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Differences in α_1-Adrenoceptor Subtype-Mediated Vasoconstriction by Tyramine and Nerve Stimulation in Canine Splenic Artery

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Abstract. This study was designed to clarify the α_1-adrenoceptor subtypes mediating the vasoconstrictor response to tyramine in isolated and perfused canine splenic artery. It was shown that tyramine potentiated the nerve stimulation-induced second peaked vasoconstriction that was readily suppressed by prazosin treatment. A bolus injection of tyramine (0.01 – 0.3 μmol) caused a vasoconstriction in a dose-related manner. The tyramine-induced vasoconstriction was inhibited by WB 4101 (10 and 100 nM), an α_{1A}- and α_{1D}-adrenoceptor antagonist, in a concentration-related manner. Neither BMY 7378 (100 nM), a selective α_{1D}-adrenoceptor antagonist, nor chloroethylclonidine (60 μM), an α_{1B}- and α_{1D}-adrenoceptor antagonist, affected the tyramine-induced response. The results indicate that the noradrenaline released by tyramine may diffuse to the extrajunctional cleft, and thus it activates the extrajunctional α_{1A}-adrenoceptors, because nerve stimulation-evoked second peaked vasoconstrictions were markedly inhibited by chloroethylclonidine but not by WB 4101.

Keywords: α_{1A}-adrenoceptor, tyramine, splenic artery, canine, vascular neuroeffector transmission

Introduction

It is well accepted that tyramine activates the postjunctional α-adrenoceptors in sympathetically innervated tissues by releasing intraneuronally stored noradrenaline into the junctional cleft (1 – 4). The subtype of α-adrenoceptors involved in the tyramine-induced response was generally recognized to be an α_1-adrenoceptor subtype in many blood vessels (3, 5). Several lines of evidence have suggested that there is heterogeneity of α_1-adrenoceptor subtypes located in the neuroeffector junction and extrajunctional region (6 – 9). The α_{1B}-adrenoceptor subtype is demonstrated to be critical in sympathetically mediated vasoconstriction and involved in the control of blood pressure (10, 11). Studies on the canine splenic artery have indicated that the postjunctional α_{1B}- and in part α_{1D}-adrenoceptor subtypes received a sympathetic adrenergic innervation, whereas the extrajunctionally located α_{1A}-adrenoceptor was activated by exogenously administered noradrenaline (8, 9). In the canine splenic artery, α_1-adrenoceptors have been identified to be a functional subtype in mediating vasoconstriction in response to tyramine (12). The purpose of this study was to clarify whether or not the specific subtypes of α_1-adrenoceptors mediate the effects of tyramine in the canine splenic artery, using BMY 7378, a selective α_{1D}-adrenoceptor antagonist (13, 14); WB 4101, an α_{1A}- and α_{1D}-adrenoceptor antagonist (15, 16); and chloroethylclonidine, an α_{1B}- and α_{1D}-adrenoceptor antagonist (14, 17).

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Materials and Methods

Arterial preparations

Mongrel dogs of either sex, weighing 10 – 15 kg, were anaesthetized with sodium pentobarbital (30 mg /kg, i.v.). After treatment with sodium heparin (200 units/kg, i.v.), the dogs were killed by rapid exsanguination from the right femoral artery. The protocol of the experiments was approved by the Committee of Animal Use and Welfare of Shinshu University School of Medicine. The arterial main branches of the splenic artery were isolated and side branches of the artery were tied with silk threads. Then, the artery (1 – 1.2 mm in outer diameter) was cut into segments (15 – 20 mm in length). Four segments were obtained from each splenic

