# CHAPTER IV

## DISCUSSION AND CONCLUSION

Effects of 5-HT on contractility and rate of the mammalian heart have received considerable attention. However, species variations have been noted in the cardiac responses to 5-HT of the dog, cat, guinea-pig and rabbit. (Schneider and Yonkman, 1954; Trendelenburg, 1960). Intravenous infusion of 5-HT in man has been shown to directly increase heart rate (Le Messurier, *et al.*, 1959). Relatively little is known about the mechanism of action of 5-HT on isolated rat heart. This discussion focuses on the effect of 5-HT on the isolated rat left and right atria. Results of Fig.1 and Fig.2 show the dose-dependent effect of 5-HT on both positive chronotropic and inotropic responses, which in general confirm previous reports by other investigators (Benfey, *et al.*, 1974; Higgins, *et al.*, 1981 a,b.). It is generally known that in rat atria, the contractile force varies inversely with heart rate ($F \alpha \frac{1}{HR}$). The modified technique have been employed in this study. The rat atria were dissected into right and left sides. By this method, the effects of 5-HT on the rate (right atria) and contractile force (left atria) are discrete since there is no interference between the right and left atria. It is seen that the time to peak effect on right atrial rate was longer (about 5-10 min) than that observed on left isometric tension (about 3-5 min). Higher doses of 5-HT caused reduction of left isometric tension within 15 min after addition of the drug (see

Fig. 2) which differed from the effect on right atrial rate (see Fig. 1). This result may suggest different mechanism of 5-HT actions on right and left atria. The doses of cyproheptadine and methysergide selected for the present investigation were 0.02 and 0.47 µg/ml respectively because they produced only slight depression on both right atrial rate and left atrial isometric tension. As shown in Fig. 3 and Fig. 5 cyproheptadine and methysergide reduced the positive chronotropic effect of 5-HT approximately 50% from control values. The pattern of the time to peak effects on right atrial rate between control group and cyproheptadine treatment were similar. However, the different effect of 5-HT antagonists on the left atrial isometric tension (see Fig. 4 and Fig. 6) was clearly observed. Both cyproheptadine and methysergide abolished the positive inotropic effect of 5-HT on left atrial isometric tension. These results strongly implied that the positive inotropic effect of 5-HT on the left atria was mediated directly by 5-HT itself. Thus, the 5-HT-induced increase in right atrial rate could be caused by direct effect of 5-HT and other positive chronotropic agent which was not antagonized by 5-HT antagonists. Two alternative mechanisms by which 5-HT stimulation is achieved have been suggested. The first is an indirect action of 5-HT on presynaptic receptors which located on the sympathetic neurons innervating the heart. Such stimulation results in the local release of catecholamine. The other mechanism is the direct stimulation on 5-HT receptors which could be blocked by 5-HT antagonists (Higgins, *et al.*, 1981 b).

Thus the effects of cyproheptadine and methysergide in this study support most of the previous results and also clearly showed

that the positive inotropic effect of 5-HT on the left atria was mostly due to direct effect of 5-HT. In contrast, the positive chronotropic effect on the right atria appeared to involve both direct and indirect compartments. In order to determine whether an increase in positive chronotropic and inotropic of 5-HT are due to catecholamine release, the effects of prior administration of propranolol 5 min before addition of 5-HT have been studied. The reduction of right atrial rate (about 50%) with propranolol implied the participation of beta-stimulating catecholamines in the action of 5-HT on the right atria. The remaining positive chronotropy should be due to the direct effect of 5-HT. The result observed in Fig.8, indicated that an increase in left atrial isometric tension was mainly due to the direct effect of 5-HT without beta-agonist action. Although the individual antagonizing effect of cyproheptadine, methysergide or propranolol could reduced the positive chronotropic about 50% from control value, the remaining positive chronotropic effect obviously could not be antagonize by individual antagonist. Thus, the combined effects of two antagonists, 5-HT antagonist and beta-adrenergic antagonist, have been studied. Results in Fig.9 and Fig.11 indicated that the positive chronotropic effect of 5-HT on right atrial rate was the combined actions of two agonists, 5-HT agonist and beta agonist, the latter could be blocked by propranolol. However, no different effect on left atrial isometric tension was observed with either combination of 5-HT antagonist and propranolol or individual antagonist (see Fig.4,6,10 and 12). These results further support our earlier suggestion that the positive inotropic effect of 5-HT on the left atria was mainly

due to the direct action of 5-HT.

