

CHAPTER 5

DISCUSSION

The present study demonstrated that isolated atria and tracheas from guinea-pigs received chronic pretreatment with cocaine for 14 days, developed supersensitivity to both epinephrine and salbutamol. As shown in Fig. 19-20, the concentration-response curves of the cocaine-treated groups for positive inotropic and chronotropic effects of both agonists shifted to the left of those of saline-treated groups. Similar results were found for the tracheal relaxing effects (Fig. 23). The pD_2 values for all effects of epinephrine and salbutamol on the cocaine-treated tissues were significantly higher than the saline-treated tissues (Table 9). According to D_{max50} ratios (Table 11) the atria from cocaine-treated groups were more sensitive to epinephrine and salbutamol by about 7-8 and 9.5-10.5 folds, respectively, whereas in the tracheas the sensitivity increased by about 8.5 and 13 folds, respectively. In addition to causing the leftward shift of the curves, the maximum inotropic effect of epinephrine and chronotropic effect of salbutamol of cocaine-treated atria were significantly higher than those of saline-treated atria, while the tracheal relaxation effect of the cocaine-treated was not significantly different. The results of our study are consistent with that reported by Chess-Williams (1985) and Broadley et al (1986) that salbutamol exhibited a partial agonist activity in guinea-pig atria. Broadley et al (1986) also demonstrated that salbutamol also acted as a partial agonist in guinea-pig trachea. We didn't observe the partial agonist activity of salbutamol in our study; this might be due to the carbachol concentration used in this study produced only partial contraction of the trachea.

Cocaine has been known to sensitize responses of autonomic effector organs to catecholamines. The sensitization is first hypothesized to be due solely to presynaptic mechanism.

This classical accepted mechanism of action involves inhibition of peripheral neuronal amine reuptake. Later, many investigators have questioned this unitary hypothesis of cocaine action and provided evidences for other neuronal or nonneuronal or postsynaptic sites of sensitization unrelated to amine disposition

1. **Presynaptic mechanism**, cocaine blocks the neuronal uptake of catecholamines by the adrenergic nerve terminals; resulting in increase the synaptic concentration of this amine available for binding to adrenoceptors and enhancing the effect of exogenously administered norepinephrine and epinephrine (Muscholl, 1961; Kopin, 1964, Iversen, 1967 and reviewed by Kalsner, 1992).

2. **Postsynaptic mechanism**, many studies suggested that the supersensitivity involved postsynaptic mechanism. Cocaine has been shown to enhance the maximum responses of rat and guinea-pig vasa deferentia to norepinephrine (reviewed by Greenberg & Long, 1970). When the neuronal uptake mechanism for norepinephrine is inhibited, less exogenous amine is required in the medium to occupy the same number of tissue receptors and the dose-response curve is shifted to the left. However, the maximum response of the tissue to the amine should not be altered if the sole event mediated by cocaine is blockade of neuronal reuptake mechanism (Greenberg & Long, 1970). According to the report of Reiffenstein and Triggle (1974), cocaine in the concentration of 3.3×10^{-7} - 3.3×10^{-5} M (101.12 ng/ml - 10.11 μ g/ml) had no effect on the uptake of norepinephrine by smooth muscle of human umbilical arteries , but it was found to potentiate response of the tissue to exogenous applied norepinephrine. In addition, cocaine also produced the leftward shifts of the dose-response curves to oxymetazoline, which is not a substrate for neuronal uptake, in isolated splenic capsular strip of cat (Summer & Tillman, 1979). Many investigators suggested that cocaine increased influx of Ca^{2+} into the cells (Greenberg & Long, 1970; Kalsner, 1992; Premkumar, 1999 and Sabra et al, 2003). Premkumar (1999) reported that cocaine potentiated the L-type calcium channel

currents by cardiac myocyte with the EC_{50} value of 274 nM. He suggested that cocaine directly bound and facilitated the opening of L-type calcium channels resulting in elevation of intracellular Ca^{2+} and this mechanism of cocaine played an important role in many of the actions in the cardiovascular and central nervous systems.

As epinephrine is also a substrate of neuronal uptake (Varmar and Nickerson, 1981), therefore, the presynaptic mechanism may play a role in the supersensitivity to epinephrine observed in this study. However, it might also involve postsynaptic mechanism, since the level of cocaine detected in plasma, and isolated trachea at 24 hr after cocaine cessation was 5.08 ± 0.63 ng/ml and 2.80 ± 0.41 ng/g whereas the levels in atria and ventricle were less than 17.5 ng/g and 3.8 ng/g, respectively. According to Trendelenburg et al (1972), the level of cocaine that caused neuronal uptake blockade of [3H]-norepinephrine in cat nictitating membrane was higher than 10^{-7} M (30.34 ng/ml). Thus, the level of cocaine found in plasma and tissues in the present study was too low to inhibit the neuronal uptake. Moreover, the supersensitivity seen with salbutamol in this study is unlikely to be due to the presynaptic mechanism since salbutamol is not a substrate for neuronal uptake (Varma and Nickerson, 1981 and Chess-Williams, 1985). Therefore, the observed supersensitivity to salbutamol must therefore originate postsynaptically.