artery. Each segment was cannulated and set up for perfusion as described previously (18, 19). Briefly, a stainless steel cannula was inserted into the arterial segment from the distal to the proximal end. A proximal portion of the segment was fixed to the distal portion of a needle-type cannula with silk threads. The cannula was 3 – 4-cm-long and 0.8 – 1.0 mm in outer diameter with small side holes 5 mm from the distal sealed end. The cannulated arterial segment was placed in a cup-shaped glass bath and was perfused by a roller pump (Tokyo Rikakikai, Tokyo) with Krebs-Henseleit solution gassed with 95% O_2 and 5% CO_2. The solution contained 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, and 10 mM glucose. The flow rate was kept at approximately 2 ml/min. The perfusion pressure was continuously measured with an electric manometer (MPU-0.5A; Nihon Kohden, Tokyo) and recorded with a rectigraph (WT-685G, Nihon Kohden). The preparation was perfused at a constant flow rate during the experiment. The basal perfusion pressure was within 40 – 80 mmHg.

After a stabilization period of 1 h, the preparation was removed from the bath solution and fixed in a horizontal position. Then, two platinum electrodes were placed on the extraluminal side of the arterial wall. Periarterial electrical nerve stimulation (PNS) was delivered by an electric stimulator (SEN-7203, Nihon Kohden) using 30-s trains of pulses at 10-V amplitude, 1-ms pulse duration, over a frequency range of 1 and 4 Hz, as previously reported (18). The organ bath was sealed with plastic film to maintain the preparation at 37°C and the suitable humidity. Ten-minute intervals between electrical stimulation periods were needed to obtain reproducible response. The intervals between frequency-response curves were over 1 h. The preparations were incubated for 1 h with tyramine or other antagonists before the next frequency-response curves were made for nerve stimulation.

In other experiments, tyramine (0.01 – 1 μmol) was bolusly administered into the rubber tubing close to the cannula in a volume of 0.01 – 0.03 ml using micro-injections at intervals of 30 min (Terumo, Tokyo) for establishing its dose-response curves. The preparations were incubated for 1 h with WB 4101 or BMY 7378 before the next dose-response curves for tyramine were made.

The pretreatment of preparations with chloroethyl-clonidine was performed by the perfusion with drug-containing physiological solution for 30 min followed by a perfusion with drug-free solution for 30 min before the next frequency-response curves or the dose-response curves to tyramine were obtained.

Drugs

Drugs used were BMY 7378 (8-[2-[4-(2-methoxy-phenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride), chloroethylclonidine dihydro-chloride, WB 4101 (2-(2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4-benzodioxane hydrochloride) (Research Biochemicals, Inc., Natick, MA, USA); α,β-methylene adenosine 5'-triphosphate lithium salt, prazosin hydro-chloride (Sigma, St. Louis, MO, USA); tyramine hydro-chloride (Wako, Osaka). All drugs used were dissolved in distilled water. The stock solutions were kept at −20°C until used.

Statistical analyses

Vasoconstrictor responses to PNS or the agonist are expressed as the maximal changes in perfusion pressure (mmHg) from their basal levels. The data are shown as the mean ± S.E.M. An analysis of variance with Bonferroni's test was used for the statistical analysis of multiple comparisons of data. P values less than 0.05 were considered statistically significant.

Results

Effects of tyramine on the vasoconstrictor responses to PNS and blocking effects of prazosin

PNS at frequencies of 1 and 4 Hz induced a double peaked (two phases) vasoconstriction consisting of an initial transient constriction followed by a prolonged contractile response in the isolated, perfused canine splenic artery (Fig. 1A), as reported previously (20). It has been reported that the first peaked response is due to purinergic P2X receptor activation and the second one is mainly due to α_1-adrenoceptor activation, because the first one was inhibited by α,β-methylene ATP, a purinergic P2X receptor desensitizer, and the second one was mostly depressed by prazosin (20, 21).