In order to confirm some previous results that 5-HT can induce the release of noradrenaline from the storage sites in sympathetic nerve (Benfey, 1974), the effects of reserpine pretreatment have been studied. Reserpine is known to be a potent cardiac depressant (Richmond, *et al.*, 1975), as well as a depleter of neural storage of nordrenaline (Goodman and Gilman, 1980). The results depicted in Fig.13 and Fig.14 indicated that with atria from reserpinized rats, a dose of 2.0 µg/ml 5-HT caused only slight reduction on both atrial rate and isometric tension but these were not statistically different from non-treated rats. It can also be seen that one minute after the addition of drugs, the increase on right atrial rate between reserpinized and non-reserpinized rats are relatively equal (109.9 ± 1.1 and 108.7 ± 0.9, see Fig.13) which was rather different from the left atrial isometric tension (110.1 ± 1.3 and 108.9 ± 1.3). As show in Fig. 13, 15 and Fig. 17, the peak effects of 5-HT in reserpinized rats were relatively the same (108-109%) during 1 to 5 min after addition of 5-HT and then gradually declined to about 106-107%. The characteristic of the peak responses were rather differ from non-reserpinized rats which produced the maximum effect during 5-10 min. The longer peroid of the peak effect in non-reserpinized rat could be due to the combination of primary direct effect followed by indirect action of catecholamine released from the sympathetic nerve by 5-HT.

To further support the previous suggestions that 5-HT caused a release of catecholamine, experiments were performed in which

cyproheptadine and methysergide were added into the chamber 5 min before the addition of 5-HT. As shown in Fig.15 and Fig.17, cyproheptadine and methysergide could significantly antagonized the chronotropic effect of 5-HT ($P < 0.05$) which was clearly seen during the time to maximum response (1-5 min). The percentage changes in reserpinized rats were about 100-101% (see Fig.15 and Fig.17) which resembled the effects produced by cyproheptadine plus propranolol (see Fig.9) and methysergide plus propranolol (see Fig.11). These results strongly indicate that catecholamine is partly involved in the 5-HT-induced positive chronotropy. No different effect of 5-HT on left atrial isometric tension was observed between non-reserpinized rats (see Fig.4 and Fig.6) and reserpinized rats (see Fig.16 and Fig.18). The positive chronotropic responeses to 5-HT in reserpinized right atria which was abolished by 5-HT antagonist and were similar to those effects found with the combination of 5-HT antagonist plus propronolol in nonreserpinized rats apparently indicated that the positive chronotropic response to 5-HT was partly mediated by the release of catecholamine in the nerve endings. Ehinger, *et al.* (1968) reported the presence of adrenergic and cholinesterase-containing neurons in the heart. The adrenergic as well as acetylcholinesterase-containing fiber were detected in all part of the heart, most abundantly in the atria. Dense nerve plexa supply the sinoatrial and atrioventricular nodes. Histochemical studies on rabbit tissues show that the catecholamines are present within nerve structures. (Angelakos, *et al.*, 1963). A high density of fluorescent fibers and fiber bundles was found in the SA node region. In addition, noradrenaline was

more concentrated in the right than the left atria of rabbit and guinea-pig hearts (Angelakos *et al*; 1963). Thus these chemical and histochemical results agree with the pharmacological effects of 5-HT reported in this study that the positive chronotropic response to 5-HT in the right atria was due to both direct stimulation on 5-HT receptors and indirect effect through endogenous catecholamine release.

In conclusion, the results of the present study suggest that the positive chronotropic effect of 5-HT on the isolated rat right atria is due to the combination of direct effect and the release of endogenous catecholamine. However, the positive inotropic response on the left atrial isometric tension is mainly due to a direct activation of 5-HT receptors in the left atria. The results obtained in this study may further improve our understanding on the role of 5-HT in rat heart. However, more studies are required to elucidate the detailed mechanism of 5-HT actions on the rat heart.