CHAPTER IV

DISCUSSION AND CONCLUSION

In this thesis, the actions of piperine on blood pressure and isolated right and left rat atria have been studied.

EFFECT OF PIPERINE ON BLOOD PRESSURE

It has heen reported that intravenous injection of piperine in cats and rats produced effects of the Bezold-Jarisch reflex (hypotension, bradycardia, apnea) followed by a rise in blood pressure. In cat, these reflexes were abolished by bilateral vagotomy (Szolcsanyi and Janossy, 1971). Conversely, 5 mg/kg of intravenously administered piperine produced only insignificant effect in dog (Singh et al. 1973). In this thesis some more details of the Bezold-Jarisch reflex and the pressor response have been studied in rat. An intravenous administration of 1 mg/kg piperine produced a transient reduction in blood pressure, bradycardia and apnea followed by a rise in tension and by subsequent delayed fall in blood pressure (see Fig. 2). The sudden drop in blood pressure combined with bradycardia and apnea indicate with great probability that the symptom are due to the Bezold-Jarisch reflex (Zipf and Miestereck, 1953). The afferent branch of the Bezold-Jarisch reflex being furnished by the vagus nerve. Fig.3 shows that with the vagus cut on both sides, 1 mg/kg of intravenous administered piperine no longer elicited a fall in blood pressure, bradycardia and apnea; instead the pressor effect was obtained. These results indicate that an intact vagus nerve was neccessary for the onset of apnea, fall in blood pressure and bradycardia in response to piperine, substantiating

our assumption that piperine elicited such interoceptive reflex to which the vagus constitutes the afferent branch. These confirmed the results obtained by Szolcsanyi and Janossy (1971) in cat. In atropinized animals (0.2 mg/kg), piperine failed to cause hypotension and bradycardia, but it still elicited apnea. This will be obvious if we consider that atropine inhibits the function of the efferent vagus only,bringing about a fall in blood pressure and bradycardia, but has no such effect on the afferent branch, which in this case conveys the impulse eliciting apnea. Also, following application of 5 mg/kg hexamethonium, a ganglionic blocking agent, piperine no longer elicited hypotension and bradycardia (see Fig. 5). Fig. 6 shows the effect of piperine injected into the carotid artery. In this manner, 1 mg/kg piperine produced a sudden rise in blood pressure, while depression, bradycardia and apnea concomitant to intravenous administration failed to set in. This observed effect can also be seen even when cocaine applied directly to carotid sinus. Additionally, hexamethonium (5 mg/kg) caused a dramatic reduction in this pressor effect of intracarotidal piperine. These could be attributed to a direct stimulating effect of piperine on the vasomotor center.

In vagotomized rats, 1 mg/kg of intravenous piperine also produced the pressor effect. It is therefore possible that the pressor effect of piperine in vagotomized rats may be due to its central action. In order to determine whether the pressor effect of piperine which may act on the vasomotor center, a ganglionic blocking drug was used to prevent the impulse from central nervous system. As shown in Fig. 9 hexamethonium which block transmission of impulse from the pre-ganglionic axon by occupying receptor sites at the post-ganglionic axon (Paton and Zaimis, 1952., Taylor, 1982) partially reduced this pressor effect.

This observation implied that the pressor effect of piperine in vagotomized rats is partially due to its direct stimulation on the vasomotor center. An addition argument in favour of this interpretation is that the pressor effect of piperine was partly antagonized by the non-selective β-adrenoceptor propranolol as well as that by the non-selective α-blocking agent phentolamine. These results strongly indicate that the pressor effect of piperine is partially due to the stimulation of the vasomotor center by the release of norepinephrine in addition to epinephrine from the adrenal gland and sympathetic nerve ending.