The perfusion with tyramine (10 μM) alone induced a slight increase in perfusion pressure (15 – 80 mmHg, n = 6). During a continuous infusion of tyramine, the second peaked constriction was markedly potentiated, but the first one was unaffected significantly. Both the potentiating effect by tyramine and the prolonged contractile response to PNS were antagonized by prior administration of prazosin (0.1 μM) (Figs. 1 and 2). Figure 1 shows an original tracing of double peaked responses from typical experiments, showing the effects of tyramine and blocking effects of prazosin. The summarized data of the potentiating effects of tyramine and blocking effects of prazosin are shown in Fig. 2. The potentiating effects of tyramine were not significantly influenced by α,β-methylene ATP (1 μM) in the concentration that readily inhibited the first peaked vaso-

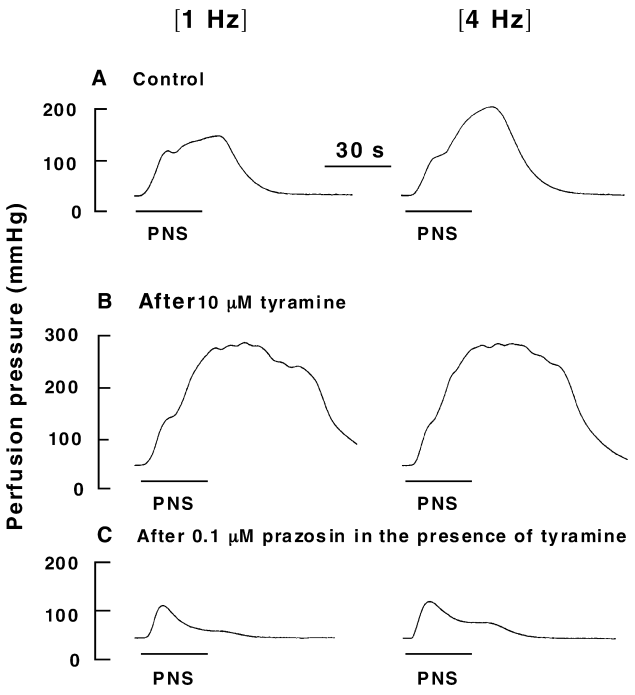

Fig. 1. Double peaked vasoconstrictor responses to periarterial electrical nerve stimulation (PNS) (A) and their change by tyramine (B) and prazosin (C) in an isolated, perfused canine splenic artery. The double peaked vasoconstrictions were induced by 30-s trains of pulses at 10-V amplitude and 1-ms pulse duration, with a frequency of 1 (left panel) or 4 Hz (right panel).

constriction (n = 4, data not shown).

Effects of α_1-adrenoceptor subtype antagonists on the vasoconstrictor responses to tyramine and PNS

When tyramine (0.01 – 0.3 μmol) was bolusly injected, marked vasoconstrictions were induced in a dose-related manner. Tyramine did not show tachyphylaxis when administered at intervals of 30 min. The dose-response curves for tyramine were inhibited by WB 4101 (10 and 100 nM) in a dose-dependent manner (Fig. 3A). Chloroethylchlonidine (60 μM) only produced a slight, but insignificant inhibition of the tyramine-induced vasoconstrictions (Fig. 4A). The vasoconstrictor responses to tyramine were not affected by an α_{1D}-adrenoceptor antagonist, BMY 7378 (100 nM) (Fig. 5A).

As demonstrated previously (20), the second peaked responses were mostly mediated by α_1-adrenoceptors. Hence, we attempted to analyze and compare the effects of α_1-adrenoceptor subtype antagonists on the second peaked constrictions. As reported previously (8), the nerve-stimulated second peaked responses were not inhibited, but rather frequently potentiated by WB 4101 (Fig. 3B). On the other hand, the second peaked vasoconstrictions were markedly inhibited by chloroethylclonidine (Fig. 4B) and slightly but significantly depressed by BMY 7378 (Fig. 5B).

