

CHAPTER 4
CARDIOVASCULAR EFFECTS OF TYRAMINE, A POSITIVE CHRONOTROPIC AND
INOTROPIC EFFECTOR
SUBSTANCE ISOLATED FROM FRESH
FRUITS OF *RANDIA SIAMENSIS*

4.1 Abstract

Tyramine (0.01-1 mg/kg, i.v.) caused an increase in both the mean arterial blood pressure (MAP) and heart rate in anesthetized rats. Propranolol (0.6 mg/kg) did not modify the hypertensive effect of the tyramine, whether it was given to the animals alone or in combination with atropine and/or phentolamine. Phentolamine (2 mg/kg) significantly inhibited the hypertensive effect of tyramine. This effect was not modified when propranolol and/or atropine were also injected into the animals at the same time. Atropine and propranolol potentiated the positive chronotropic effect of tyramine. Atropine produced about a 2 fold potentiation of the positive chronotropic effect of the tyramine compared to that produced by propranolol. When these two antagonists were combined, the sensitivity of the positive chronotropic responses by the tyramine were similar to those in the presence of propranolol alone, whereas the maximal positive chronotropic effect was similar to that in the presence of atropine alone, and no further modification was found when phentolamine was also given. However, both the hypertensive and positive chronotropic effects of the tyramine were significantly suppressed by pretreatment of the animals with reserpine. In the *in vitro* preparations, tyramine caused an increase in both the rate of spontaneous contraction of the right atrium and the strength of the electrical field-stimulated contraction of the left atrium. Both these effects were significantly inhibited by propranolol or by pretreatment of the animals with reserpine. In addition, the strength of the basal contraction driven by electrical field-stimulation was also inhibited by higher concentrations of the tyramine. Tyramine produced a dose-dependent contraction on the isolated thoracic aortic ring, and this was potentiated by N^G-nitro-L-arginine (LNA), or by removal of the vascular endothelium, but inhibited by phentolamine or pretreating the animals with reserpine. These results indicate that the hypertensive and positive chronotropic effects of tyramine are due to tyramine exerting its effects

via the α - and β -adrenergic receptors respectively of the cardiovascular system by stimulating the release of endogenous catecholamines, most likely from the nerve endings and the adrenal medulla. Tyramine may also have some effects on the central regulated pathway of the pacemaker activity of the heart.

4.2 Introduction

Tyramine is a trace amine naturally found in peripheral tissues as well as in the central nervous system of vertebrates (Tallman et al., 1976; Paterson et al., 1990). It can be present in multiple dietary sources: aged cheeses, aged meat, alcoholic beverages, chocolate, some fruits and vegetables. In the present study it was found at about 0.52 % in an n-butanol extract from fruits of *Randia siamensis*. The peripheral effects of tyramine are generally attributed to its action as a "false neurotransmitter" by promoting the efflux of catecholamines from the sympathetic neurons and the adrenal medulla (Schonfeld and Trendelenburg, 1989; Mundorf et al., 1999). This results in the indirect stimulation of adrenergic receptors (Black et al., 1980). However, the cardiovascular profile of tyramine has not yet been studied.

In chapter 2, it was found that *R. siamensis* extract caused an increase in MAP and heart rate in anesthetized rats in a similar manner to those produced by isoproterenol. Using bioguided-fractionation, 11 pure compounds were isolated from the *R. siamensis* extract, but only tyramine caused an increase in both MAP and heart rate in a similar manner to that produced by *R. siamensis* extract. Thus, it is of interest to explore further whether the mechanisms responsible for the hypertensive and positive chronotropic effects of tyramine, as a single compound, are similar to or different from those of the *R. siamensis* extract, a mixture of several compounds.

4.3 Objectives

To study the cardiovascular profile of tyramine in rats and assess the mechanisms involved in its hypertensive and positive chronotropic effects.

4.4 Materials and Methods

The protocols used for studying the effects of tyramine (Sigma, USA.), for direct comparison purposes, are the same as those described in Chapter 2 for studying both the *in vivo* and *in vitro* effects of the *R. siamensis* extract.

4.5 Results

4.5.1 Effects of adding atropine, propranolol and/or phentolamine or reserpine pretreatment on the tyramine induced effects on mean arterial blood pressure and heart rate of anesthetized rats

The basal MAP and heart rate of anesthetized rats were 135 ± 6.56 mmHg and 404.17 ± 8.00 bpm, respectively.

