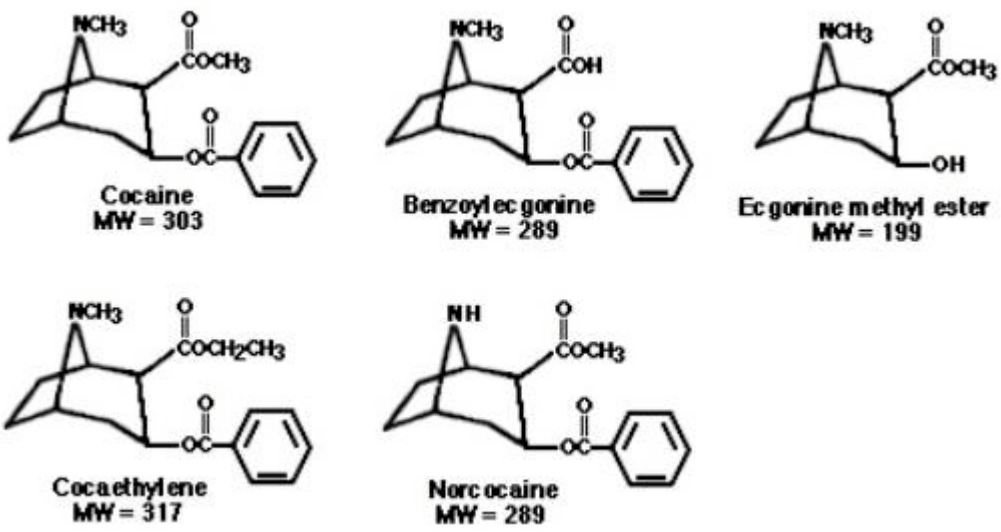

Figure 2 Cocaine and metabolites

4.3.4 Half-life and Route of Exposure

The biological half life depends on the route of administration and individual subject. It is of the order of 0.5 to 1.5 hours (Clarke, 1986 and Kuczkowski, 2004). After oral administration, it appears to be 0.8 hours (Baselt, 1989), nasal administration, 1.25 hours (Baselt, 1989; Ellenhorn & Barceloux, 1988), parenteral administration 0.7 to 0.9 hours (Ambre et al., 1988; Ellenhorn & Barceloux, 1988; Burnat & Le Brumant-Payen, 1992). The elimination half-life of cocaine in plasma has been determined to be between 40 and 90 min in humans, in dogs 72 min, and monkeys 72-78 min are roughly similar. In contrast, elimination is considerably faster in rats and mice (12-18 min). Therefore, rodents are capable of tolerating greater doses and may require these to mimic adverse effects in humans (Knuepfer, 2003). The other study, (Mets, et al., 1999) compared the

pharmacokinetics of bolus dose cocaine administration with that of its three most important metabolites; norcocaine, ecgonine methylester, and benzoylecgonine and assessed whether kinetics are dose dependent at two equimolar doses equivalent to cocaine hydrochloride 2.5 and 5 mg/kg respectively. They found that the elimination half-lives of cocaine and norcocaine were similar, 28-33 min, ecgonine methylester, 60-71 min, benzoylecgonine was 40-44 min and cocaine clearance 155-158 ml/kg/min. So they concluded that the pharmacokinetic profile of these congeners was not dose dependent when the two doses administered were compared (Kuczkowski, 2004).

4.3.5 Excretion

Cocaine has a systemic clearance of 2 L/min. 1 to 9 percent of cocaine is eliminated unchanged in the urine, with a higher proportion in acid urine. Ecgonine methyl ester accounts for about 30-50 percent of cocaine urinary disposition and benzoylecgonine for most of the remainder. At the end of 4 hours, most of the drug is eliminated from plasma, but metabolites may be identified up to 144 hours after administration (Ellenhorn & Barceloux, 1988). The serum half-life of cocaine is 45 to 90 minutes; only 1 percent of the parent drug can be recovered in the urine after it is ingested. Thus, cocaine can be detected in blood or urine for only several hours after its use. However, its metabolites are detectable in blood or urine for 24 to 36 hours after ingestion, thereby providing a useful indicator of recent drug ingestion. Hair analysis provides an extremely sensitive marker of cocaine use in the preceeding weeks or month, depending on the length of the hair analyzed (Ness, et al. 1999). Unchanged cocaine is also excreted in the stool and in saliva (Clarke, 1986 and Cone & Weddington, 1989).

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 (Karch, 1991). Cocaine and benzoylecgonine can be detected in maternal milk up to 36 hours after administration and in the urine of neonates for as much as 5 days (Chasnoff, et al, 1987). Freebase cocaine crosses the placenta, and norcocaine persists for 4 to 5 days in amniotic fluid, even when it is no longer detectable in maternal blood (Stinus, 1992). Only a very small amount (about 1-5 percent) of cocaine appears unchanged in the urine and it is cleared in 4-6 hours. Benzoylecgonine and ecgonine methyl ester may persist in the urine for a very long times, they are detectable for 24-60 hours after cocaine use (Kuczkowski, 2004).

Cocaine can be excreted in sweat. Alternative biological matrices such as sweat provide a less invasive method of monitoring illicit drug use over a longer period of time in treatment, workplace, military, and criminal justice settings. Identification of the parent drug and its metabolites, analyte concentrations, time course of detection, and dose-concentration relationships are important aspects of evaluating sweat testing for cocaine. The mechanisms by which drugs are incorporated into sweat are not fully understood. The primary mechanism appears to be passive diffusion of nonionized drug from capillaries into sweat glands. At the lower pH of sweat, drugs may ionize and accumulate in sweat (Kacinko, et al., 2005).

4.4. Pharmacodynamic of Cocaine

Cocaine is substance that typically causes heightened feelings of well-being and euphoria, and an increased state of arousal. As with most drugs abused by humans, the psychostimulants are similarly self-administered by animals, and most of the information regarding their mechanism of action has been derived from animal research. The importance of these pharmacodynamic effects within the brain with respect to drug-taking behaviour has been clearly demonstrated in animal models. Although research in humans is more limited, the findings confirm that similar pharmacodynamic effects occur following psychostimulant administration in humans. Cocaine is classified as a psychomotor stimulant and it produces potent central nervous system stimulatory effects. It is also used therapeutically as a local anesthetic.

4.4.1 Mechanisms of Action

Cocaine has two well-defined pharmacological actions: it is local anesthetic and a monoamine reuptake blocker. In addition, there are several other purported actions of cocaine that may be responsible for its unusual euphoric, addictive, and toxic effects (Knuepfer, 2003).

4.4.1.1 Local Anesthetic Action

Cocaine has long been known to be a local anesthetic, and it is believed to be the only naturally occurring agent used for this purpose. Therapeutic local anesthetics depend on the ability of agents to interfere with Na^+ channel activity, thereby reducing or

blocking nerve conduction. Cocaine is known to pass into the Na^+ channel and to bind on the inside of the membrane to inhibit further conduction of Na^+ ions through the membrane in electrically active cells, such as myocardial and nerve cells (Crumb & Clarkson, 1990 and Knuepfer, 2003).

4.1.1.2 Monoamine Reuptake Blockade Action

Cocaine acts as a powerful sympathomimetic agent. It blocks the presynaptic reuptake of norepinephrine and dopamine, producing an excess of these neurotransmitters at the site of the postsynaptic receptor (Lange & Hillis, 2001). Cocaine produces a dose dependent increase in blood pressure and heart rate, which, in recreational doses, usually remains within the physiological range. The sympathomimetic actions of cocaine, at cellular level, are mediated by stimulation β-adrenergic receptors (Egred & Davis, 2005). The cardiovascular effects of cocaine occur predominantly secondary to increased levels of plasma catecholamines. Hypertension, tachycardia, malignant arrhythmias, myocardial ischemia and infarction are all life-threatening cardiovascular complications of catecholamine accumulation following acute cocaine intake. Cocaine-induced cardiovascular complications do not seem to be dose-dependent and even small recreational doses can lead to significant mortality and morbidity in an otherwise healthy parturient. It is important to note that cocaine-abusing patients are at risk of cocaine-related complications, even if the last drug intake occurred more than 24 hr earlier. The euphoric effects of cocaine also result from prolongation of dopaminergic activity in the limbic system and the cerebral cortex (Kuczkowski, 2004). The use of cocaine rapidly leads to physical dependence. Sudden discontinuation of cocaine intake results in fatigue, mental depression and craving for the drug.

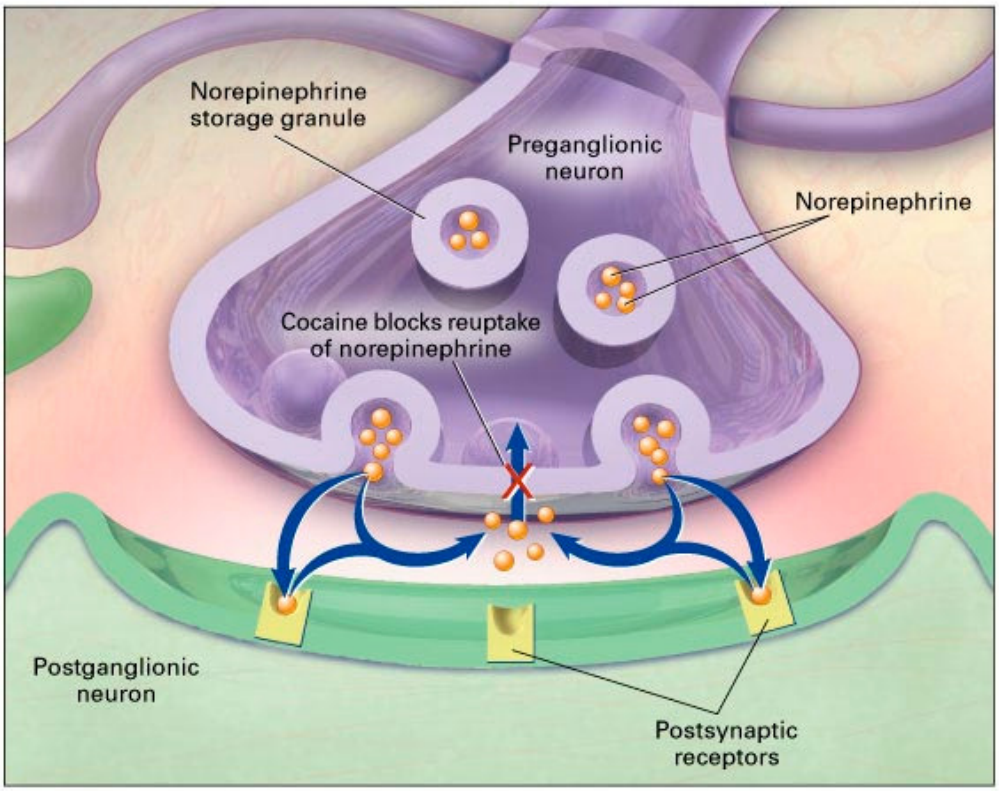

Figure 3 The mechanism by which cocaine alters sympathetic tone. Cocaine blocks the reuptake of norepinephrine by the preganglionic neuron, resulting in excess amounts of this neurotransmitter at receptor sites on the postganglionic neuron (Lange & Hillis, 2001).