According to O'Donnell and Wanstall (1979), the guinea-pig atria contain only β_1 -adrenoceptors and trachea contains a mixture of β_1 - and β_2 -adrenoceptors. However, Brodde (1989) reported that in guinea-pig atria, the ratio of β_1 : β_2 subtypes was 75:25, whereas this ratio in trachea was 15:85. Epinephrine acts on both β_1 - and β_2 -adrenoceptors with the relative potency of $\beta_1 \geq \beta_2$, whereas salbutamol is a selective β_2 -adrenoceptor agonist (U'Prichard et al, 1978). It has been reported that the relaxation of guinea-pig trachea caused by epinephrine was shown to be accompanied by suppression of the slow wave and membrane hyperpolarization through activation of β -adrenoceptor

(probably β_2 -subtypes) (Bulbring and Tomita, 1987). Based on the main population of β -adrenoceptor subtypes, it is reasonable to determine the difference in the degree of supersensitivity between β_1 - and β_2 -adrenoceptors induced by cocaine using isolated guinea-pig atria and trachea as models for β_1 - and β_2 -adrenoceptors, respectively. It is interesting to study the responsiveness of atria and trachea isolated from chronic cocaine-treated guinea-pigs. to epinephrine which acts on both β_1 - and β_2 -adrenoceptors (but preferential acts on β_2 -adrenoceptors in trachea) and salbutamol, a selective β_2 -adrenoceptor agonist. The results of this study demonstrated that cocaine-induced the supersensitivity to epinephrine and salbutamol in both atria and trachea. As shown by the higher pD_2 of both agonists in all cocaine-treated groups than those of control groups (Table 9), it is indicated that the responsiveness of atria and trachea to exogenously administered epinephrine and salbutamol significantly increased. Thus, it seems likely that supersensitivity occur in both adrenoceptor subtypes.

The enhanced maximum responses of the atria of cocaine-treated guinea-pigs to epinephrine cannot be explained by using the concept of blockade of neuronal uptake. It is generally assumed that the maximum response to catecholamines occurs when that fraction of the available receptors essential for the contractile process are occupied. When the uptake mechanism for elimination of the amine is blocked, the concentration of amine in the medium necessary to saturate the same number of receptors is diminished and the dose-response curve for the amine is shifted to the left. Thus, the enhanced maximum response should be produced by other mechanism of cocaine. As mentioned above, there were evidences showing that the postsynaptic mechanism of cocaine involved the increase in Ca^{2+} influx through voltage-gated channels. Epinephrine produces contraction in atria by activation of β_1 -adrenoceptors which couples to G_s protein and results in activation of adenylyl cyclase and cAMP production (Klabunde, 2005). The cAMP mediated cellular events are induced through a phosphorylated protein

(PKA). PKA induces the opening of voltage-gated Ca^{2+} channels and consequently increases calcium influx resulting in increased contractility and heart rate. Kalsner (1992) concluded from the study of cocaine sensitization of coronary artery contractions and induced spasm of the artery that cocaine has a potent action on the L-type calcium channels, magnifying the effects of stimuli that make use of these channels in contraction. The author demonstrated that the response of the tissues subthreshold concentrations of the calcium channels opener Bay K 8644 were increased greatly by cocaine and this magnification was antagonized by nifedipine. The spontaneous generated tone was also magnified significantly by cocaine and this enhancement also was selectively antagonized by nifedipine. As previously mentioned, Premkumar (1999) reported that cocaine potentiated the L-type calcium channel currents by cardiac myocyte and suggested that cocaine directly bound and facilitated the opening of L-type calcium channels resulting in elevation of intracellular Ca^{2+} and this mechanism of cocaine played an important role in many of the actions in the cardiovascular and central nervous systems. Thus, it is possible that these action of cocaine might enhance the action of epinephrine on atria which causing the increase in maximum response and might cause the leftward shift of the concentration-response to epinephrine.