As shown in Figure 38, tyramine caused an increase in MAP (hypertensive) and heart rate (positive chronotropic) in a dose-dependent manner. Atropine (0.6 mg/kg) or propranolol (0.6 mg/kg) did not modify the dose-response curve of the tyramine induced increase in MAP, whereas both did potentiate, instead of attenuate, the positive chronotropic activity of the tyramine, with atropine having twice the effect of propranolol. On the other hand, phentolamine (2 mg/kg) significantly inhibited the hypertensive effect of tyramine without any modification to its positive chronotropic effect. When both propranolol and phentolamine were given to the animals at the same time, the dose-response relationship to tyramine was similar to that given by propranolol alone. However, when both atropine and propranolol were given together, the positive chronotropic effect of the low doses of the tyramine were similar to those produced by propranolol alone, whereas at higher doses of tyramine the effect was similar to that produced by atropine alone. A similar result was found when the three antagonists, atropine, propranolol and phentolamine, were given simultaneously.

Figure 39 shows the effects of tyramine on the MAP and heart rate of normal and reserpinized rats. Tyramine caused an increase in MAP and heart rate in normal rats. After pretreatment with reserpine, both the tyramine induced increase in MAP and heart rate were significantly inhibited.

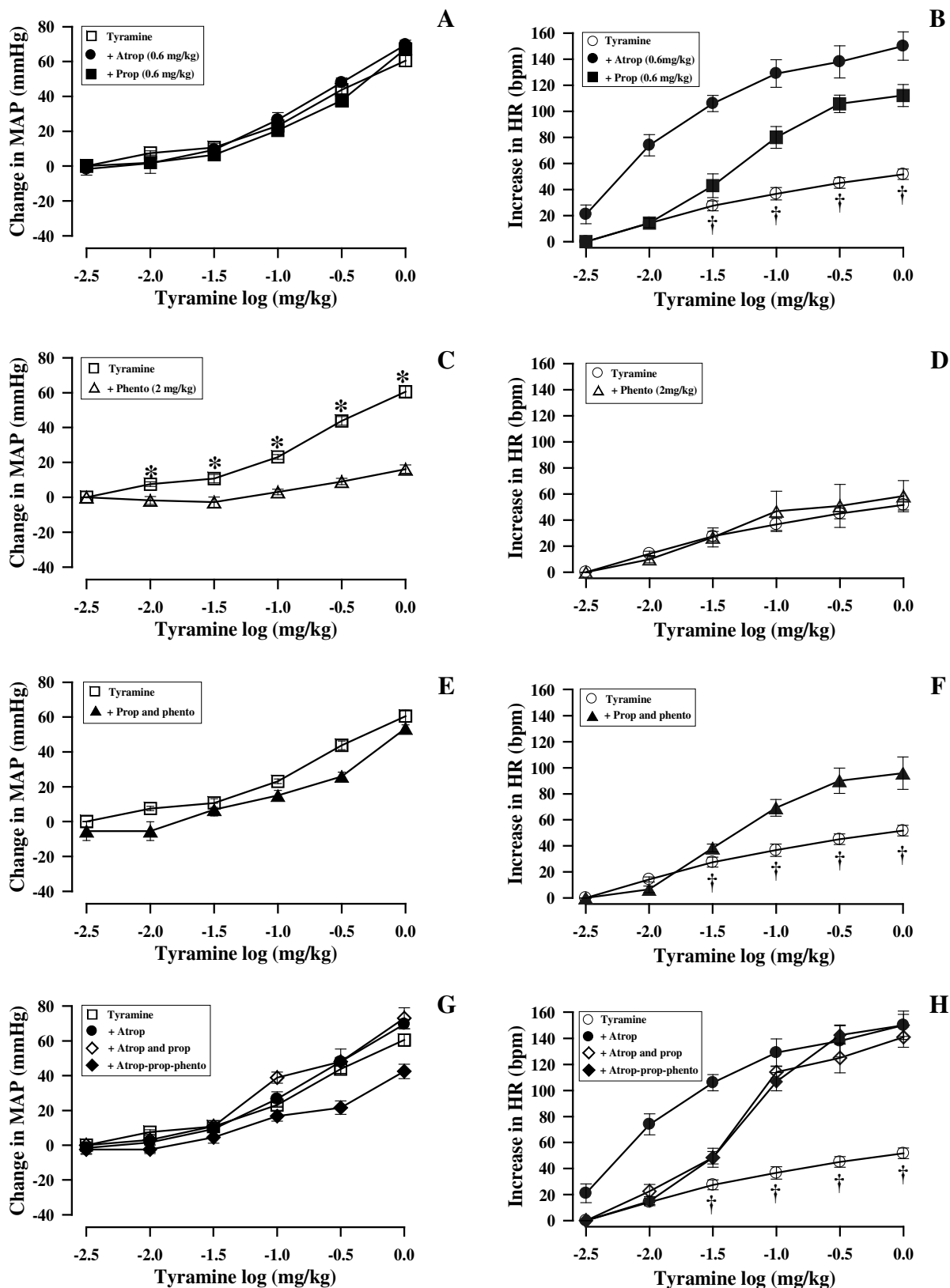


Figure 38 Effects of atropine (atrop), propranolol (prop) and/or phentolamine (phento) on changes in the mean arterial blood pressure (MAP, left), and the positive chronotropic effect (increase in heart rate, right) of tyramine in anesthetized rats. Each point represents mean \pm S.E.M. of 6 experiments *Significantly higher

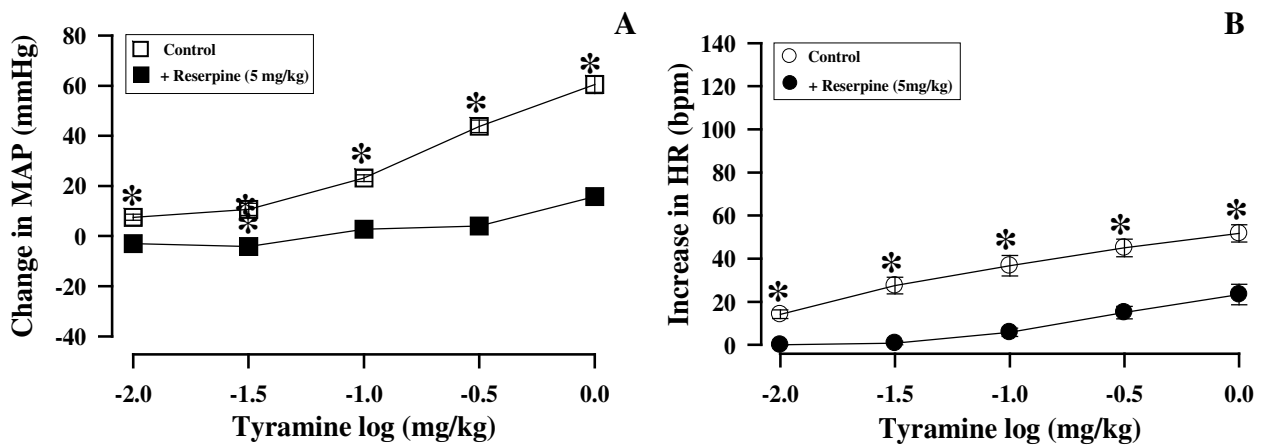


Figure 39 Effects of the depletion of sympathetic neurotransmitters by reserpine on the increase in mean arterial blood pressure (MAP, A) and the positive chronotropic effect (increase in HR, B) of tyramine in anesthetized rats. Each point represents mean \pm S.E.M. of 6 experiments. *Significantly higher than those in reserpinized rats, $p < 0.05$.

4.5.2 *In vitro* effects of propranolol on the positive chronotropic and positive inotropic effects of tyramine

The basal rate of spontaneous contraction of the right atrium was 276.67 \square \square 6.67 bpm, and the basal strength of the electrical field-stimulated contraction of the left atrium was 0.41 \square \square 0.07 g. Tyramine (10^{-8} - 10^{-4} M) caused a concentration-dependent increase in both these parameters. In both cases the tyramine dose-response curves made a parallel shift to the right in the presence of 10^{-8} M propranolol. At a concentration of 10^{-7} M propranolol, the dose-dependent responses were further suppressed and with a decrease in the maximal response (Figure 40).