4.4.2 Summary of the Effects of Cocaine

From the above mentioned was shown action of cocaine, it can be divided the effects into physiological and psychological effects (Table 2). Cocaine has complex actions on the sympathetic nervous system, nerve conduction, and on the central nervous system (reviewed by Boghdadi, et al, 1997; Harrison, et al, 2000; and White & Lambe, 2003). It is the most

potent central nervous system stimulant of natural origin. While different forms of cocaine do not result in different types of physiological or psychotropic effects, the route of administration does impact, as discussed above, the immediacy, intensity, and duration of cocaine's effects. The sections below discuss cocaine's physiological and psychotropic effects.

4.4.2.1 Physiological Effects of Cocaine

Cocaine, like other central nervous system stimulants such as amphetamine, caffeine, and nicotine, produces alertness and heightens energy. Cocaine acts on the central nervous system by inhibiting the re-uptake of the neurotransmitter; norepinephrine, dopamine and serotonin. The augmentation of norepinephrine results in increased motor activity, with slight tremors and convulsions in the user's extremities. In the cardiovascular system, the augmentation of norepinephrine results in increased heart rate, elevated blood pressure, and other symptoms similar to hypertension. The rate of increase in these physiological responses varies by route of cocaine administration, with the most efficient absorption routes (inhalation and injection) producing the most rapid increases. Cocaine's vasoconstrictive properties reduce the size of the blood vessels, causing the air sacs in the lungs to dilate and the capillaries in the nasal passages to constrict. Because cocaine permits less body heat to be lost, cocaine users generally experience an increase in body temperature. In cases involving cocaine overdoses, body temperatures as high as 114^{0} F have been reported (White & Lambe, 2003).

4.4.2.2 Psychotropic Effects of Cocaine

Cocaine also inhibits the re-uptake of dopamine, a neurotransmitter that controls the pleasure centers in the central nervous system, causing a sense of euphoria, decreased anxiety and social inhibitions, and heightened sexuality. Increased dosages of cocaine and use of the most rapid drug administration routes produce euphoric experiences that create vivid, long-term psychological memories that form the basis for subsequent craving of the drug (Prakash & Das, 1993). Psychosis and hallucinations have been reported with increased doses of cocaine, including foraging and "skin picking" (a slang term for a condition in which addicts mistakenly believe that bugs are crawling on their skin). In addition to producing euphoria and psychoses, cocaine use causes the user to crave other drugs, including alcohol. Polydrug use is particularly significant because concurrent use of cocaine and other drugs is associated with increased toxicity (Jatlow, 1987 and Harrison, et al, 2000).

Table 2 The physical and psychological effects of cocaine

Dose	Physiological Effects	Psychological Effects
Initial Low Doses	Tachycardia, tachypnea, hypertension, dilated pupils, sweating, reduced appetite, reduced need for sleep, reduced lung function, dry mouth, impaired motor control & performance of delicate skills and driving	Euphoria, sense of well being, impaired reaction time and attention span, impaired learning of new skills
Increased	Seizures, cardiac arrhythmias,	Anxiety,

doses	myocardial infarction, stroke, respiratory arrest	irritability, insomnia, depression, paranoid, aggressiveness, impulsivity, delusions, agitated/ excited delirium, reduced psychomotor function
Chronic Use	Erosions, necrosis and perforation of nasal septum, anosmia, rhinorrhoea and nasal eczema, chest pains, muscle spasms, sexual impotence, weight loss, malnutrition, vascular disease	Dependence, disturbed eating and sleeping patterns

(Wetli, 1985; Stark et al 1996; and Stark 1999)

4.5 Drug Dependence

Drug dependence can be both physiological and psychological. Psychoactive substance dependence has been described as a cluster of cognitive, behavioral, and physiologic symptoms that indicate that the person has impaired control of psychoactive substance use and continued use of the substance despite adverse consequences not limited to the physiologic symptoms of withdrawal and tolerance (withdrawal symptoms)

vary greatly across classes of substances. Marked and generally easily measured physiologic signs of withdrawal are common with alcohol, opiates, sedatives, hypnotics, and anxiolytics. Such signs are less obvious with amphetamines, cocaine, nicotine, and cannabis, but intense subjective symptoms can occur upon withdrawal from heavy use of these substances. The nature and severity of dependence has been shown to be primarily influenced by the individual's drug tolerance and the immediacy and duration of the drug's effect (Lago & Kosten, 1994).

4.5.1 Physiological Dependence

Unlike some drugs, cocaine is not physiologically addicting. Examples of drugs that cause physiological dependence include: opiates (e.g., heroin, morphine, codeine, and methadone), barbiturates (e.g., phenobarbital, secobarbital), anxiolytics (e.g., diazepam, meprobromate), nicotine (e.g., tobacco products), caffeine (e.g., coffee and tea), and alcohol. For drugs that cause physiological dependence, the nature of withdrawal symptoms varies with the type of drug. For example, opiate withdrawal is characterized by restlessness, sweating, extreme anxiety, fever, chills, and extreme diarrhea; alcohol withdrawal is characterized by hyperexcitability, hallucinations, psychomotor agitation, confusion, and delirium tremens - a syndrome characterized by a variety of discomforts. While cocaine is not physiologically addicting, users may experience anxiety and depression when cocaine is not available for use. These sensations, while possibly affecting physical systems in the body, have not been demonstrated to be related to bodily function; rather, these sensations have been classified as psychological manifestations resulting from psychological dependence (Lago & Kosten, 1994).

4.5.2 Psychological Dependence

Psychological dependence is a compulsion for repeated use of a drug for its euphoric effects despite any adverse effects that may occur. Cocaine exhibits powerful reinforcing properties that cause users compulsively to misuse the drug resulting in psychological addiction. The psychological craving for cocaine is the most important contributor to its abuse potential. Cocaine users discover that higher doses intensify the euphoria. Therefore, unless the user has imposed a limit on the quantity of drug used during a fixed period, or an external limit on supply exists, some users will gradually increase the frequency of use and quantity of the dose. The pursuit of euphoria becomes so great that users may often ignore all signs of physical and psychological risk, either to the individual or to others. With continued use, elation and self-confidence associated with the euphoria diminish, and depression and irritability set in. Often, in an attempt to ward off depression and/or the "crash" from the high, cocaine users further intensify their pattern of use, resulting in cocaine binges lasting for several hours or even days. The psychological components of dependence are the same across all categories of psychoactive drugs. For example, persons dependent on psychoactive drugs may exhibit a compulsion to use a drug over a longer period than originally intended. The individual uses larger amounts of the drug while enjoying the drug experience less. Because the user is unable to reduce or discontinue use and behavior associated with procuring, preparing, or being intoxicated, drug use consumes increasing amounts of the individual's life. Once the individual seeks treatment for dependence, the distinction between physiological and psychological dependence becomes irrelevant: physiological dependence becomes merely one factor in the diagnosis of psychoactive substance dependence (White & Lambe, 2003).

4.5.3 Factors Affecting Cocaine Dependence

The level and severity of cocaine dependence is affected by two factors: route of administration and drug tolerance (Lago & Kosten, 1994).

4.5.3.1 Route of Administration

As stated earlier, cocaine, regardless of how it is administered (injection, inhalation, nasal insufflation, or ingestion), produces the same type of psychotropic effects but with different levels of immediacy, intensity, and duration. Because of its relationship with immediacy, intensity, and duration, the route of administration plays an important role in determining the likelihood that use will lead to dependence and abuse. First, the intensity of the psychotropic effects is greater for those methods of administration that deliver the drug most rapidly to the brain. Consequently, routes of administration that result in the most rapid increases in blood concentration will provide the maximum levels of psychotropic effects. Second, the duration of the effect is inversely related to its intensity: methods of administration that bring about the most intense effects also will have the shortest durations. Consequently, routes of cocaine administration that result in more rapid increases in the blood's drug concentration - such as injection and inhalation - are more likely to lead to drug dependence. For the injection and inhalation administration methods, cocaine's effects are quick in onset, short-acting, and carry a greater likelihood that the user will administer the drug more frequently (e.g., daily or more often). Inhalation also carries a greater likelihood that users will administer the drug

in binges. For the insufflation or ingestion administration methods, the cocaine effects are slow in onset, longer acting, and less likely to involve administering the drug frequently (e.g., daily or more often) or in binging episodes (White & Lambe, 2003).

4.5.3.2 Drug Tolerance

Drug tolerance is the process by which the effectiveness of a drug diminishes over time such that increasing doses are necessary to achieve effects comparable to prior doses. Acute tolerance is defined as a change in responsiveness to a drug's effects in the short-term, even within the course of a single dose. Cocaine's physiological and psychotropic effects dissipate quickly, but the drug continues to be present in the bloodstream after the effects are no longer being experienced. Therefore, acute tolerance to the physiological and psychotropic effects of cocaine develops rapidly. When tolerance occurs, users need increasing amounts of the drug to achieve comparable levels of physical and psychological euphoria. Consistent with the development of drug tolerance, experienced users are often able to administer doses that would otherwise be fatal to a first-time user (Johanson & Fiscman, 1989; and Brotto, et al. 2007).

4.6 Pathophysiology of Cocaine

Cocaine is the commonest illicit drug used and the most frequent cause of drug related death. Its use is associated with both acute and chronic complications that may involve any system, the most common being are as follow:

4.6.1 Cardiovascular System

Cocaine use has been associated with both acute and chronic cardiovascular diseases. These include acute myocardial infarction, myocardial ischemia (both silent ischemia and ischemia associated with angina), acceleration of the development of atherosclerosis, myocarditis, cardiomyopathy (both dilated and hypertrophic), arrhythmias, hypertension, aortic dissection, and endocarditis. Cardiac complications are the commonest cause of death among cocaine users, and can occur after acute or chronic abuse (White & Lambe, 2003 and Kloner & Rezkalla, 2003).