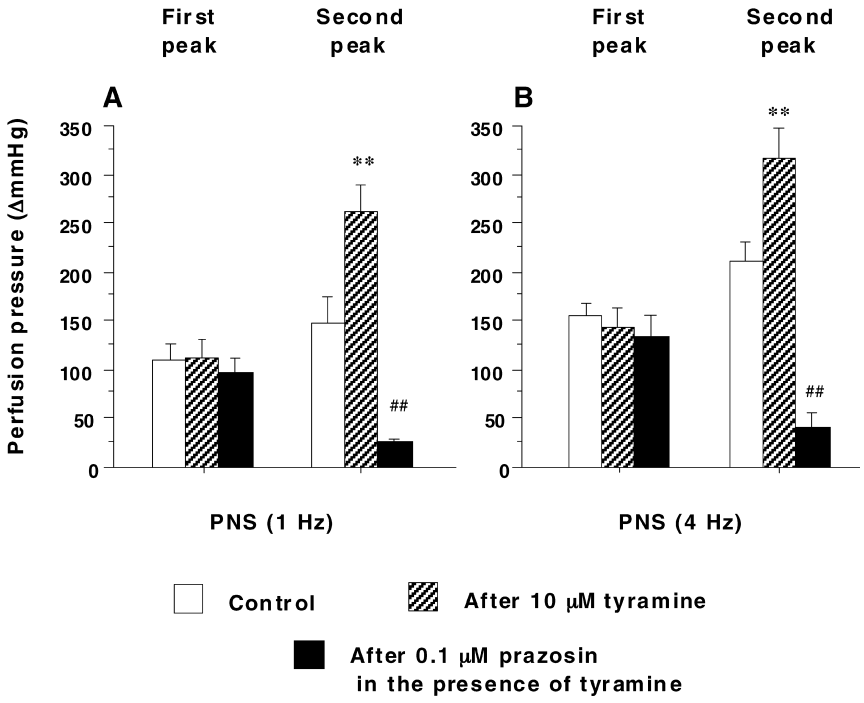

Fig. 2. Effects of tyramine and prazosin on vasoconstrictor responses to periarterial electrical nerve stimulation (PNS) at 1 Hz (A) and 4 Hz (B) in the canine splenic arteries. The vessels were electrically stimulated by 30-s trains of pulses at 10-V amplitude and 1-ms pulse duration, with a frequency of 1 and 4 Hz. Data are presented as the mean ± S.E.M (n = 6). $^{**}P<0.01$ *vs* the control group. $^{\#\#}P<0.01$ *vs* the preceding group.