Furthur, the effect of piperine on blood pressure was investigated in pithed rats inwhich the entired central nervous systems had been destroyed (Shipley and Tilden, 1959). On 1 mg/kg piperine given into the femoral vein, both systolic and diastolic blood pressure increased sharply. This observation demonstrated a peripheral mechanism of action. Reserpine pretreatment almost abolished the pressor effect of piperine. Reserpine is known to be a substance that causes a depletion of catecholamines from adrenergic tissues (Carlsson, 1965; Sjostrand and Swedin, 1967). Thus, the blockade by this drug of the piperine effect is attributed to the release of catecholomines from sympathetic nerve terminal. Additionally, this pressor effect was effectively reduced by the non-selective β-adrenoceptor blocker propranolol (0.70 mg/kg) as well as by phentolamine (1 mg/kg), the non-selective α-adrenoceptor antagonist. These results suggest that the pressor effect of piperine is mainly due to catecholamine action at cardiac β-adrenoceptor and vascular α-adrenoceptor.

It has been reported that propranolol could block the action of tyramine both by blocking β-receptors and by reducing the release

of noradrenaline (Foo et al., 1968). In general, drugs that block noradrenaline release by indirect acting agonist would be expect to block its vasoconstrictor action as well as its action on the heart. Some experimental data suggest that the amounts of the β-receptor blocking drug required to block vasoconstrictor response would be appreciably higher than those required to block β-adrenoceptors (Aramendia and Kaumann, 1967). Thus, propranolol at concentration of 0.70 mg/kg used in the present experiment would rather block action of noradrenaline on the heart than its vasoconstrictor action.

EFFECTS OF PIPERINE ON HEART - RATE AND FORCE OF CONTRACTION

The isolated right and left rat atria were used separately to investigate the effect of drugs on the rate and isometric force respectively. This technique eliminates the possible interference between rate and contractile-force. The results of this study show clearly that piperine at five doses, 3,6,12,24,48 ug/ml, produces initial stimulation of right atrial rate and left atrial isometric force and with high doses (24 and 48 ug/ml) these initial stimulations were followed by depression of both rate and contractile force.

In order to investigate the possible mechanisms of action mediating the positive chronotropic and inotropic effects of piperine, it should be studied with pharmacological tools in isolated right and left atria. The sites or modes of positive chronotropic and inotropic effects of piperine may be due to

: action like beta-adrenergic agonist or mediating release of catecholamine

: action like 5-HT or mediating release of 5-HT

: action mediated via alpha-adrenergic receptor

Beta-adrenergic agonist such as isoprenaline has powerful effect on β-adrenergic receptors which caused positive chronotropic and inotropic effects on a variety of isolated heart preparations (Wasserman & Levy, 1981; Kalsner, 1980). Propranolol is known to antagonize these positive chronotropy and inotropy (Blinks, 1967, Bristow et al.,1970; Alder-Grachinsky & Langer, 1975; Ebner, 1981). It is seen in Fig. 24 that the positive chronotropic effect of piperine could be reduced approximately 50 % by propranolol, suggesting, at least in part, β-mediated. However, propranolol at concentrations of 0.03, 0.07 and 0.15 ug/ml could not antagonize the positive inotropic effect of piperine, thus negates the involvement of β-adrenergic receptors.

To furthur determine whether an increase in right atrial rate and left atrial contractile force are due to catecholamine release, the effects of reserpine pretreatment have been studied. Reserpine is known to be a potent cardiac depressant (Richmond et al., 1974) as well as depleter of neural storage of catecholamine (Sedvall, 1964; carlsson, 1965; Sjostrand & Swedin, 1967). The results depicted in Fig. 27,28 indicated that with atria from reserpinized rats, piperine caused approximately 58 % and 90 % on right atrial rate and isometric tension less than those observed in non-treated rats respectively. Reserpine pretreatment reduced the positive chronotropy resembled the effect produced by propranolol. The results obtained indicated that the positive chronotropy of piperine is partly due to β-stimulating catecholamines. On the left atria, piperine-evoked positive inotropy was greatly reduced by reserpine pretreatment, suggesting that the positive inotropy is mainly due to indirectly release of endogenous catecholamines.

5-Hydroxytryptamine is known to present in rat atria and also stimulate isolated atria of the rat hearts (Benfey et al., 1974; Fozard & Mwaluko, 1976; Higgin, 1981). In our preparation, 5-HT has positive chronotropic and inotropic actions. It is thus possible that it can mediate action of piperine in our isolated atria. It has been reported that 5-HT stimulated the heart by direct action on the myocardium and indirectly by releasing endogenous catecholamines (Higgin, 1980, 1981). In order to determine whether the positive chronotropy and inotropy of piperine are due to indirect release of 5-HT, the effects of 5-HT antagonists, methysergide and cyproheptadine, have been studied. Methysergide as well as cyproheptadine was found to be ineffective in blocking the positive chronotropy and inotropy of piperine, suggesting that piperine does not indirectly stimulate the atria by releasing intracardiac store of 5-HT.