4.6.1.1 Myocardial Infarction and Chest Pain

The most common presenting manifestations of cocaine abuse in cardiac complications must include chest pain. The pain is commonly described as sharp, stabbing or pleuritic and may represent ischemia secondary to coronary vasospasm. Chest pain is invariably present prior to cocaine-related infarction. Ischemia and infarction usually occur in chronic abusers, but infarction can occur after initial use of cocaine. Myocardial ischemia results from the imbalance between oxygen supply and demand. The occurring after cocaine use, involves several mechanisms. It is related to the block of the re-uptake of norepinephrine that leads to α and β adrenergic effects. These include increased heart rate and blood pressure and simultaneous coronary vasospasm with reduced oxygen delivery leading to myocardial ischemia (Hollander & Hoffman, 1992; Rump**,** et al., 1995; and White & Lambe, 2003). In addition, there is evidence that cocaine activates platelets, increases platelets aggregability, and potentiates thromboxane production promoting thrombus formation

(Kugelmass, et al., 1995)

4.6.1.2 Arrhythmias

The arrhythmias are usually transient and resolves when cocaine is metabolised. Sinus tachycardia and bradycardia, supraventricular arrhythmias, bundle branch block, ventricular fibrillation or asystole, ventricular tachycardia, all been reported. The electrical stability of the heart may be impaired by ischemia as well as by the enhanced endogenous sympathetic activity induced by cocaine. Particularly during ischemia, cardiac arrhythmias may result from an alteration of the autonomic activity and from any intervention eliciting sympathetic nerve activity enhancement (Rump, et al., 1995). Several mechanisms contribute to cocaine induced arrhythmia: myocardial ischemia, raised plasma catecholamine concentrations, centrally upregulated sympathetic nervous system outflow, hyperpyrexia, metabolic acidosis and cardiac myocyte membrane stabilization by cocaine (by interference with transmembranal Na^+ and Ca^{2+} flux). Intracardiac nerve impulses undergo slowed, heterogeneous depolarisations, with prolonged repolarisations (Fernandez, et al. 1983)

4.6.2 Respiratory System

The deleterious effects of cocaine on the upper airway have been described above. Other respiratory complications associated with the smoking of crack cocaine include pneumonitis, pulmonary hemorrhage, vascular lesions, and pulmonary edema (Haim, et al., 1995).

4.6.2.1 Barotrauma

Inhalation of cocaine involves deep, sustained inspiratory effort, often followed by frequent and prolonged Valsalva manoeuvres. Pneumomediastinum or rarely pneumopericardium occurs as a consequence of increased intra-alveolar pressure followed by rupture of alveoli and centripetal dissection of air along bronchovascular sheaths into the mediastinum. Pneumothorax, which is rarely fatal, results from either extension of the pneumomediastinum through the parietal pleura or, less commonly, from peripheral dissection of air with rupture of visceral pleural blebs. The three most common symptoms of barotrauma are pleuritic chest pain, sore throat, and dyspnoea (Tomashefski, & Felo, 2004).

4.6.2.2 Asthma

The smoking of cocaine is temporally associated with severe exacerbation of asthma. There are no distinctive histological features of cocaine-associated asthma. The walls of mucus-filled bronchi are thickened by oedema, eosinophil-rich inflammation, dystrophic basement membrane, and smooth muscle hyperplasia. The mechanism(s) by which cocaine increases the risk of death in asthmatics is incompletely understood. Cocaine may act as a non-specific irritant of the bronchial mucosa to precipitate an asthmatic attack. A direct cardiovascular effect of cocaine or its interaction with β-adrenergic agonists used to treat asthma may possibly induce a fatal cardiac arrhythmia (Tomashefski, & Felo, 2004).

4.6.3 Central Nervous System

The pathological effects of cocaine on the central nervous system can be subdivided into three groups: cerebrovascular effects, neurological effects, and psychological effects.

4.6.3.1 Cerebrovascular Effects

Cocaine may cause either ischemic or hemorrhagic strokes. Ischemic and hemorrhagic strokes occur with approximately equal frequency after smoking crack cocaine, whereas hemorrhagic strokes are more common after cocaine hydrochloride use (Johnson, et al. 2001)

(1) Intracranial Hemorrhage and Cerebral Infarction

Intracranial hemorrhage and cerebral infarction have been widely reported to occur after cocaine abuse. The rapid rise in blood pressure that occurs shortly after administration, together with impaired cerebral autoregulation, may precipitate spontaneous hemorrhage. Subarachnoid hemorrhage is more common in the presence of preexisting cerebral pathology, such as cerebral aneurysm or arteriovenous malformations. The mechanisms for cocaine-induced cerebral ischemia and infarction include cocaine-induced vasospasm, cerebral vasculitis, or cerebral artery thrombosis caused by increased thromboxane production and enhanced platelet aggregation (Kibayashi & Hirsch, 1995 and Herning, et al., 1999). Moreover, cocaine-induced changes in dopamine level are most prominent in mesocorticolimbic neurons. Augmentation of mesocorticolimbic

dopamine function may be associated with cerebral ischemia by means of two mechanisms. First, dopamine may control local blood flow by inducing vasospasm of smooth muscles lining the cerebral vessels, particularly those of the middle cerebral artery. Second, cocaine-induced reductions in cerebral metabolism may lead to feedback down-regulation of blood flow. Rapid reperfusion of these previously ischemic areas may, conversely, result in hemorrhage (Bankole, et al., 2001)

4.6.3.2 Neurological Effects

The acute and long term neurologic complications have been reported with cocaine use, the most common being headache. They may occur during cocaine use or during withdrawal. Cocaine acts as a CNS stimulant via inhibition of presynaptic reuptake of norepinephrine, dopamine, and serotonin. It also causes release of epinephrine by the adrenal medulla. The intensity and duration of cocaine's stimulant effects are mediated by the rate of rise and the peak of cocaine blood levels. Catecholamine release in the CNS also increases motor activity, which may be manifested by abnormal involuntary movements and convulsions (White & Lambe, 2003).

(1) Seizures

Seizures are also associated with acute or chronic cocaine use and in overdose. Overall, intravenous dosing or smoking crack cocaine is more likely to precipitate seizures. In patients with a known seizure disorder, cocaine use yields twice the likelihood of seizure, and intranasal use is more likely to cause seizures (Koppel, et al. 1996). Seizure activity is related to the

peak plasma concentration of cocaine, and usually occurs within minutes of dosage, although it can occur up to 24 h after ingestion; one study in rats suggested that delayed seizure activity is related to the cocaine metabolite, benzoylecgonine, but this is yet to be demonstrated in humans. Generally, self-terminating tonic-clonic seizures are usual, but multiple seizures or status epilepticus can occur, which can be lethal, particularly in association with hyperpyrexia, cardiac dysrhythmia, and cerebral haemorrhage. Cocaine reduces the seizure threshold by chronic subthreshold stimulation of the limbic system - the 'kindling effect' (reverse tolerance). Intrasynaptic accumulation of excitatory neurotransmitters occurs, which is thought to precipitate epileptiform activity (Brady, et al., 1991).

(2) Hyperthermia

Hyperthermia results from increased motor activity, increased heat production, and reduced heat dissipation due to constricted blood vessels (Glauser & Queen, 2007). Central mediated hyperthermia results from brainstem D_1 dopamine receptor down regulation. Body temperature may rise by up to $1^{o}C$ after cocaine use. Severe hyperthermia, in temperature excess of $40^{o}C$, is associated with a poor outcome and may be the first sign of progression to excited delirium (White & Lambe, 2003).

(2) Movement Disorders

Cocaine causes dopamine to accumulate in the synaptic clefts in the basal ganglia and other areas of the brain, as which produces intense stimulation and can result in

movement disorders. Tourette's syndrome, idiopathic dystonia, tardive dyskinesia, choreoathetosis, akathisia, and acute dystonic reactions may be caused by cocaine (White & Lambe, 2003).

4.6.3.3 Psychological effects

(1) Euphoria

Elevated dopamine levels in the mesolimbic and mesocortical areas of the brain gives rise to intense euphoria. Simultaneously, cocaine suppresses the activity of the pontine nucleus and locus coeruleus, producing anxiolysis. However, dopamine depletion, caused by repetitive cocaine use, produces tolerance to the euphoric effects of cocaine, as well as physiological cocaine addiction (Ellison, 1994).

4.6.4 Gastrointestinal System

Smoking cocaine has been reported to induce intestinal perforations. It is known that cocaine blocks the re-uptake of norepinephrine. This may lead to mesenteric ischemia and focal tissue ischemia, which may lead to perforation. Cocaine injected intravenously has been shown to cause bowel ischemia without evidence of thrombosis, emboli, or atherosclerosis. The intestinal vasculature contains alpha-adrenergic receptors, which are stimulated by norepinephrine, leading to mesenteric vasoconstriction and focal ischemia (Glauser & Queen, 2007). Gastric mucosal cells and duodenal villi rapidly become ischaemic

in response to reductions in mesenteric blood flow, leading to gastritis and ulceration; this may theoretically be potentiated by cocaine-mediated gastric hypomotility and delayed gastric emptying (which prolongs mucosal exposure to gastric acid). Mesenteric vasoconstriction can result in ischaemia and acute inflammation or infarction along the length of the bowel. Gangrenous perforation may be unrecognised for a hazardous length of time, but should be considered in the presence of acute, persistent abdominal pain and leukocytosis. Acute and subacute hepatocellular necrosis, acute and chronic hepatic failure, ischaemic and viral hepatitis, centrilobular necrosis, and hepatic haemorrhage have been reported (Sarper, et al., 1993).

4.6.5 Renal System

It is generally accepted that cocaine has potent vasoconstrictive effects on vascular smooth muscle. Inhibition of synaptosomal uptake of catecholamines and blockage of reuptake of norepinephrine in sympathetically innervated tissues, as well as other vasoconstrictive factors, such as endothelins, may be involved in the vascular dysfunction seen in cocaine intoxication. Acutely, cocaine can cause acute renal failure and renal infarctions (Glauser & Queen, 2007). Cocaine may directly damage the kidneys by causing renal infarction (secondary to renal artery spasm, atherosclerosis, and thrombosis) or focal segmental glomerulosclerosis (by increasing mesangial cell proliferation). More commonly, however, cocaine exerts a nephrotoxic effect by causing rhabdomyolysis. Rhabdomyolysis can occur by several mechanisms: skeletal muscular ischaemia secondary to prolonged arterial vasoconstriction, hyperthermia, direct muscle cell apoptosis, tissue trauma (whilst intoxicated), and tonic-clonic

seizures. Muscular pain and hyperkalaemia occur. Myoglobin precipitates in renal tubules, causing obstruction and inflammation, which is aggravated by ischaemic renal tubular disease. Acute renal failure may ensue (Sharff, 1984 and Mattana, et al. 1994)

SECTION II: PHARMACOLOGY OF ADRENOCEPTORS

1. Adrenoceptors

Adrenergic receptors or adrenoceptors are expressed on virtually every cell type in the body and are the receptors for epinephrine and norepinephrine within the sympathetic nervous system. They serve critical roles in maintaining homeostasis in normal physiologic settings as well as pathologic states. These receptors are also targets for therapeutically administered agonists and antagonists. The endogenous catecholamines; norepinephrine and epinephrine; are involved in the regulation of virtually every organ system (Bylund, et al., 1994 and Badino, et al., 2005).