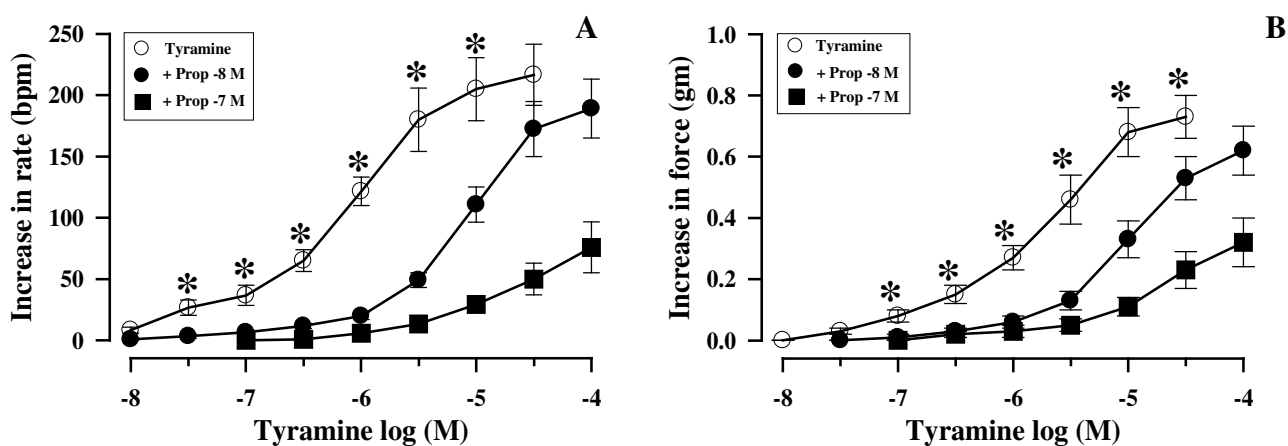


Figure 40 Effects of propranolol (prop) on the positive chronotropic effects of spontaneous contraction of the right atrium (A) or on the positive inotropic effects of the electrical field-stimulated contraction of the left atrium (B) induced by

tyramine. Each point represents mean \pm S.E.M. of 6 experiments. *Significantly higher than those in the presence of propranolol, $p < 0.05$.

4.5.3 Effects of reserpine on the positive chronotropic and positive inotropic effects of tyramine with isolated right and left atria of reserpinized rats

In reserpine pretreated rats, the basal rate of spontaneous contraction of the right atrium was 264 ± 16 bpm and the basal strength of the electrical field-stimulated contraction of the left atrium was 0.68 ± 0.02 g. This was higher than that of normal rats (0.41 ± 0.07 g).

Figure 41 shows the effects of reserpine pretreatment on the rate of spontaneous contraction of the right atrium and the strength of electrical field-stimulated contraction of the left atrium induced by tyramine. Tyramine (10^{-8} - 3×10^{-5} M) caused a concentration-dependent increase in the rate of spontaneous contraction of the right atrium and the strength of the electrical field-stimulated contraction of the left atrium. Both of these effects were completely suppressed by pretreatment the rats with reserpine. In addition, the basal strength of the electrical field-stimulated contraction of the left atrium was also suppressed by tyramine after treatment with reserpine.