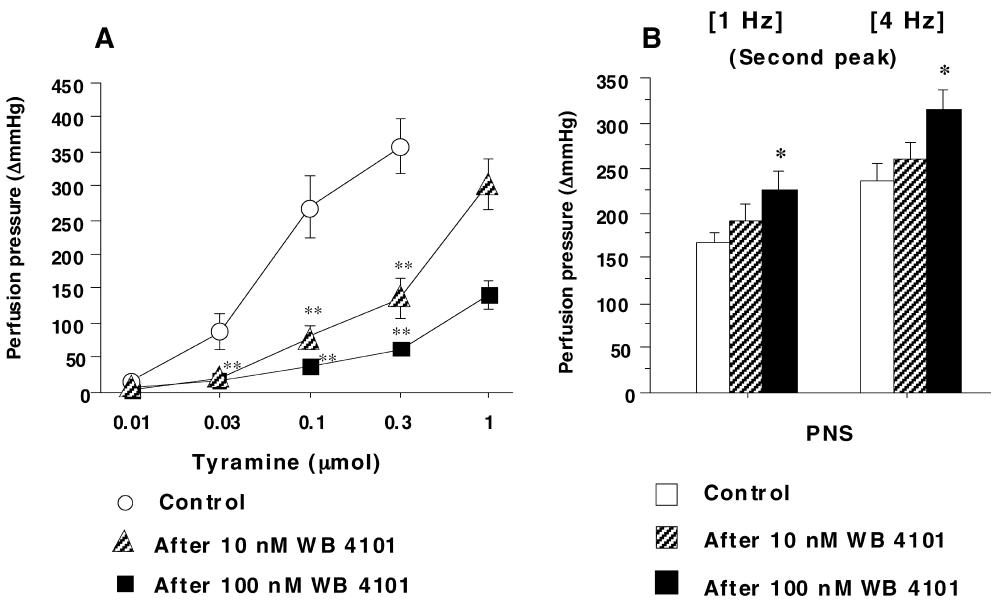

Fig. 3. Effects of WB 4101 on tyramine-induced vasoconstrictions (A, n = 12) and PNS-evoked second peaked responses (B, n = 6) in canine splenic arteries. The vessels were electrically stimulated by 30-s trains of pulses at 10-V amplitude and 1-ms pulse duration, with a frequency of 1 and 4 Hz. Data are presented as the mean ± S.E.M. **P*<0.05, ***P*<0.01 *vs* the control group.

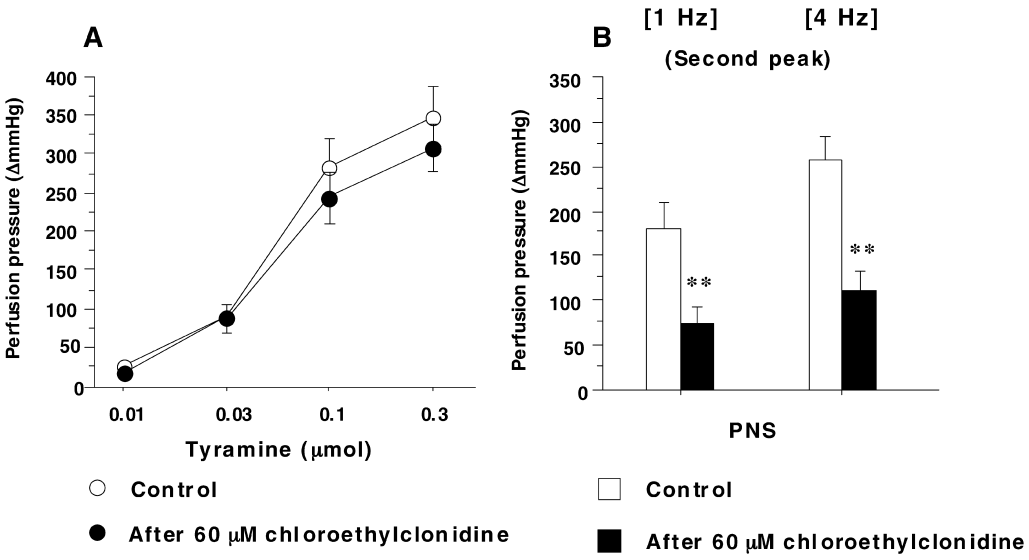

Fig. 4. Effects of chloroethylclonidine on tyramine-induced vasoconstrictions (A, n = 8) and PNS-evoked second peaked responses (B, n = 6) in canine splenic arteries. The vessels were electrically stimulated by 30-s trains of pulses at 10-V amplitude and 1-ms pulse duration, with a frequency of 1 and 4 Hz. Data are presented as the mean ± S.E.M. ***P*<0.01 *vs* the control group.

Discussion

It has been recognized that indirect sympathomimetic amines, such as tyramine, produce their postjunctional effects in sympathetically innervated tissues by releasing intraneuronally stored noradrenaline into the junctional cleft (1). Previously, we observed that tyramine evoked a marked vasoconstriction of the canine splenic artery, but it was unable to induce a constrictive response in reserpinized preparations (22). The prolonged cold storage of the canine splenic artery, which causes irreversible degeneration of adrenergic nerve fibers and thus abolishes adrenergic transmission, markedly attenuated the tyramine-induced response (12, 23). In addition, we observed that the vasoconstriction of the canine splenic artery by tyramine was strongly reduced