Adrenoceptors are a heterogeneous group of hormone/neurotransmitter receptors that mediate the central and peripheral actions of the natural adrenergic amines, epinephrine, and norepinephrine. These receptors constitute a subfamily of the seven transmembrane domains/G-protein-coupled receptors, and have been divided into two major types based on their affinities for agonists and antagonists, their coupling to signaling pathways, and their amino acid sequences. The major types are the α_1-, the α_2- and the β_1- β_2-adrenoceptors (Hieble et al., 1995).

2. Typs and Subtypes of Adrenoceptors

The adrenoceptor family was first divided into subtypes, the α-and β-adrenoceptors as determined by pharmacological study in isolated tissues. A quarter of a century later, the α-adrenoceptors were further subdivided based on their anatomical location, with α-adrenoceptors located on peripheral sympathetic nerve terminals designated as α_2-adrenoceptors and those located post-synaptically designated as α_1-adrenoceptors. This anatomical classification rapidly gave way to the identification of pharmacological differences between the α-adrenoceptors, notably the ability of yohimbine and rauwolscine to act as α_2-adrenoceptors antagonists. Subsequent studies using pharmacological and molecular biological techniques have further subdivided the α-adrenoceptors family; three subtypes within each group have now been cloned and pharmacologically characterised. The α_1-adrenoceptor subtypes have been classified as the α_{1A}, α_{1B} and α_{1D}-adrenoceptor and the α_2-adrenoceptor have been classified as the α_{2A}, α_{2B} and α_{2C}-adrenoceptor (α_{2D} species variation of the human) (Bylund et al., 1998 and Robinson & Hudson, 1998).

β-adrenoceptors are also heterogeneous in nature and were again initially subdivided into β_1-and β_2-adrenoceptors, on the basis of the relative potencies of a series of catecholamines in *vitro* and in *vivo* systems. Subsequently the β-adrenoceptors have been classified using functional studies, receptor binding and genetic techniques. The β-adrenoceptor family is subdivided into three distinct subtypes, the β_1-and β_2-adrenoceptors and the atypical β_3 -adrenoceptors. There is an additional β-adrenoceptor subtype which has been identified in cardiac tissue and is a putative, atypical subtype classified as the β_4 -adrenoceptors (Robinson & Hudson, 1998).

The sites of action for β_3-adrenoceptor agonists appear to be skeletal muscle, brown adipose tissue and white adipose tissue.

β_3- adrenoceptor mRNA is expressed in lower amounts in human than in rodent adipose tissue and the promoter for the human β_3-adrenoceptor appears to drive expression of the mRNA predominantly in brown adipose tissue. Since there is relatively little brown adipose tissue in adult humans, one would not expect as marked effects of β_3-adrenoceptor agonists in humans as in rodents. There are, nevertheless, a number of arguments which suggest that β_3-adrenoceptor agonists might be of value in the treatment of human obesity and Type 2 diabetes (Ito et al., 1998).

The β_4-adrenoceptor is an atypical state of the β_1-adrenoceptor because responses displaying the two pharmacologies desensitised and resensitised in parallel in a rat model of cardiac failure. Although as yet there are no selective compounds for this particular subtype (Kaumann et al., 2001).

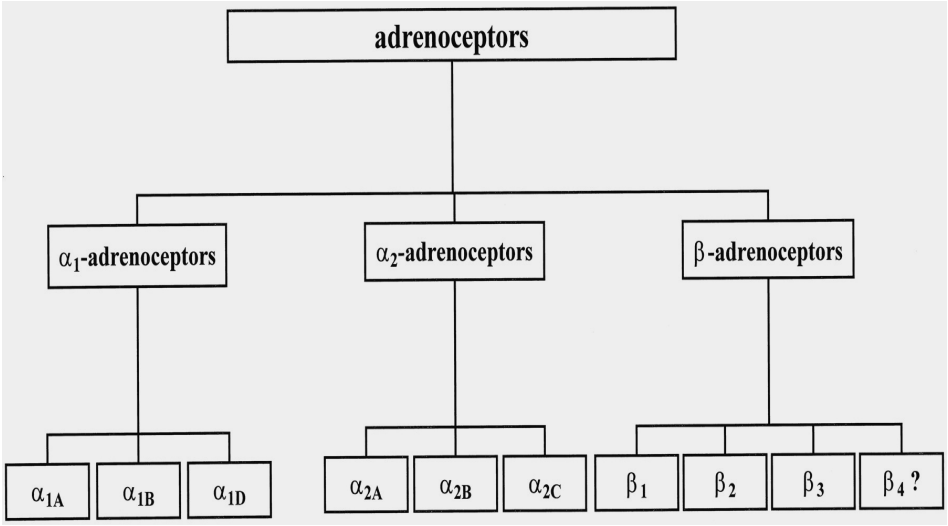

Figure 4 The adrenoceptor family is divided into three subfamilies based on pharmacological properties, structural homology, and signaling mechanisms (Brodde, & Martin, 1999).

1. Location and Function

1. α_1-Adrenoceptors

The α_1-adrenoceptors are located in the central and peripheral nervous system. In the CNS they are predominantly located post-synaptically where they mediate an excitatory role. Following cloning of the α_1-adrenoceptor subtypes, mRNA studies have shown α_1-adrenoceptor adrenoceptor mRNA in the hippocampus and cortex. Peripheral α_1-adrenoceptors are located on both vascular and non-vascular smooth muscle where activation of the receptor results in contraction. On vascular smooth muscle the α_1-adrenoceptors are located intrasynaptically where they mediated the response to endogenous neurotransmitter release. They are are also located on the heart where they mediated a positive inotropic effect and on the liver where they activate glycogen phosphorylation. With the availability of subtype selective compounds the α_{1A}-subtype has been shown to be responsible for the contraction of vas deferens smooth muscle. Vasoconstriction in some blood vessels has been shown to be α_{1B}-mediated and contraction of the rat aorta is α_{1D} -mediated (Hieble et al., 1995 and Sven et al., 2004).

2. α_2-Adrenoceptors

The α_2-Adrenoceptors are found in both the central and peripheral nervous system, and located on both pre and post synaptic neurons. In the CNS these receptors play an important role in regulating neurotransmitter release through autoreceptors located on norepinephrine nerve terminals and heteroreceptors located on other neurotransmitter terminals. Their importance in

regulating the release of both norepinephrine and serotonin has resulted in the investigation and development of α_2 -antagonists such as idazoxan for use in treatment of depression. They also mediate central cardiovascular responses and α_2-agonists such as clonidine cause hypotension and bradycardia. Furthermor, The sedative properties of α_2-agonists, mediated by somatodendritic autoreceptors on the locus coeruleus, have resulted in the development of α_2-adrenoceptor agonists such as dexmedetomidine as veterinary sedatives and anesthetics. The α_2-agonists also have the advantage of being analgesic, a response mediated by α_2-Adrenoceptors in the spinal cord. Other central effects of α_2-Adrenoceptors include the regulation of blood pressure, hypothermia, pupil diameter and a role in cognitive function. Peripheral functions include contraction of vascular smooth muscle, inhibition of lypolysis through α_2-adrenoceptors located on fat cells and hyperpolarisation of sympathetic ganglia (Bylund et al., 1998).

3. β-Adrenoceptors

β_1-and β_2- Adrenergic receptors can be found in the heart and lungs as well as peripheral tissues throughout the body in varying densities and affinities. β_1-Adrenoceptors are much more prevalent in the heart while β_2- adrenoceptors are prevalent in bronchial and vascular smooth muscle, peripheral leukocytes, adipose tissue, adrenergic nerves, and striated muscle. β -adrenergic stimulation results in positive inotropic and chronotropic responses, bronchodilation, vasodilatation, lipolysis, stimulation of adrenergic neurons, and protection against the release of circulating bronchoconstrictors. A summary of the biological responses mediated by β-adrenergic receptors is found in 3. β- Adrenoceptors can be stimulated endogenously through an increase in adrenergic

drive or exogenously through the use of β-agonists and other sympathomimetic agents (Shelley and Nicholas, 2007).

Table 3 Biological responses mediated by β -adrenergic receptors

Organs	Biological responses	β_1	β_2
Heart	Myocyte growth, toxicity	++ +	+
	Positive inotropic, chronotropic response	++	+
Lung	Relax bronchial smooth muscle		++
	Inhibit mast cell release		++
Neuromuscular system	Stimulate adrenergic neurons	+	++
	Muscle contractions, catabolism		++
Peripheral tissues	Increase lymphocyte release of immune mediators		++
Electrolytes	Induce hypokalemia	+	++

(Badino, et al., 2005)

The β_3-adrenoceptor is predominantly found in white adipose tissue of rats where it stimulates lipolysis, and brown adipose tissue where it stimulates lipolysis and thermogenesis (Arch & Kaumann, 1993). In addition to β_3-adrenoceptors, rat adipocytes express β_1-adrenoceptors that can also stimulate lipolysis. Stimulation of the β_3- adrenoceptors result in significant weight loss in obese rodents without adverse effects on β_1-adrenoceptor-mediated inotropic and chronotropic effects. In humans, the β_3-adrenoceptor is present in abundance in brown

adipocytes of newborns. Adult humans lack significant quantities of this thermogenic tissue (Danforth & Himms-Hagen, 1997). However, recent evidence suggests that adrenergic stimulation of white adipocytes can result in activation of dormant brown adipocytes or the white adipocytes themselves take on a brown adipocytic phenotype (Yoshida et al., 1998).

Putative β_4-adrenoceptors exist in tissues, such as fat and heart, which are known to express high levels of β_1-AR with activation causing an increase in heart rate and force. The β_4-adrenoceptor has never been cloned despite serious attempts to do so (Strosberg and Arch, 2000), and now that the full human genome is known, it is clear that it is not a distinct molecular entity, but rather a form of the β_1-adrenoceptor. However, definitive evidence for this putative β_4-adrenoceptor is still lacking, and there is some evidence that it may be a 'state' of the β_1-adrenoceptor (Kompa & Summers, 1999).