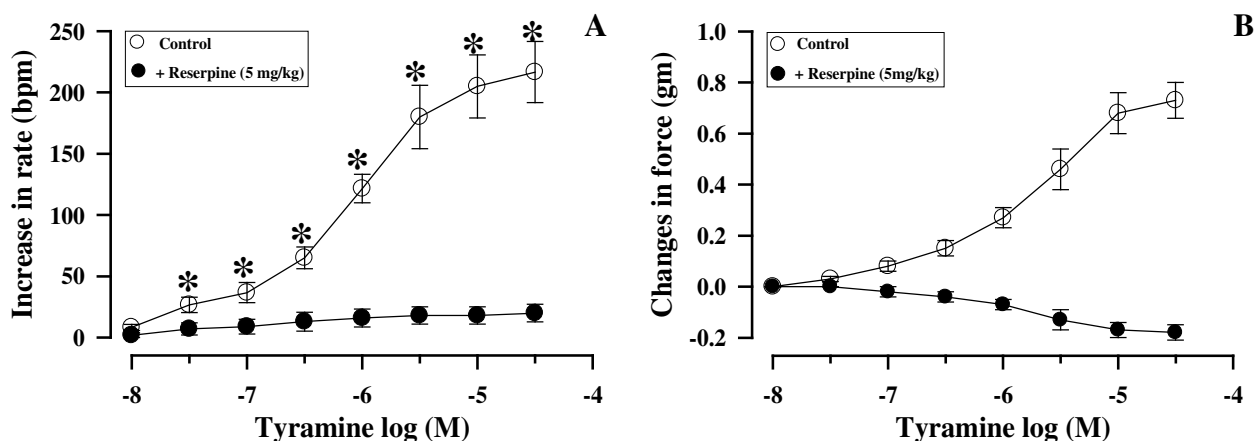


Figure 41 Effects of the depletion of the sympathetic neurotransmitters by reserpine on the positive chronotropic effect of spontaneous contraction of the right atrium (A) or on the positive inotropic effect of the electrical field-stimulated contraction of the left atrium (B) induced by tyramine. Each point represents mean \pm S.E.M. of 6 experiments. *Significantly higher than those of reserpinized tissues, $p \leq 0.05$.

4.5.4 Effects of the tyramine on thoracic aortae *in vitro*

As shown in Figure 42, tyramine caused only a very small increase of vasoconstriction of the endothelium-intact thoracic aortic rings. When the vascular endothelium was removed, or nitric oxide synthase was blocked by LNA, the vasoconstriction responses increased significantly (Figure 42A). However, removal of the endothelium caused a bigger response than did treatment with LNA. 10^{-7} M phentolamine abolished the effect of removal of the endothelium and significantly inhibited the effect of LNA (Figure 42B and C), as did reserpine pretreatment (Figure 42D).

The vasorelaxing activities of tyramine on the thoracic aortic rings precontracted with phenylephrine (3×10^{-6} M) are shown in Figure 43. Tyramine at concentrations $> 3 \times 10^{-5}$ M caused a relaxation of the endothelium-intact thoracic aortic that was virtually unaffected by removal of endothelium, pretreatment with reserpine or the presence of LNA. However, the removal of endothelium could significantly attenuate the relaxant effect of tyramine only at the highest concentration.

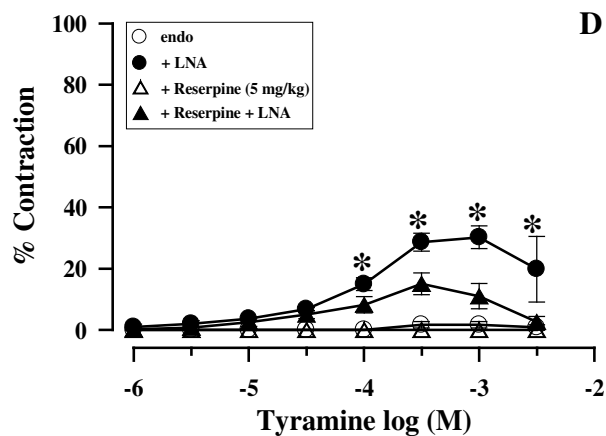
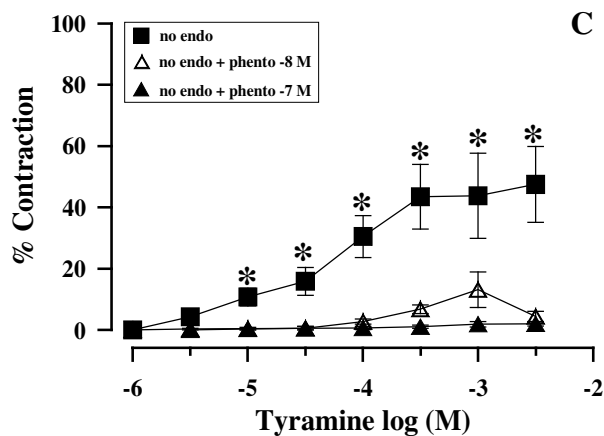
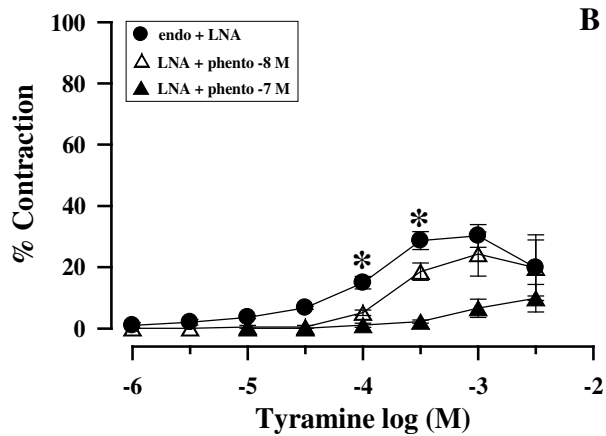
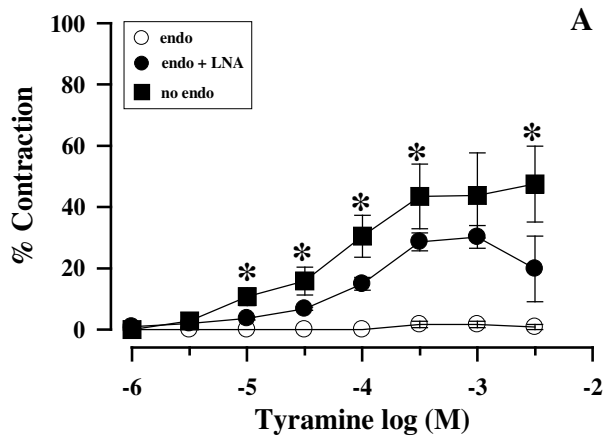


