RESPONSES OF ISOLATED DOG CORONARY ARTERIES TO TYRAMINE

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Abstract—In isolated dog coronary arteries contracted with prostaglandin $F_{2\alpha}$, tyramine in concentrations of 10^{-6} and 5×10^{-6} M caused relaxations, but it produced contractions at 2×10^{-5} M or higher. The relaxant response to tyramine was attenuated, but the contractile response was enhanced at the second trial as compared with the responses at the first. Relaxations induced by low concentrations of tyramine were reversed to contractions by treatment with propranolol (10^{-6} M) or sotalol (10^{-5} M), and were abolished by cocaine (3×10^{-6} M) or bretylium (2×10^{-5} M). In coronary arteries isolated from reserpine (0.5 mg/kg)-pretreated dogs, tyramine produced only a contraction. Under resting conditions, contractions induced by tyramine (5×10^{-6} to 2×10^{-3} M) were potentiated by cocaine and propranolol, and were inhibited by phentolamine. Norepinephrine produced a dose-dependent relaxation in the arteries contracted with prostaglandin $F_{2\alpha}$. In the presence of propranolol, the arteries under resting conditions were contracted by norepinephrine, the contraction being suppressed by treatment with phentolamine. It may be concluded that relaxations of dog coronary arteries induced by tyramine are mediated by liberation of norepinephrine from adrenergic nerves which stimulates beta-adrenoceptors in the smooth muscle. It seems likely that the tyramine (2×10^{-5} M or higher)-induced contraction is not mediated by norepinephrine released, but it is partly due to a direct action on alpha-adrenoceptors.

Tyramine releases norepinephrine from organs and tissues innervated adrenergically. In a variety of arteries isolated from different regions (e.g., aorta (1), ear artery (2), mesenteric artery (3)), tyramine produces a contraction mediated by the release of norepinephrine from adrenergic nerves, whereas the tyramine-induced contraction of dog cerebral arteries is derived mainly from direct alpha-adrenoceptor stimulation (3). Vasodilatation in response to stimulation of adrenergic nerves or to norepinephrine has

been demonstrated in rabbit facial vein (4) and sheep and dog coronary artery (5, 6). Exogenous norepinephrine preferentially stimulates excitatory alpha-adrenoceptors in large coronary arteries but inhibitory beta-adrenoceptors in small arteries (6-9). Dog coronary arteries are adrenergically innervated (10, 11). However, little is known concerning the effect of endogenous norepinephrine on the coronary arterial smooth muscle.

The present study was thus undertaken to determine actions of tyramine on coronary arteries isolated from dogs and to analyze

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the mechanism of action of tyramine, particularly in relation to the release of norepinephrine. Responses of isolated coronary arteries to norepinephrine were also investigated.

MATERIALS AND METHODS

Mongrel dogs of either sex, weighing 6 to 15 kg, were anesthetized with intraperitoneal injections of 50 mg/kg of sodium thiopental and were sacrificed by bleeding from common carotid arteries. Thirteen dogs were pretreated 20 to 24 hr before the start of experiments with intramuscular injections of 0.5 mg/kg reserpine; norepinephrine contents in mesenteric and cerebral arteries are diminished by this pretreatment (12). The heart was removed. The distal portion of circumflex branches of the left coronary artery was isolated; the outside diameter was in a range of 0.7 to 2.0 mm. Coronary arteries used were of intermediate sizes in comparison with small (0.25 to 0.5 mm) and large (1.5 to 2.4 mm) arteries reported by Zuberbuhler and Bohr (6). These arteries were expected to show both contractile and relaxant responses to endogenous norepinephrine. The arteries were helically cut into strips approximately 20 mm long. The helical strip was fixed vertically between hooks in a muscle bath containing the nutrient solution which was gassed with a mixture of 95% O₂ and 5% CO₂ and was maintained at 37±0.5°C. The hook anchoring the upper end of the strip was connected to the lever of a force-displacement transducer (Nihon Kohden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 1.5 to 2.0 g, which has been reported to be approximately optimal for inducing the maximum contraction (13). Constituents of the solution were as follows (mM): Na⁺ 162.1, K⁺ 5.4, Ca⁺⁺ 2.2, Mg⁺⁺ 1.0, Cl⁻ 159.0, HCO₃⁻ 14.9, and dextrose 5.6. The pH of the solution was 7.2 to 7.3. Osmotic adjustment was not

made when K⁺ up to 30 mM was added. Before the start of experiments, preparations were allowed to equilibrate for 90 to 120 min in the bathing media, during which time the fluids were replaced every 10 to 15 min.