4. Signal Transduction Mechanism

1. α_1-Adrenoceptors

The α_1-adrenoceptors mediate their response via G-protein coupled receptors through a G_p/G_q mechanism. All the subtypes are coupled to phospholipase C and activation of the receptor results in the production of the second messengers, inositol triphosphate (IP_3) and diacylglycerol (DAG). The production of these second messengers results in an activation of both voltage dependent and independent Ca^{2+} channels as well as stimulation of proteinkinase C, phospholipase A2 and D, arachidonic acid release and cyclic AMP formation (Hein & Martin 2007).

2. α_2-Adrenoceptors

The α_2-adrenoceptors are part of the large family of G-protein coupled receptors and mediate their functions through a variety of G-proteins including G_i/G_o. All the subtypes have been shown to be negatively coupled to adenylate cyclase and mediated an inhibitory effect through the inhibition of cyclic AMP production. In addition there is now evidence linking the α_2-Adrenoceptors to stimulation of Ca^{2+} influx and also activation of K channels, phospholipase A2 and Na^+/H^+ exchange (Hein & Martin, 2007).

3. β- Adrenoceptors

For all β-adrenoceptors transduction is via G-proteins coupled to the intracellular second messenger adenylate cyclase. All β-receptors are positively coupled to adenylate cyclase via activation of G_s G-protein, however activation of the β_2 and β_3-adrenoceptors results in stimulation or stimulation and inhibition of adenylate cyclase. Activation of the β_1 and β_4 receptor results in an increase in the formation of cAMP and the subsequent stimulation of cAMP-dependent protein kinase (Ma & Haung, 2002).

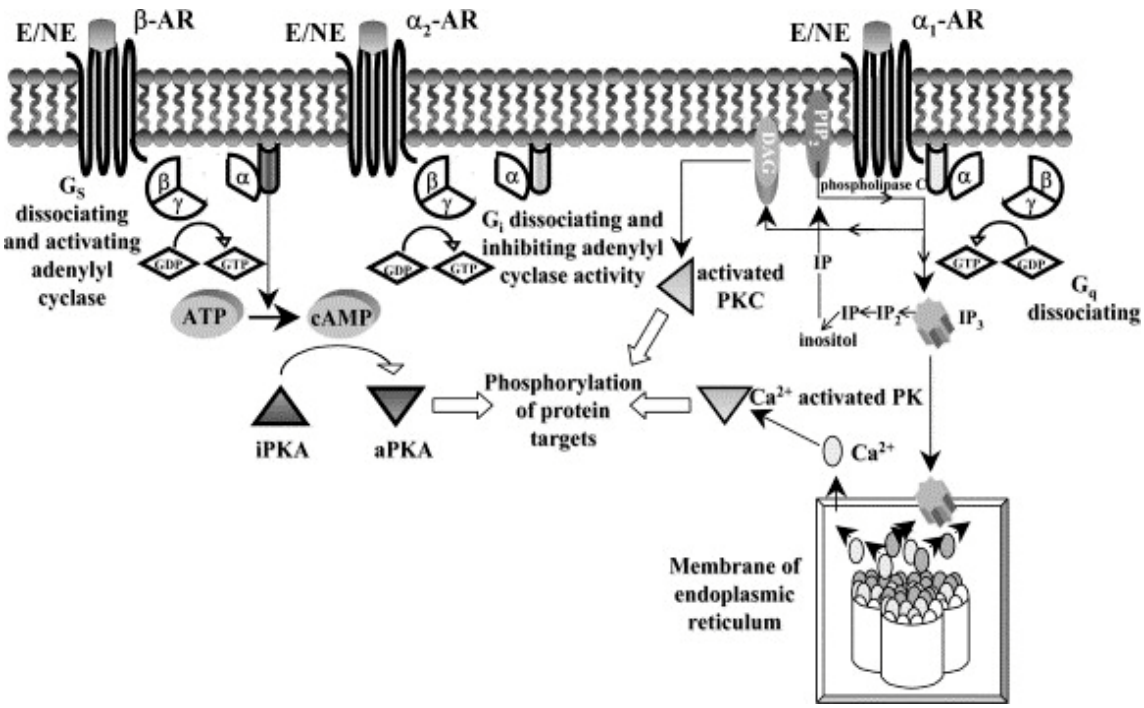

Figure 5 Signal transduction of adrenergic receptors binding of adrenergic receptors to agonists (epinephrine = E and norepinephrine = NE) activates many G protein-coupled receptors, leading to inhibition or activation of second messenger production. This figure shows how α_2-AR inhibits cAMP production via G_i protein or β-AR enhances cAMP levels via G_s protein pathway. Cyclic AMP activates protein kinases that participate in the cellular response. Stimulation of α_1-AR produces activation of the enzyme phospholipase C and production of the second messengers, inositol triphosphate (IP_3) and diacylglycerol (DAG). Intracellular IP_3 releases intracellular $Ca^{2+,}$ whereas DAG remains in the cytoplasmatic membrane where it activates protein kinase C. IP_3 is transformed in phosphatidyl inositol (PI) by subsequent dephosphorylation and then in phosphatidyl inositol diphosphate (PIP_2) via ATP. The last step in the signal

transduction is phosphorylation of protein target by protein kinase that results in the cellular response (Badino, et al. 2005).

Table 4 Comparison of adrenoceptor subtypes

Type	Agonists	Antagonists	Location	Transduction mechanism	Physiological function
α_1	oxymetazoline cirazoline methoxamine phynylephrine epinephrine	phentolamine prazosin tamsulosin terazosin phenoxybenzamine	smooth muscle	activates $G_p/_q$, ↑PI turnover, ↑ $[Ca^{2+}]$ i.e., activates voltage-gated Ca^{2+} channels	smooth muscle contraction myocardial contraction vasoconstrictionGI relaxation
α_2	clonidine, lofexidine, xylazine	yohimbine	pre/ post synaptic nerve terminals	activates G_i, inhibits adenylate cyclase, ↓ cAMP, inhibits voltage-gated Ca^{2+}channels,activates Ca^{2+} - dependent K^+ channels	vasoconstrictionhypotension sedation analgesia anaesthesia inhibitiom of neurotransmitter release
β_1	norepinephrine	betaxolol bisoprolo	heart and cerebral	↑adenyl cyclase (via G_s)	↑heart rate and force of

Type	Agonists	Antagonists	Location	Transduction mechanism	Physiological function
	isoproterenol dobutamine xamoterol	l atenolol practolol metoprolol	cortex		contraction
β_2	albuterol bitolterol mesylate clebuterol formoterol isoproterenol levalbuterol metaproterenol salmeterol procaterol terbutaline	butoxamine propranolol	lung smooth muscle cerebellum	↑adenyl cyclase (via G_s)	smooth muscle relaxation bronchodilatation
β_3	L-796568	SR 59230A	adipose tissue	↑adenyl cyclase ↓adenyl cyclase	Lipolysis and thermogenesis
β_4	None	bupranolol		↑ cAMP levels, stimulation of cAMP-dependent protein kinase (via Gs)	↑heart rate and force of contraction

(Badino, et al., 2005)

4. Clinical Uses

1. α-Adrenoceptors

The clinical uses of adrenergic compounds are vast. The treatment of many medical conditions can be attributed to the action of drugs acting on adrenergic receptors. α- adrenoceptor ligands can be used in the treatment of hypertension. Drugs such as indoramin and prazosin are α_1-adrenoceptor antagonists and have antihypertensive effects, as is clonidine an α_2 adrenoceptor agonist. α_1-adrenoceptor antagonists are also employed in the control of benign prostatic hypertrophy. However there can be cardiovascular side effects associated with α_1 block. α_2-adrenoceptor agonists such as clonidine are often used as an adjunct to general anaesthetics (Robinson & Hudson, 1998).

2. β- Adrenoceptors

Adrenergic drugs are used in the treatment of a wide range of medical conditions. Including the use of β_2-receptor selective agonists in the treatment of asthma and other related bronchospastic conditions examples of these drugs include salbutamol and salmeterol. Beta-blocker drugs are commonly used in the treatment of angina pectoris, cardiac arrhythmia and for the long-term treatment of patients who survive myocardial infarction. β-receptor antagonists have also been used as anti-hypertensive for a number of years. β -blockers have also proven useful in the treatment of conditions such as migraine, anxiety disorders,

hyperthyroidism, alcohol withdrawal and when applied topically are useful in the treatment of glaucoma and ocular hypertension (Robinson & Hudson, 1998).

SECTION III: PHARMACOLOGY OF ADRENOCEPTOR AGONISTS AND ANTAGONIST

1. Adrenoceptor Agonists

1.1. Catecholamines

Catecholamines are a subgroup of sympathomimetic amines and represent the agents of choice for inotropic and vasopressor support through interaction with adrenergic receptors. The endogenous forms (dopamine, epinephrine, norepinephrine), released as hormones and neurotransmitters influence a multitude of physiological functions while the synthetic forms (i.e. dobutamine, isoproterenol) are designed to target specific receptors resulting in selected effects on hemodynamics (Steinberg & Notterman, 1994).

1.1.1 Biosynthesis and Inactivation of Catecholamines

The catecholamines are derived from tyrosine. Synthesis occurs in the nerve terminals and in the adrenal gland. Tyrosine hydroxylase catalyzes the first step and is the major site of regulation (inhibition by dopamine and noradrenaline, activation by cAMP). This step gives rise to 3, 4-dihydroxyphenylalanine (L-

DOPA), which in turn is a substrate for L-aromatic acid decarboxylase. Decarboxylation yields the first mediator, dopamine. Further hydroxylation of dopamine leads to norepinephrine, and methylation to epinephrine (Stevenson, 1998).

Catecholamines are rapidly inactivated in the blood by either methylation through the action of catechol-*O*-methyltransferase (COMT) or oxidized by monamine oxidase (MAO) with inactive metabolites excreted by the kidneys (Allen et al., 1997). There is also evidence that some catecholamines are partly cleared by the lungs; particularly norepinephrine, epinephrine and dopamine. In addition to metabolism and elimination the action of catecholamines may be terminated by uptake into neuronal and non-neuronal tissue (Notterman, 1991).