The mechanism of action of salbutamol in atria is similar to epinephrine (Westfall and Westfall, 2006), so the sensitizing mechanism caused by cocaine should be similar. However, the effect of cocaine induced Ca^{2+} influx can not be explained for the increase responsiveness of trachea to both agonists. Since in the trachea, the agonist caused the decreasing in intracellular Ca^{2+} and results in muscle relaxation. Agonist produces relaxation of trachea by activation of β_2 -adrenoceptors which couples to G_s protein and results in activation of adenylyl cyclase and cAMP production. The cAMP mediated cellular events are induced through a phosphorylated protein (PKA). PKA causes the relaxation of trachea by several mechanism: (1) lowering of

free intracellular Ca^{2+} by active removal of free Ca^{2+} from the cell and into intracellular stores; (2) opening calcium-activate potassium channels, thus hyperpolarizing the cell membrane; and (3) inhibition of myosin phosphorylation (Barnes and Mueller, 1995). Premkumar (1999) demonstrated that cocaine at low concentration ($<2 \mu\text{M}$) selectively potentiated L-type Ca^{2+} currents and had no effect on voltage-gated Na^+ and K^+ currents. At higher concentrations, all of these currents were blocked (produced a nonselective block of cationic conductances). In the present study, it is demonstrated that cocaine produced supersensitivity in trachea in response to epinephrine and salbutamol so it is possible that the blocking effect of cocaine might potentiate the relaxing action of epinephrine and salbutamol in the trachea. And it is estimated that detected level of cocaine in this study was high enough to block the currents.

Beside the above mechanism of cocaine-induced supersensitivity, there were some studies demonstrated that low doses of cocaine (0.1-2 mg/kg, i.v.) were associated with an increased in sympathetic outflow from the central nervous system, while higher dose (3-5 mg/kg, i.v.) resulted in decrease sympathetic nerve activity (reviewed by Sabra et al, 2000). Rhee et al (1990) reported that, in rats and rabbits, cocaine doses of 0.3-1 mg/kg raised the blood pressure, while doses of 3-5 mg/kg decreased blood pressure and other cardiac parameters. Sabra et al (2000) reported in their study in the cat that cocaine (5 mg/kg, i.v.) caused the potentiation of cardiac response to adrenergic stimuli and involved presynaptic mechanism to block norepinephrine uptake and postsynaptic mechanism to raise the maximal responses. The latter may result from inhibition of central sympathetic outflow or from activation of cardiac Ca^{2+} channels, leading to increased cardiac sensitivity to noradrenaline. They suggested that the decreased sympathetic output to the heart might sensitize it to the effects of agonist acting on adrenergic receptors, by inducing receptor or post-receptor modifications. Sabra et al (2000) also demonstrated that

pretreatment with verapamil prevented the cocaine-induced enhancement of the maximum response to norepinephrine, but it did not inhibit potentiation of submaximal doses. They concluded that verapamil, by not interfering with the inhibition of reuptake, did not prevent cocaine from potentiating the submaximal effect of norepinephrine. Verapamil, however, inhibited the potentiation of the maximum effect, i.e., the other component of potentiation due to postsynaptic mechanisms which dependent on mobilization of calcium ion from the extracellular space through voltage-dependent channels. The studies mentioned above involved the acute effect of cocaine. In contrast, our studies performed on chronic cocaine treatment, guinea-pigs received cocaine (2.5 mg, i.p., b.i.d.) for 14 days. Even though dose of cocaine used in the present study was lower than the others; however, chronic treatment schedule might produce sensitization by the same postsynaptic mechanism.

In this study, the tissue concentration of epinephrine was not determined, so there is no evidence to prove whether the concentration of epinephrine was high or low at the synaptic site. Sunbhanich (1980) determined the K_D values of epinephrine under non-equilibrium condition compare to $[D]_{\max 50}$ obtained under equilibrium condition in guinea-pig isolated atria. The K_D values obtained under non-equilibrium condition was assumed to represent the concentration of catecholamine in the biophase and $[D]_{\max 50}$ represented the concentration in the organ bath. In control experiment, the ratio of $K_D/[D]_{\max 50}$ was more than unity, suggested that the concentration of epinephrine in the biophase was higher than that in the bathing solution. In contrast, the ratio of $K_D/[D]_{\max 50}$ decreased toward unity in the presence of cocaine, suggesting that the biophase concentration of epinephrine was decrease. This suggested that in the present of cocaine the concentration of agonist in the biophase was lower than that of the control. Therefore, potentiation of epinephrine in guinea-pig isolated atria was due to postsynaptic action rather than its neuronal uptake blocking action. Kneuepfer et al (1993) determined whether there is a relationship between

the different effects of cocaine administration on cardiovascular responses and atrial norepinephrine (also, a substrate of neuronal uptake) concentrations were not affected by cocaine. Alburges and Wamsley (1993) and Alburges et al (1996) demonstrated that chronic cocaine treatment (5, 10, 15, 20, and 25 mg/kg, intraperitoneally, twice a day for 14 days) did not change the concentration of norepinephrine and its metabolites at certain areas of the brain. Thus, these evidences support that the chronic cocaine treatment in our study might not involve with the inhibition of uptake process.