It has been proposed widely that α-adrenergic receptors exist in the heart and mediate positive chronotropic and inotropic effects (Govier, 1968; Nakashima et al., 1972; Yamanaka et al., 1981). Thus, the effect of α-antagonist, phentolamine, has been studied. In the presence of phentolamine (0.32 ug/ml) the positive chronotropy and inotropy remained totally unaffected. This finding demonstrated that piperine does not act through α-adrenergic receptor.

To furthur confirm the effects of reserpine pretreatment that piperine can induce the release of catecholamines from storage sites in sympathetic nerve, the effects of desipramine (0.27 ug/ml or 10^{-6}M) and cocaine (9.1 ug/ml or $3x10^{-5}$M) have been studied. The concentrations of desipramine and cocaine used in the present experiment are the median effective concentrations in inhibiting the neuronal uptake of noradrenaline in the rat heart (Dart et al., 1983). It is seen in Fig. 35,36,37

and 38 that both desipramine and cocaine effectively reduced the positive chronotropy and inotropy elicited by piperine 60 % and 90 % respectively. Desipramine and cocaine antagonized the positive chronotropic and inotropic effects similar to the results obtained from reserpine pretreatment. Thus, the blockade by these drugs of piperine effects is probably attributed to failure of release of the endogenous noradrenaline by piperine. These results suggest the sympathomimetic action of piperine in the isolated heart depend upon an intact noradrenaline uptake mechanism. Since piperine can be taken up by neurons in the central nervous system and release 5-HT from nerve endings (Liu et al., 1984), its sympathomimetic action of piperine may possibly be linked to its entry into the terminals of the adrenergic neuron by a process related to the uptake mechanism for noradrenaline.

Since the results obtained in the present experiment clearly show that piperine produced the positive inotropy indirectly by release of endogenous catecholmines, propranolol in concentrations of 0.03, 0.07, 0.15 ug/ml (0.1, 0.24 and 0.5 uM) could not antagonized this effect. However, the highest and intermediate concentrations used in the present work seem to reduce the positive inotropy. It has been postulated that an indirect antagonism required a precise dose ratio of indirectly acting agonist and antagonist (Black, 1973, Black et al., 1980). Thus, it is possible that the concentrations of propranolol used in this experiment is not appropriate to the concentration of piperine. In addition, there is a complication that could underlie the observed results : Low concentrations of propranolol can reduce the electrically stimulated release of noradrenaline from adrenergic nerve endings by inhibiting a positive feedback mechanism mediated by presynaptic β-adrenoceptors (Adler-Graschinsky and Langer, 1975). This would

complicate the interpretation of our experiments should the release of noradrenaline by piperine by subjected to the same control.

In conclusion, the results of the present study demonstrated the cardiovascular effects of piperine in anesthetized rat and on cardiac tissue preparation. Intravenous administered of piperine in anesthetized rat produced an initial short fall in blood pressure, bradycardia and apnea followed by a rise in tension. A fall in blood pressure, bradycardia and apnea were abolished after bilateral vagotomy and is therefore mediated by afferent fibres in the vagus nerve. The rise in blood pressure, consequent of the triad responses, may be due to central activation of sympathetic nervous system; and peripheral mechanism which depend on cardiac and vascular response to the released catecholamine. In isolated right and left rat atria, piperine produced the positive chronotropy and inotropy. The mechanism is mainly due to its indirect effect which is most likely the release of catecholamine from adrenergic nerve in atria. This evidence support the pressor effect of piperine in pithed rats showing that piperine produced such an effect by indirect releasing of catecholamines; which stimulates β-adrenergic receptor on the hearts and α-adrenoceptor on the vascular system. The results obtained from the present study may demonstrate the cardiovascular effects of piperine in rat. However, more studies are required to elucidate the detail mechanism of piperine on cardiovascular system.