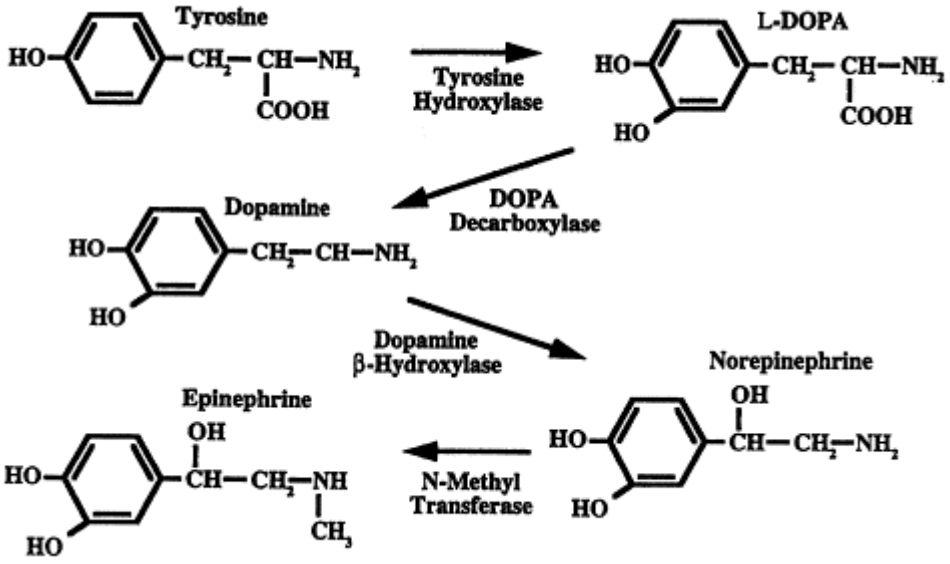

Figure 6 Synthesis of endogenous catecholamines

1.1.2. Pharmacokinetic Aspects

All three catecholamines are rather polar due to both the hydroxyl groups and the amino group, which will be mostly protonated at physiological pH. They will thus not cross the blood brain barrier easily, so that the catecholamine pools in the brain and in the periphery will not interfere with each other (Stevenson, 1998).

1.1.3. Mechanism of Action

Catecholamines exert their effects through interaction with specific cell surface receptors. Adrenoceptors are complex glycoproteins that loop across the cell membrane with the N-terminus extracellular and the C-terminus intracellular. Each receptor is approximately 410-450 amino acids in length with three extracellular and three intracellular loops. Activation of the adrenoceptor triggers a complex cascade of events through interaction with plasma membrane G proteins (guanine nucleotide binding proteins) resulting in altered levels of adenylate cyclase and ultimate the intracellular concentration of Ca^{2+}. The degree of adenylate cyclase activity is dependent upon the receptor affinity for stimulatory or inhibitory G proteins (Padbury et al., 1990).

1) Interaction with the appropriate agonist triggers binding of β- adrenoceptors to G_s proteins (stimulatory) thus increasing the proteins' affinity for GTP. The G protein complex stimulates the adenylate cyclase production of cyclic AMP (cAMP) which then activates cAMP-dependent protein kinase. The protein kinase goes on to phosphorylate intracellular proteins responsible for modulating intracellular Ca^{2+} levels.

1.1 β_1- adrenoceptors activity is linked to phosphorylation of Ca^{2+} channel proteins and promoting the influx of Ca^{2+} into the cell. β_1-adrenoceptors stimulation also promotes the

uptake of Ca^{2+} by the myocyte sarcoplasmic reticulum thus increasing the amount released during the next systole.

1.2 β_2- adrenoceptors trigger activation of adenylate cyclase and cAMP but the end result is removal of intracellular Ca^{2+} and uptake by the sarcoplasmic reticulum, thus leading to vasodilatation of the peripheral vasculature.

1.3 The dopaminergic receptor, DA_1 also couples with the G_s protein and activating the same cascade as stated above. This results in smooth muscle relaxation and dilation of the renal and splanchnic vascular beds.

2) Agonist-bound α_2-adrenoceptors and DA_2 receptors bind with inhibitory G proteins (G_i) which limit adenylate cyclase activity and lowers intracellular cAMP levels. The net result is inactivation of Ca^{2+} channels and limited release of intracellular Ca^{2+} from the sarcoplasmic reticulum. The physiologic response is seen as vasoconstriction and decreased norepinephrine and hormone release.

3) Agonist bound α_1-receptors also bind to a G protein (possibly G_q) which activates membrane-bound phospholipase C. This complex, in turn, hydrolyzes phosphatidylinositol-4,5-biphosphate (PIP_2) to second messengers diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP3). DAG leads to activation of protein kinase C and movement of extracellular Ca^{2+} into the cell. IP3 plays a similar role but also promotes the release of Ca^{2+} from the sarcoplasmic reticulum (Padbury et al., 1990).

1.1.4. Pharmacological Effects.

4.1 Epinephrine (EP)

Epinephrine is a potent stimulator of both α-and β-adrenoceptors, therefore, its effects on target organs is complex (Fisher et al., 1993 and Stevenson, 1998).

1.) Effects of EP on blood pressure are dose dependent. When given in large doses intravenously, EP gives a rapid increase in blood pressure. As the response wanes, the mean pressure falls below normal before returning to control levels. The pressor effects are due to (1) the positive inotropic effect of EP, (2) the positive chronotropic effect, and (3) vasoconstriction in many vascular beds. The depressor effect is due to the activation of vasodilator β_2-adrenoceptors in the vasculature perfusing skeletal muscle. When given in small doses, there is little or no effect on the mean blood pressure because the increase in blood pressure resulting from increased heart rate and contractility is counteracted by the decrease in total peripheral resistance due to vasodilatation in blood vessels perfusing skeletal muscle. When EP causes an increase in mean arterial pressure (high doses), it activates a compensatory vagal baroreceptor mediated bradycardia which also helps to return blood pressure toward normal (Hoffman, et al., 1996 and Stevenson, 1998).
2.) Effects of EP on vascular smooth muscle are variable, resulting in a substantial redistribution of blood flow. That is, EP causes a marked reduction of blood flow through the skin by activating its α1-adrenoceptor, while simultaneously redistributing flow through the muscles by causing vasodilatation there through the activation of β_2 receptors. This has obvious utility in survival of the organism by preparing it for fight or flight. EP can reduce renal blood flow by 40 percent in doses that do not effect to mean blood pressure (Hoffman, et al., 1996 and Stevenson, 1998). No significant constrictor action on cerebral blood vessels. It is

shown that the blood flow to the brain is not restricted during responses to stressors.

3.) Effects of EPI on cardiac muscle are mediated primarily by β_1-adrenoceptors, although some β_2 and α-adrenoceptors are also present in the heart. As indicated before, EP has a powerful chronotropic and inotropic effect. The chronotropic action of EP is due to its ability to accelerate the slow depolarization of pacemaker cells of the SA node that takes place during diastole. Large doses may provoke cardiac arrhythmias (Hoffman, et al., 1996).

4.) Effects of EPI on other smooth muscles. In general GI muscle is relaxed, and resting tone and peristaltic movements are reduced. This is due to the inhibitory effect mediated by activation β_2 -adrenoceptors, and possibly also due to inhibition of release of ACh by activation of inhibitory presynaptic α_2-adrenoceptors on cholinergic nerve terminals. The response of the uterus is variable depending on phase of the sexual cycle, state of gestation, and dose of the drug. During the last month of pregnancy, EP inhibits uterine tone and contractions, by activating β_2 -adrenoceptors. As a result, selective β_2 agonists are used to delay the onset of premature labor. Bronchial smooth muscle is powerfully relaxed by EP via activation of β_2 -adrenoceptors. Selective β_2 agonists are used in the treatment of asthma. EP relaxes the detrusor muscle of the bladder by activating β-adrenoceptors, and contracts the sphincter muscles due to alpha agonist effects, the result is urinary retention (Hoffman, et al., 1996).

4.2 Norepinephrine (NE)

NE is a potent agonist at α and β_1-adrenoceptors, and has little action on β_2 -adrenoceptors, therefore when given by

intravenous infusion of low dose, NE causes a pronounced increase in total peripheral resistance. This is combined with its direct inotropic effect on the heart to cause a substantial increase in mean blood pressure, and a reflexly mediated bradycardia (Notterman, 1991 and Keller et al., 1996).

4.3 Dopamine (DA)

At low doses DA activate D_1 receptors in renal, mesenteric, and coronary vascular beds. This leads to vasodilation. Increased flow through renal blood vessels is useful in cardiogenic and septicemic shock when perfusion of vital organs is compromised (Padbury et al., 1990). DA activates β_1 - adrenoceptors at higher concentrations leading to a positive inotropic effect. Total peripheral resistance is usually unchanged, although at higher concentrations DA can cause activation of α_1-adrenoceptors mediating vasoconstriction (Notterman, 1991 and Moran et al., 1993).

4.4 Isoproterenal (ISO)

ISO is primarily a β-adrenoceptor agonist, therefore intravenous infusion of ISO leads to a substantial reduction of total peripheral resistance. Simultaneously, ISO causes a direct inotropic and chronotropic effect on the heart. The net result is a reduction in mean pressure. ISO relaxes almost all varieties of smooth muscle, but particularly bronchial and GI smooth muscle. Its effectiveness in asthma may also be due to inhibition of the release of histamine by activation of β_2-adrenoceptor. ISO used in emergencies to stimulate heart rate in patients with bradycardia or heart block. Its use in asthma and shock has been discontinued due to development

of more selective sympathomimetics (Notterman, 1991 and Kulka et al. 1993).

2. Non-catecholamine

2.1 Salbutamol

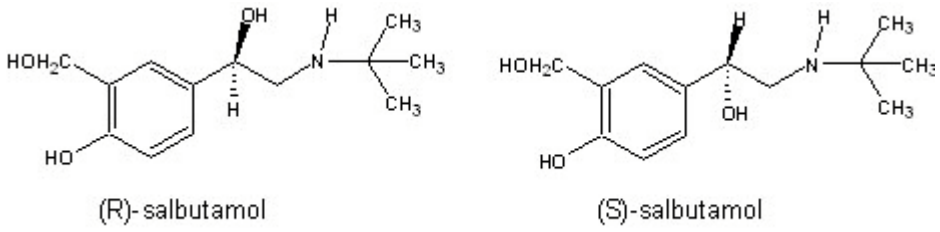

Figure 7 Chemical structure of salbutamol

Salbutamol is structural analogues of the catecholamines which have been modified so that it is not substrate of COMT and is poor substrate for MAO, this results in a longer duration of action compared to catecholamines and varies from 3 to 6 hours when administered by inhalation. It is mainly utilized for treatment of asthma. The advantage over non-selective beta agonists is that it does not cause undesirable cardiovascular effects by stimulating β_1 -adrenoceptors of the heart (Grainger et al. 1991).