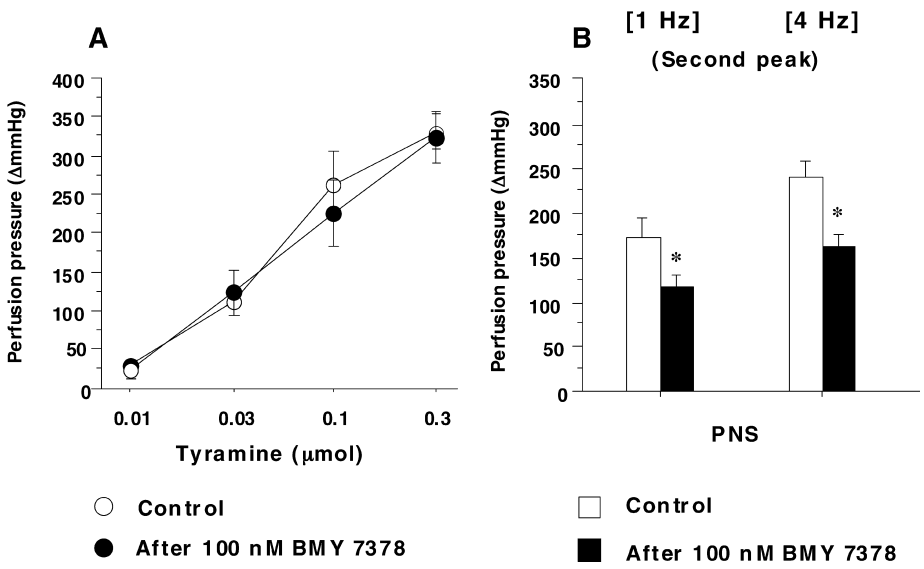

Fig. 5. Effects of BMY 7378 on tyramine-induced vasoconstrictions (A, n = 6) and PNS-evoked second peaked responses (B, n = 6) in canine splenic arteries. The vessels were electrically stimulated by 30-s trains of pulses at 10-V amplitude and 1-ms pulse duration, with a frequency of 1 and 4 Hz. Data are presented as the mean ± S.E.M. **P*<0.05 *vs* the control group.

by imipramine, a catecholamine uptake inhibitor (24). These observations confirm that tyramine contracts the canine splenic artery via its indirect sympathomimetic action.

ATP has been considered as a co-transmitter with noradrenaline in peripheral sympathetic nerves (25). The ability of tyramine to release noradrenaline from sympathetic nerve terminals is well documented. However, tyramine has no capacity to release the neuronal ATP from postganglionic sympathetic nerve terminals (26).

We recently demonstrated that the neurogenic vasoconstriction of the canine splenic artery appeared to be a double peaked response. The first phase may contain mostly a P2X receptor-mediated purinergic component and the second phase, mainly an α_1-adrenoceptor-mediated adrenergic component (20). Tyramine is capable of facilitating outflow of noradrenaline during nerve excitation, in addition to an increase in the basal outflow of noradrenaline (3). In the present study, we were able to confirm that tyramine facilitated the second phase of double peaked vasoconstrictions, but having little effect on the first one. The facilitating effect of tyramine was suppressed by prazosin, an α_1-adrenoceptor antagonist, but not by α,β-methylene ATP, a P2X receptor desensitizing agent. These results indicate that tyramine is mainly able to release the adrenergic transmitter noradrenaline from nerve terminals, and the released noradrenaline exerts its neuroeffector response via an activation of α_1-adrenoceptors.

In the present functional experiments, the dose-response curves for tyramine were shifted to the right by the α_{1A}- and α_{1D}-adrenoceptor-selective antagonist WB 4101 in a concentration-dependent manner, whereas they were nearly unaffected by either chloroethylclonidine, which inactivates both α_{1B}- and α_{1D}-adrenoceptors, or by the α_{1D}-adrenoceptor-selective antagonist BMY 7378. Thus, the inhibitory effect on the tyramine-induced response by WB 4101 possibly indicates that the subtype of α_1-adrenoceptors involved in tyramine-mediated response is solely the α_{1A}-adrenoceptor, and not concomitantly the α_{1D}-adrenoceptor, since the latter should be inactivated by the relatively high concentration of chloroethylclonidine used (60 μM). This is also corroborated by the fact that the tyramine-induced response was not modified at all by blockade of the α_{1D}-subtype with BMY 7378. Thus the subtype of α_1-adrenoceptors contributing to the neuroeffector response of tyramine is presumed to be mostly the α_{1A}-subtype.