Figure 42 Effects of N^G-nitro-L-arginine (LNA, 3×10^{-4} M) or removal of vascular endothelium (A), phentolamine (B and C), or LNA and/or reserpine induced depletion of the sympathetic neurotransmitters (D) on the contractile response of the thoracic aortic rings to tyramine. Each point represents mean \pm S.E.M. of 6 experiments. *Significantly higher than those other groups, $p \leq 0.05$. 'endo' = intact endothelium

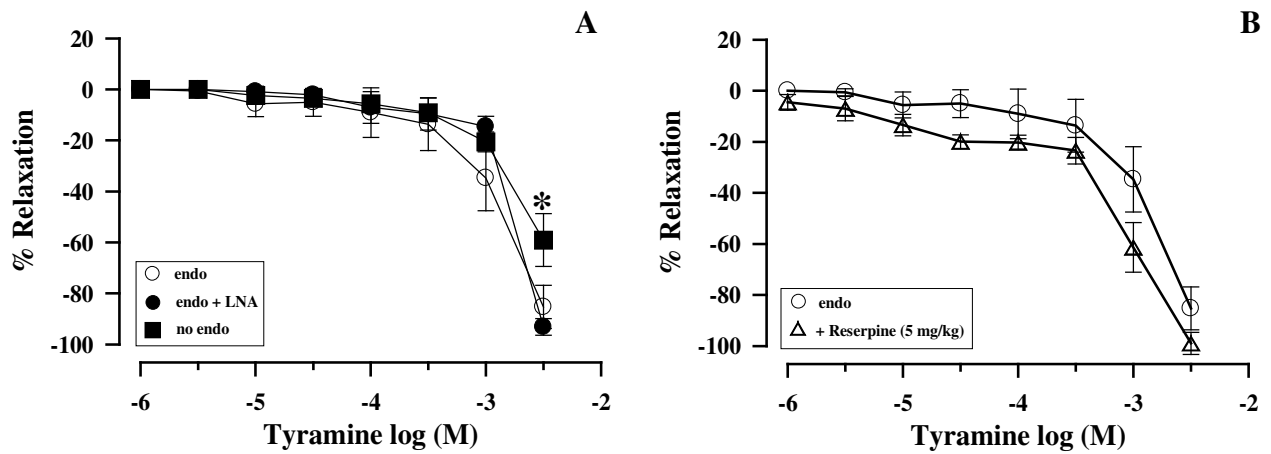


Figure 43 Effects of 3×10^{-4} M LNA and removal of vascular endothelium (A) or the depletion of the sympathetic neurotransmitters by reserpine (B) on the dilatory response (precontracted with 3×10^{-6} M phenylephrine) of the thoracic aortic rings to tyramine. Each point represents mean \pm S.E.M. of 6 experiments. *Significantly higher than those other groups, $p < 0.05$. 'endo' = intact endothelium

2.6 Discussion

The present study demonstrates that tyramine exerts both hypertensive and positive chronotropic effects in anesthetized rats. These effects are similar to those produced by *R. siamensis* extract or epinephrine, and this confirms that tyramine is the substance responsible for the hypertensive and positive chronotropic effects of the *R. siamensis* extract. Further investigations explored whether the mechanisms responsible for the hypertensive and positive chronotropic effects of the tyramine, added as a single substance, were similar to those of the *R. siamensis* extract, which is a mixture of at least 11 compounds (chapter 3).