All selective β_2-agonists (salbutamol, terbutaline and fenoterol) have a similar molecular structure based on the isoproterenal molecule, but rimiterol is not. All 4 agents are selective β_2 -adrenoreceptor agonists with effects on smooth and skeletal muscle, which include bronchodilatation, relaxation of the uterus and tremor. Both salbutamol and terbutaline are highly β_2 -adrenoceptor selective and may stimulate the β_1 -receptors as well,

causing an increase in heart rate and myocardial contractility (Tandon, & Kailis, 1991) but this may be due to a reflex response following relaxation of vascular smooth muscle resulting in vasodilatation rather than stimulation of β_1 -adrenoceptors. Smooth muscle relaxation is thought to occur following stimulation of the β_2 -adrenoceptor in the cell membrane causing conversion of ATP to cAMP, which then activates protein kinase. This leads to phosphorylation of proteins which then bind intracellular calcium reducing its availability for actin-myosin cross-linkage and therefore relaxation of the muscle. β_2-adrenoceptor agonists also have mild anti-inflammatory activity because they have been shown to inhibit the release of bronchoconstrictor mediators from mast cells in vitro and the release of mediators into the circulation following provocation challenge testing in vivo.

β_2-adrenoceptor agonists have also been shown to enhance mucociliary clearance and have metabolic effects such as raising free fatty acid, glucose and insulin concentrations (Neville et al. 1991). Hypokalaemia also occurs commonly, especially following intravenous administration, and is thought to be related to linkage of β_2-receptors to Na^+/K^+-ATPase (Neville et al., 1991 and Grainger et al., 1991).

3. Adrenoceptor Antagonist

3.1 Propranolol

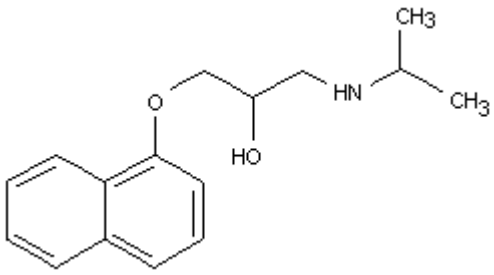

Figure 8 Chemical structure of propranolol

Propranolol is a non-selective β -adrenoceptor blocking agent. It has no other autonomic nervous system activity. Propranolol is a competitive antagonist which specifically competes with β -adrenoceptor stimulating agents for available β -adrenoceptors sites. When access to β -adrenoceptors site is blocked by propranolol, the chronotropic, inotropic, and vasodilator responses to beta-adrenergic stimulation are decreased proportionately (Heidenreich et al., 1997).

β-adrenergic blockade is useful in some clinical conditions in which sympathetic activity is excessive or inappropriate, and therefore, detrimental to the patient. Sympathetic stimulation is however, vital in some situations (e.g. in patients with AV block or with a severely damaged heart) and should be preserved. The basic objective of beta-adrenergic blockade is to decrease adverse sympathetic stimulation but not to the degree that impairs necessary sympathetic support. Beta-blockade results in bronchial constriction by interfering with endogenously or exogenously induced bronchodilation (Exner et al., 1999 and Hoffman, 2006). The mechanism of the antihypertensive effects of propranolol has not been well established. Among the factors that may be involved are decreased cardiac output, inhibition of renin release by the kidneys, and diminution of tonic sympathetic nerve outflow from vasomotor centers in the brain. It has been suggested, but not established, that

propranolol may achieve a better antihypertensive effect in patients with normal or elevated plasma renin activity (PRA) than those with low PRA (Heidenreich et al., 1997 and Hoffmann, 2006).

Propranolol may reduce the oxygen requirement of the heart at any level of effort by blocking catecholamine induced increases in the heart rate, systolic blood pressure, and the velocity and extent of myocardial contraction. On the other hand, propranolol may increase oxygen requirements by increasing left ventricular fiber length, end diastolic pressure, and systolic ejection period. When the net effect is beneficial in anginal patients, it manifests itself during exercise or stress by delaying the onset of pain and reducing the incidence and severity of anginal attacks (Gay et al., 1990). Propranolol exerts antiarrhythmic effects in concentrations producing beta-adrenergic blockade, which appears to be its principal antiarrhythmic mechanism of action. Beta-adrenergic blockade is of importance in the management of arrhythmias caused by increased levels of circulating catecholamines or enhanced sensitivity of the heart to catecholamines (Bart et al., 1997 and Exner et al., 1999).

SECTION V: SUPERSENSITIVITY

1. Pharmacology of Supersensitivity

The ability of nerve to modulate the activity of the cells which they innervate is well known. The long-term interactions

between nerve and effector cells have been classified as trophic phenomena. The trophic influence of innervation on the chemical sensitivity of muscle cell has been extensive studied (reviewed by Abel et al., 1981). It appears that many neural and hormonal agents have the ability to influence adrenergic neurotransmission by increasing or decreasing the release of norepinephrine during nerve stimulation. Adrenergic neurotransmission to cells can be interrupted in a variety of ways that reduce in the normal impulse traffic and trend to increase cellular responsiveness; a phenomenon called supersensitivity. It has been examined in a wide variety of tissue types, including skeletal muscle, smooth muscle, cardiac muscle, exocrine gland, the pineal gland and central neurons (Flemming, et al., 1973 and reviewed by Schulz, et al., 1984). It has become clear that multiple cellular changes may contribute to supersensitivity in a given type of cell and, futhermore, that the cellular change which is of primary importance varies from one cell type to anther.

1.1.Definition and Types of Supersensitivity

Supersensitivity is defined as a pheonmenon in which the amount of a substance required to produce a given biological response is less than “normal”, i.e. the dose-response curve is shifted to the left (Fleming, et al., 1973 and Goto, et al., 1985) or an increase in functional affinity (that is, a decreased EC_{50}) for agonists (reviewed by Taki, et al., 2004). Supersensitivity of muscle to transmitter substance at adrenergic and cholinergic synapses has been experimentally induced by both surgical and phrmacological methods.

Most studies of supersensitivity in adrenergic systems have been carried out on the nictitating membrane of the cat, where at least two qualitatively different types of supersensitivity appear

to be involved. One of these, found after surgical denervation, is specific for norepinephrine and closely related amines and it is very similar to the supersensitivity caused by cocaine. It is generally belived to be due to failure of active uptake of catecholamines by adrenergic nerve terminals, which is the main mechanism responsible for inactivating catecholamines in the vicinity of adrenergic receptors. Greater amounts of norepinephrine accumulate in the vicinity of the receptors over a prolonged period, accounting for the supersensitivity. This called “presynaptic supersensitivity” (reviewed by Evans, et al., 1973) or “deviation supersensitivity”, develops 24 to 48 hours after surgical denervation and has been correlated with the degeneration of the postsynaptic adrenergic fibers. Most investigators suggest that it is the result of altered disposition of an agonist within a tissue, such as with inhibition of neuronal uptake of norepinephrine (Fleming, 1976 and Goto, et al., 1985). In addition, it can be caused by a variety of mechanisms that increase the amount of neurotransmitter available for interaction with membrane receptors. These mechanisms include decrease adrenergic neuronal uptake, decrease degradative enzyme activity or decrease extraneuronal uptake (reviewed by Nasseri, et al., 1985).

A another type of supersensitivity found after both surgical denervation and decentralization is non-specific in that it increases the response not only to sympathomimetic drugs but also to acetylcholine and other agonists. It appears to be at a postsynaptic level and develops slowly over a period of two or four weeks. There is evidence to suggest that it is arises when receptor sites on the effector cells are exposed to reduced levels of transmitter (reviewed by Evans, et al., 1973). It is called nondeviation or postjunctional supersensitivity that is a change in the responsiveness of cells which develops to compensate for chronic changes in the net stimulus they receive. Chronic change in the net stimulus to cells is experimentally achieved by interfering

with neurotransmission to the cells (Fleming, 1976 and reviewed by Goto, et al., 1985). When it causes by chronic interruption of the normal stimulatory (by denervation, depletion of transmitters, etc.) to an effector cells which results in an enhanced responsiveness of the effector cells, demonstrable as leftward shifts of dose-response curves to agonists (Abel, et al., 1981 and Schulz, et al., 1984). There is usually a delay in the development of this phenomenon of 3 to 7 days after reduction of neuronal input or develops slowly with maximum increases in sensitivity usually found 3 to 14 days after interruption of neurotransmission (Abel, et al., 1981).

In addition, postjunctional supersensitivity is usually non-specific, with increases in sensitivity seen for a variety of drugs and ions. The mechanisms underlying postjunctional supersensitivity has been suggested that changes in receptor density may contribute to the enhanced responsiveness in supersensitive cells (reviewed by Nasseri, et al., 1985). More evidence indicates that there may be a number of cellular changes which contribute to the development of this phenomenon. These include an increased number of receptors, partial membrane depolarization, changes in the mobilization of calcium and alteration in adenylate cyclase (Fleming, 1976 and Abel, et al., 1981). It has become clear that multiple cellular changes may contribute to supersensitivity in a given type of cell and, furthermore, that the cellular change which is of primary importance varies from one cell type to another (reviewed by Schulz, et al., 1984).

In skeleton muscle, a spread of receptors outward from the end-plate contributes markedly to the shift of the dose-response curve to cholinomimetic agonists, but a partial depolarization of the cell membrane, altered calcium movements and loss of cholinesterase activity also contribute. In contrast, supersensitivity occurs in a variety of smooth muscles without any measurable

changes in receptor binding. Evidence from the guinea-pig vas deferens and the rabbit saphenous artery have established that a decrease in electrogenic sodium pumping and the associated partial depolarization are important contributory change to supersensitivity in those smooth muscles. An association between supersensitivity and partial membrane depolarization also has been suggested in the rat portal vein (reviewed by Schulz, et al., 1984). Postjunctional supersensitivity has been demonstrated in several cardiac preparations. In view of the marked toxic effects of large doses of reserpine on cardiac tissue, it is desirable to use doses of reserpine just large enough to produce almost complete depletion of endogenous norepinephrine. For guinea-pig atria, this treatment produces chronoptropic, arrythmogenic and inotropic supersensitivity to catecholamines that was well developed within 7 days of the initiation of treatment with reserpine. Not all portions of the myocardium develop supersensitivity with this treatment in the guinea-pig. For example supersensitivity does not develop in the left atrium, but the right atrium displays both chronotropic and inotropic supersensitivity. Postjunctional supersensitivity is much more specific in the guinea-pig right atrium than in smooth muscle. There is supersensitivity to isoproterenal but not to calcium, histamine, aminophylline or pilocarpine (Torphy et al., 1982).