The sympathetic-innervated subtypes of junctional α_1-adrenoceptors in the canine splenic artery have been shown to be mainly α_{1B}-, and in part, α_{1D}-subtype, rather than the α_{1A}-subtype (8, 9, 21). It was demonstrated that the neurogenic second peaked response was much reduced by chloroethylclonidine and was partially inhibited by BMY 7378, but was not affected by WB 4101 (8, 9), as confirmed even in the present study. However, there is possibility that chloroethylclonidine inhibits the PNS-induced response by activating the α_2-adrenoceptors in sympathetic nerve terminals (27). It has been suggested that a prejunctional α_2-adrenoceptor mechanism may modulate the release of noradrenaline and ATP in the canine splenic artery (28). In the present

study, chloroethylclonidine selectively reduced the PNS-induced second, adrenergic vasoconstriction, but did not affect the first, purinergic response (data not shown, n = 6). Thus, the antagonistic effect of CEC is likely mediated by a postjunctional mechanism.

On the other hand, the vasoconstrictor response to administered noradrenaline was antagonized by WB 4101, whereas they were not inhibited by either chloroethylclonidine or BMY 7378 (8, 9). It is therefore hypothesized that α_{1B}-, and α_{1D}-adrenoceptors may be located in the junctional region, whereas α_{1A}-adrenoceptors may reside in the extrajunctional region of the neuroeffector junction in the canine splenic artery (8, 21, 29). Tyramine is recognized to activate the postjunctional α-adrenoceptors in sympathetically innervated tissues by releasing intraneuronally stored noradrenaline into the junctional cleft (1 – 4). The present results dispute this notion and likely indicate that noradrenaline released by tyramine from nerve terminals of the canine splenic artery may activate the extrajunctional α_{1A}-subtype rather than junctional subtypes of α_1-adrenoceptors, that is, α_{1B}- and/or α_{1D}-receptors.

It has been well known that nerve excitation and tyramine stimulate the release of noradrenaline from sympathetic nerve terminals, but the noradrenaline releasing mechanism is different. Tyramine-induced release of noradrenaline is not dependent upon the neuronal depolarization and the influx of extracellular Ca^{2+}, and thus the exocytosis process is not believed to be involved (30). Tyramine enters nerve terminals presumably via the uptake$_1$ mechanism, initially displacing noradrenaline from the axoplasm and later mobilizing the release of noradrenaline from vesicular stores (2, 4). The distinct releasing mechanism of noradrenaline between nerve excitation and tyramine may be considered to have a differential releasing route for sympathetic nerve transmitters. In addition, it is considered that tyramine causes noradrenaline release from nerve terminals to junctional and extrajunctional areas, but totally less release to the junctional area. Moreover, it is possible that tyramine exerts its action on different noradrenaline stores from excitation-sensitive stores. This may explain the extrajunctional effects of noradrenaline released by tyramine.

From the results of previous studies (8, 9), and the present study, it is postulated that the vasoconstriction of the canine splenic artery by nerve excitation and tyramine may be mediated by different α_1-adrenoceptor subtypes. The noradrenaline released by nerve excitation may activate mainly the α_{1B}-, and in part, α_{1D}-subtype, while that released by tyramine may activate α_{1A}-adrenoceptors via extrajunctional α_1-receptors in the canine splenic artery. The distinct subtypes of α_1-adrenoceptors activated by nerve excitation and tyramine support a new notion that the adrenergic transmitter released by tyramine may extrajunctionally exert its neuroeffector action.

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