As shown in Figure 38, the hypertensive activity of the tyramine was not modified by propranolol, but it was inhibited by phentolamine. This indicates that the hypertensive activity of the tyramine is exerted via the α -adrenergic receptors of the cardiovascular system. On the other hand, phentolamine had no effects on the positive chronotropic activity of the tyramine whether the animals were treated by phentolamine alone or in combination with propranolol and/or atropine. These results indicate that the positive chronotropic activity of the tyramine is unlikely to be mediated by the α -adrenergic receptors of the cardiovascular system.

Propranolol did not modify the hypertensive effect of the tyramine, whether the animals were treated with propranolol alone, or in combination with phentolamine, or even when atropine was also added. These results indicate that the β -adrenergic receptors or muscarinic receptors of the cardiovascular system may not play a role in the hypertensive effect of the tyramine.

Poller et al. (1997) reported that tyramine caused an increase in blood pressure in humans with only a slight increase in heart rate. They also found that pretreatment of the subjects with atropine did unmask the positive chronotropic effect of the tyramine. This led them to suggest that the positive chronotropic effect of the tyramine was buffered by a central reflex control caused by the increase in blood pressure. In the present study we also found that the magnitude of the positive chronotropic responses of the tyramine was not as big as that of its hypertensive effect. The tyramine induced increase in blood pressure possibly acting as a reflex buffer. In order to reveal this possibility, the rats were injected with atropine before studying the

dose-response relationship to tyramine. As shown in Figure 38B, atropine did potentiate the dose-response curve to tyramine in a dose-dependent manner. These results indicate that the positive chronotropic response to tyramine might be partially buffered by the central reflex of the hypertension.

It has long been known that tyramine behaves as a “false neurotransmitter”, by stimulating release of endogenous catecholamine at the sympathetic nerve terminals and adrenal medulla. Thus, the positive chronotropic effect of the tyramine would be due to an indirect action on the endogenous catecholamines (Schonfeld and Trendelenburg, 1989; Mundorf et al., 1999). In order to confirm that the positive chronotropic activity of the tyramine observed in the present study involved the β -adrenergic receptors by the catecholamines, a further study was carried out by treating the rats with propranolol before studying the dose-response relationship to tyramine. As shown in Figure 38B, propranolol did potentiate, instead of inhibit, the positive chronotropic effect of the tyramine. This result indicates that the positive chronotropic activity of the tyramine is not simply an indirect action following release of the catecholamine. The other possibilities would be (1) tyramine also has a direct action on the heart, (2) it may stimulate release of some other substances besides the catecholamines, and (3) it may have a direct or indirect action via the central pathway which regulates the pacemaker activity.

Because we found that atropine potentiates the positive chronotropic activity of the tyramine, if this effect was solely caused by the hypertensive reflex buffer, it should be inhibited by propranolol. Therefore, giving propranolol at the same time as atropine before studying the dose-response relationship to the tyramine, would be expected to suppress the positive chronotropic potentiation produced by atropine. As shown in Figure 38H the sensitivity of the positive chronotropic response was not changed, so this compares to that produced by propranolol alone, whereas the maximum positive chronotropic effect of the tyramine was shifted by the same magnitude as that produced by atropine alone. These results confirm that the positive chronotropic effect of the tyramine is partly buffered by the hypertensive central reflex. The finding that propranolol could not suppress the maximum positive chronotropic activity of tyramine in the presence of atropine also indicates that tyramine may have some other effect besides stimulating release of endogenous catecholamines. The finding that there was no further modification of the positive chronotropic activity of the tyramine was found when phentolamine

was also given to the animals at the same time as propranolol and atropine, indicated that the positive chronotropic effect of the tyramine does not involve the α -adrenergic receptors of the cardiovascular system. However, the hypertensive and the positive chronotropic effects of the tyramine disappeared in reserpinized rats. This confirms that the hypertensive and the positive chronotropic effects of the tyramine act indirectly by stimulating release of endogenous catecholamine from the nerve terminals and the adrenal medulla (Mahon and Mashford, 1963).