1.2. Causes of Supersensitivity

Supersensitivity may be caused by a variety of procedures, including surgical and chemical denervation, decentralization, or the use of pharmacological agent (reviewed by Taki, et al., 2004). The following procedures and some agents are common used as pharmacological tools to understand the mechanisms of supersensitivity. Such as “denervation” (loss of nerve supply), stands for chronic postganglionic denervation (experiments are carried out 7 to 14 days after surgical removal of

the superior cervical ganglion). Denervation supersensitivity occurs as an increased response of an organ or tissue to a neurotransmitter or other compound with similar pharmacological actions (agonist) after the nerve supply to the structure has been cut, destroyed chemically or depleted of its store of neurotransmitter (reviewed by Bannister, et al. 1980). It has been widely described in animals especially after remove of the sympathetic nerve supply to various structures.

"Decentralization" stands for chronic preganglionic denervation (experiment performed 7 to 14 days after section of the cervical sympathetic chain), whenever the term "pretreatment" refers to the application of the agent before the beginning of the experiment (usually 24 hours prior to the experiment) i.e., pretreatment with 6-hydroxydopamine or reserpine. Both of agents cause chronic interruption of neuronal stimulation on target cells, 6-hydroxydopamine (6-OHDA), depletes peripheral tissues stores of norepinephrine and selectively destroys noradrenergic nerve terminals (Chess-Williams, et al., 1985), reserpine, deplete catecholamine in the adrenergic nerve due to inhibition of catecholamine reuptake by binding the pre-junctional storage vesicle for cathecholamine (Taki, et al., 2004). In smooth muscle these treatments cause an increase in contractile sensitivity of a variety of tissues to stimulation by the neurotransmitter norepinephrine and a number of other contractile agonists (Fleming & Trendelenburg, 1961 and reviewed by Nasseri, et al., 1985)

The comparative of denervation supersensitivity with other procedures, including preganglionic denervation (decentralization) and chronic depletion of norepinephrine with reserpine also produced nonspecific supersensitivity of the nictitating membrane. The shift to the left of the dose-response curve for norepinephrine was similar for decentralization and chronic administration of reserpine. The time courses of the development of supersensitivity were the same for postganglionic

denervation, decentralization, and chronic transmitter depletion. However, the shift of the norepinephrine curve was greater when induced by postganglionic denervation (simply called denervation) than by decentralization or chronic reserpine (Fleming, 1999).

1.3. Mechanisms underlying the Supersensitivity

In extensive studies, several mechanisms underlying the supersensitivity have been proposed; increases in the density or affinity of postjunctional receptors, changes in the resting membrane potential and alterations of calcium homeostasis (reviewed by Taki, et al., 2004). Trendelenburg (1966) had established that denervation produced supersensitivity via two distinct and independent processes. One mechanism, specific for substrates of the neuronal transport system for norepinephrine, is associated with the loss of that transport system. This mechanism is mimicked by cocaine, which inhibits the neuronal transport system. The other mechanism, due to adaptation of the postsynaptic cells, is nonspecific. Trendelenburg (1966) introduced the terms presynaptic and postsynaptic supersensitivity to distinguish between these mechanisms. Because they do not alter neuronal uptake, decentralization and chronic reserpine induce only the postsynaptic variety. Denervation, because it leads to degeneration of the nerve endings, produces both forms of supersensitivity, which are additive and, therefore, produce a greater shift in the dose-response curve for norepinephrine than for agonists that are not substrates for neuronal uptake. The above-mentioned concepts predict that, for agonists that are not substrates for neuronal uptake, the shift in the dose-response curve would be the same after either denervation or decentralization. This prediction was corroborated for barium. The existence of these two types of supersensitivity has been established in a number of noradrenergically innervated

muscles and the suggestion was made that the two mechanisms be identified by new terms, deviation supersensitivity and adaptive supersensitivity (Fleming, 1999).

2. Supersensitivity of Cocaine

Cocaine is known to cause supersensitivity to some sympathomimetic amines. At the junction between an adrenergic nerve terminal and the effector organs, cocaine has two actions: it blocks the uptake of norepinephrine into the stores and it causes supersensitivity to norepinephrine. It is believed by many investigators that the block of uptake is responsible for the supersensitivity, because norepinephrine may reach higher concentrations at the receptors of the effector organ when there is no uptake into the nerve terminals (Trendelenburg, 1965). On the other hand, several investigators have suggested that the sensitizing action of cocaine can not be attributed entirely to its ability to inhibit the uptake of norepinephrine. So 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-inervated tissues. Such postsynaptic changes may involve selective allosteric alterations of the agonist-receptor and/or non specific alteration of ion fluxes (Nakatsu and Refenstein, 1968; Kalsner and Nickerson, 1969; Reffenstein and Triggle, 1974; see reviewed by Fleming *et al*., 1990; Alburges *et al*., 1996). Thus, there is some controversy as to whether cocaine potentiates the effects of norepinephrine and related amines by impairment of the neuronal uptake of these amines (prejunctional effects) or by a direct effect on the effector cells (postjunctional effect) (Trendelenburg, 1972). Many experiments supported the mechanism are:

2.1. Blockade of Monoamine Uptake Mechanism

According to the uptake theory, the sensitivity of an effector organ is determined by the rate with which uptake into the nerve terminals removes the norepinephrine from the neighborhood of the receptors. In the present of normal uptake, the concentration of injected norepinephrine at the receptors remains low; under such conditions the sensitivity to norepinephrine is low. When uptake of this amine is impaired, the concentration at the receptors increases, i.e. supersensitivity ensues (Trendelenburg, 1966). 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 tissues, 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. Thendelenburgs *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 resepine-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 extraneuronal 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 nucleaus locus coeruleus and guinea pig submucous plexus after

cocaine exposure. These neurons exhibited inhibitory postsynaptic potentials (i.p.s.p.s.) which resulated 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 hyperoplazation 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 never 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 injectoion (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.

Cocaine blocks the uptake of monoamine neurotransmitters *in vitro*, with about equal potency for the three major monoamines, dopamine (DA), norepinephrine (NE), and serotonin (5-HT). Further, it is widely held that the behavioral effects of cocaine are related to the increase in extraneuronal levels

of monoamine neurotransmitters in the CNS. A substantial body of data implicates blockade of CNS dopamine (DA) reuptake by dopamine transporters (DAT) as a primary mechanism in the abuse-related effects of cocaine (reviewed by Wang, et al., 2007). *In vivo* assays also indicate that cocaine can increase the extracellular levels of monoamines. Research with *in vivo* microdialysis has demonstrated an increase in monoamine concentrations in rat striatum and nucleus accumbens following cocaine administration, and in monkey striatum as well. Experiments utilizing *in vivo* electrochemistry have reported that systemic administration of cocaine can decrease the clearance of locally-applied dopamine in rat striatum and accumbens, suggesting that blockade of uptake underlies the monoamine elevations (reviewed by Wong, et al., 2007)

2.2 Receptor Sensitization Mechanisms

Cocaine has been known for a long time to sensitize responses of autonomic effector organs to epinephrine and norepinephrine. This sensitization is widely attributed to blockade of neuronal uptake sites for cathecholamines with a resultant increase in their concentrations and durations at postsynaptic sites of action. Several workers, however, have questioned this unitary hypothesis of cocaine action and provided evidence for non-neuronal or postsynaptic sites of sensitization, unrelated to amine disposition (reviewed by Kalsner, 1993). Contrary to the presynaptic hypothesis, many researchers believed that the reuptake blockade alone can not portray the cocaine induced sensitzing 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).

There is an evidence that most of the uptake and storage of norepinephrine is in sympathetic nerves, and that cocaine inhibits the uptake of norepinephrine by the sympathetic nerves. It follows that, if supersensitivity is due to inhibition of norepinephrine uptake, cocaine should not sensitize a nerve-free tissue to norepinephrine (Maxwell, et al. 1968). There is some evidence that storage of tissues in the cold leads to the degeneration of intrinsic nerves before the responsiveness of the tissue is greatly altered. Varma and McCullough (1969) reported that cocaine sensitized cold stored (6-8 ^{o} C, for 7 days) aortic and splenic strips of rabbit and nictitating membrane of cats to norepinephrine without inhibitory the retention of H^{3}-norepinephrine which they suggested that the site of the sensitizing action of cocaine is postsynaptic adrenergic receptors.

Kalsner and Nickerson (1969) found that cocaine potentiates the response of the aortic strip to methoxamine and histamine, two agents which do not seem to be taken up by adrenergic neurons. They 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 it. Two parameters under various experimental conditions were 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 norepinehrine (3×10^{-5} M) and 5-hydroxytryptamine (3.3×10^{-4} M). It was clear that the 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 effects cells. This might be equivalent to the “postsynaptic” action, which was suggested to occur in innervated system (Nakatsu and Reiffenstein, 1968; Shibata et al., 1971).

The experiment of Summers and Tillman (1979) demonstrated that desmethylimipramine (desipramine), a more potent 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 were pretreated with phenoxybenzamine (POB), an irreversibly-adrenoceptor blocking agent. Pretreatment with POB and the administration of norepinephrine until the concentration of norepinephrine reached an equilibrium state, and 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 supramaximum 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.

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 concentration 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 behaviroal sensitization effects described during cocaine use might involve the up-regulation in the dopaminergic receptor.

Several studies have shown that voltage-gated calcium channel blockers can prevent the behavioral and reinforcing effects of the drug and also cocaine-induced cardiac events, including lethal ventricular fibrillation. However, the role of voltage-gated calcium channels in cocaine-induced responses is not clear. Ca^{2+}- channel blockers has been used as a treatment for the cardiotoxic of cocaine intoxication (Kneueffer, 2003). Premkumar (1999), study the effect of cocaine on the L-type calcium channels in isolated ventricular myocyte, he showed that cocaine in pharmacological dosage selectivity and potency enhances L-type calcium channel currents in the myocyte. This potentiate of cocaine is due to an increase and decrease, respectively, in the calcium channel opening and closing rate with no apparent effects on voltage-dependence or single-channel conductance. The effects of cocaine are rapidly reversible and unaffected by prior ATPγ*S*-induced channel phosphorylation. These results suggest that cocaine directly binds and facilitates the opening of L-type calcium channels. Importantly, elevated intracellular calcium levels via this mechanism triggering

second messenger pathways and gene activation may contribute to many of the cardiovascular and central nervous system effects of cocaine.

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).