In this study, propranolol, the nonselective- β -adrenoceptor antagonist, was shown to inhibit the responses of both atria and trachea to epinephrine with a high pA_2 values irrespective of cocaine treatment. The mean pA_2 values of propranolol for the inhibition of the response of the atria of saline-treated group were 8.30 ± 0.03 (for force) and 8.24 ± 0.06 (for rate) whereas those pA_2 values of the atria of cocaine-treated group were 8.42 ± 0.04 (for force) and 8.37 ± 0.06 (for rate). The pA_2 values of propranolol for blocking the tracheal relaxing effect of epinephrine of the saline- and cocaine-treated groups were 8.12 ± 0.03 and 8.31 ± 0.04 , respectively. There was no change in the maximum of the responses to epinephrine and the slope Schild regression did not differ significantly from unity. This indicated a competitive nature of the antagonism. The pA_2 values obtained in this study were in the same range with the previous reports by other investigators. The pA_2 values reported for the antagonism of propranolol on β -adrenoceptors of guinea-pig atria were 8.51 (Harms, 1976) and 7.89-8.8 (reviewed by Cohen et al, 1980) and, the pA_2 values for the antagonism of β -adrenoceptors on guinea-pig trachea were 8.25 (Harms, 1976) and 8-8.7 (reviewed by Cohen et al, 1980). Thus, the effects of epinephrine on the organs from both control and cocaine-treated groups were due to the activation of β -adrenoceptors. All pA_2 values of propranolol of all cocaine-treated groups were slightly but significantly different from those of saline-treated group.

The results suggested that cocaine might slightly increase the affinity of propranolol to β -adrenoceptors.

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

By using radioligand [^3H]-prazosin binding technique, Karliner et al (1979) demonstrated that there were α_1 -adrenoceptors in guinea-pig myocardium. Epinephrine, norepinephrine and isoproterenol competed with the binding with the rank order as follows: epinephrine > norepinephrine > isoproterenol. The role of adrenoceptor stimulation by catecholamines on cardiac muscle contractility has been studied to a great extent. Under normal conditions, tension development is modulated primarily through activation of the β -adrenoceptors. α_1 -Adrenoceptors contribute to contractility by producing a small but significant inotropic effect (reviewed by Gorostiza et al, 19995). Parr and Urquilla (1972) reported that

phentolamine shifted the inotropic curve of epinephrine in rabbit atria, but it did not modify the chronotropic response to the amine. Propranolol antagonized both the inotropic and chronotropic effects of epinephrine. The results suggested that both α - and β - adrenoceptors involved in the inotropic effect of epinephrine. As mentioned above, cocaine also induced supersensitivity in α_1 -adrenoceptors. In this study, which the α_1 -adrenoceptors were unblocked, the supersensitivity in α_1 -adrenoceptors might actually occur in atria that consist of both α_1 and β - adrenoceptors. However, the present results demonstrated that the degree of supersensitivity of epinephrine (α_1 - and β -adrenoceptor agonists) in the atria were not higher than that of salbutamol (selective β_2 -adrenoceptor agonist). Thus, it is suggested that α_1 -adrenoceptors did not play a major role in the supersensitivity observed in this study.

In summary, the present study demonstrated that supersensitivity to adrenergic agonist-induced by cocaine occurred for both β_1 - and β_2 -adrenoceptors. The responsiveness to both β_1 -and β_2 -agonist, epinephrine (acts on β_1 -adrenoceptors in the atria, and preferentially acts on β_2 -adrenoceptors in the trachea) and a β_2 -selective agonist, salbutamol of isolated atria and trachea of cocaine-treated guinea-pigs were higher than those of saline treated groups. These results the supersensitivity to salbutamol which is not a substrate for neuronal uptake support the postsynaptic mechanism of cocaine-induced sensitization. Although the result of present study cannot entirely exclude the presynaptic mechanism involving the inhibition of reuptake by cocaine from the supersensitivity to epinephrine, but the level of cocaine in plasma, trachea and cardiac tissue were lower than those of the level causing reuptake blockade reported by other investigators. In addition, the maximum increase in force of contraction produced by epinephrine in atria of the cocaine-treated guinea-pig was higher than those control groups. Therefore, the supersensitivity produced by cocaine in this study might mainly involve postsynaptic mechanism. The postsynaptic mechanism might be

due to increase in Ca^{+2} influx by cocaine through L-type calcium channels or produce receptor or post-receptor modification as suggested by other investigators. It is conceivable that such sensitization may, under conditions of sympathoadrenal discharge such as stress, exaggerate the cardiac effect of cocaine which contributes to cardiotoxic manifestation of cocaine abuse. In addition, the use of salbutamol for treatment of asthma might produce more pronounced tachycardia in patient using cocaine