As the positive chronotropic activity of tyramine is potentiated by atropine and/or propranolol, tyramine may stimulate release of another positive chronotropic substance besides the catecholamines, and/or may have a direct or indirect effect on the central pathway that regulates the pacemaker activity. To examine these possibilities, experiments were carried out with isolated preparations of the right and the left atria of both normal and reserpinized rats. We would expect that if tyramine stimulated release of another substance besides the catecholamine, its positive chronotropic effect should not be completely suppressed by propranolol. Besides this, if tyramine also produced central effects, then the positive chronotropic potentiation produced by propranolol would also disappear. As shown in Figure 40, tyramine caused an increase in both the rate of spontaneous contraction of the right atrium and the strength of the electrical field-stimulated contraction of the left atrium in a dose-dependent manner. These results are similar to those reported by Hayes et al. (1984) and Rubino et al. (1997). Both these effects are significantly inhibited by propranolol or by pretreatment of the animals with reserpine, and no potentiating effect was observed (Figure 40 and 41). These results indicate that the positive chronotropic and positive inotropic activities induced by tyramine are due to stimulation of the release of the catecholamine acting via the β -adrenergic receptors at the atria. The finding that there is no potentiation or any additive effects of the tyramine on the positive chronotropic activity in the presence of propranolol, indicates that it is unlikely that tyramine stimulated the release of another positive chronotropic effect from the nerve terminal at the atria. In addition, the positive chronotropic potentiation by the propranolol on tyramine responsiveness is unlikely to occur at a peripheral site. In the present study, tyramine also caused a decrease in the basal strength of the electrical field-stimulated contraction of the left atrium isolated from reserpinized rats. This indicates that tyramine itself may have a direct action on the left atrium to counter the voltage

induced contraction of the left atrium. However, further studies would be needed to clarify this possibility.

Tyramine produced very little vasoconstriction of endothelium-intact thoracic aortic rings, but this effect was markedly potentiated by removal of the vascular endothelium or by blocking the nitric oxide synthase with LNA (Figure 42). This indicates that tyramine also stimulated release of nitric oxide from the vascular endothelium to attenuate the vasoconstrictory response to tyramine. The finding that phentolamine significantly suppressed the vasoconstrictory responses of the tyramine on the thoracic aortae in the presence of LNA, or on endothelium-denuded thoracic aortae, or by pretreatment of the animals with reserpine, indicated that tyramine stimulated release of neuronal catecholamines (Jacob et al, 2003) that acted at the α -adrenergic receptors of the blood vessels and caused vasoconstriction.

In addition, tyramine at the highest concentration (3×10^{-6} M) that the concentration greater than those causing a maximal contraction of the aortic ring induced a vasodilatation (Figure 42). To confirm this possibility, the vasodilatory responses to tyramine were studied. Tyramine produced a vasodilator response on the thoracic aortic rings precontracted with 3×10^{-6} M phenylephrine. This effect was not inhibited by the nitric oxide synthase inhibitor, LNA, or removal of the vascular endothelium, but potentiated by the reserpine treatment (Figure 43). These results indicated that the vasodilator activity of tyramine was mediated via the way different from that of the vasoconstriction. This similar result of tyramine has been reported by Varma and Chemtob (1993). They found that at concentrations higher than those producing a maximal vasoconstriction, tyramine caused the relaxation of rat aortic strips precontracted with tyramine, norepinephrine, phenylephrine, 5-hydroxytryptamine, prostaglandin $F_{2\alpha}$, endothelin, angiotensin II, and potassium that these effects are endothelium- and α_2 -adrenergic receptor independent. Moreover, Varma and Chemtob (1993) also suggest that tyramine may act via specific tyramine receptors or nonselective.

4.7 Conclusion

Tyramine has both hypertensive and positive chronotropic effects on the cardiovascular system, by stimulating the release of endogenous catecholamines, most likely from

nerve endings and the adrenal medulla to act directly at the α - and β -adrenergic receptors of the heart and blood vessels. Tyramine also exhibited the endothelium-independent vasodilatation. These findings confirm that tyramine is the substance responsible for the hypertensive and positive chronotropic effects of the *R. siamensis* extract, and may be the vasodilator substance.

Tyramine may also have an action on the central pathway that regulates the pacemaker activity of the heart. It also has a negative inotropic effect on the electrical field-stimulated contraction of the left atrium after pretreatment the animals with reserpine. However, the mechanisms responsible for these effects would need further studies.

The effect on the central pathway was not found when the tyramine was present in a mixture with other compounds of the *R. siamensis* extract. The reason for this may be that this effect was attenuated by the negative chronotropic effect produced by the pseudoginsenoside-RT₁. However, again further study would be needed to clarify these possibilities.