Isometric contractions and relaxations were displayed on an ink-writing oscillograph (Sanei Sokki Co., Tokyo, Japan). Most of the arterial strips were contracted with prostaglandin F_{2α} (10⁻⁷ to 10⁻⁶ M) before tyramine or norepinephrine was added. Tyramine in low concentrations (10⁻⁶ and 5×10⁻⁶ M) relaxed prostaglandin F_{2α}-contracted arteries, but in concentrations of 2×10⁻⁵ M or higher contracted them. At the end of these experiments, papaverine was added in a concentration of 10⁻⁴ M, sufficient to cause the maximum relaxation (14); relaxations or contractions induced by tyramine relative to papaverine-induced relaxations are presented. Contractions induced by tyramine and norepinephrine under resting conditions were compared with those induced by 30 mM K⁺. Preparations had been exposed for 30 min to blocking agents before the dose-response curve for tyramine or norepinephrine was obtained. Results shown in the text and figures are expressed as mean values±S.E.M. Statistical analyses were made using the Student's *t*-test. Drugs used were tyramine hydrochloride, *d*-norepinephrine hydrochloride, cocaine hydrochloride, *d*-propranolol hydrochloride, sotalol hydrochloride, phentolamine mesylate, prostaglandin F_{2α} (Ono Co., Osaka, Japan), bretylium tosylate, aspirin, atropine sulfate, cinanserin hydrochloride, *d*-chlorpheniramine maleate, hexamethonium bromide and reserpine (Apoplone, Daiichi Pharmaceutical Co., Tokyo, Japan).

RESULTS

Responses of coronary arteries to tyramine:

The addition of tyramine in concentrations of 10⁻⁶ and 5×10⁻⁶ M elicited a dose-dependent

relaxation in all of the 33 helical strips of dog coronary arteries contracted with prostaglandin $F_{2\alpha}$ (10^{-7} to 10^{-6} M) (Fig. 1). Similar relaxation was obtained when the arteries were contracted with serotonin (10^{-9} to 2×10^{-8} M) or angiotensin II (5×10^{-10} to 5×10^{-9} M). Further increases in concentrations of tyramine to 2×10^{-5} M or higher

contracted these arteries. In coronary arteries isolated from 13 dogs pretreated with reserpine, tyramine (10^{-6} M or higher) did not produce a relaxation but a dose-dependent contraction (Fig. 1).

The relaxant response to tyramine of the arteries contracted with prostaglandin $F_{2\alpha}$ was significantly attenuated at the second trial as compared with the response at the first (Fig. 2, left), while the contractile response to tyramine (5×10^{-6} to 2×10^{-3} M) was significantly enhanced at the 2nd trial (Fig. 2, right). The dose-response curve of tyramine was reproducible after the 2nd trials; therefore, the 2nd dose-response curve was taken as a control in the subsequent experiments with antagonists.

Relaxations induced by tyramine were suppressed by treatment for 30 min with cocaine (3×10^{-6} M) and were reversed to contractions by propranolol (10^{-6} M) (Fig. 3). The relaxant effect was restored following repeated washing of the preparations. Sotalol (10^{-5} M) also reversed the tyramine-induced relaxation to a contraction; tyramine (5×10^{-6} M) elicited a relaxation of $42.6 \pm 4.9\%$ (relative to that induced by 10^{-4} M papaverine) in control media and a contraction of $48.1 \pm 11.6\%$ ($N=7$) in the presence of sotalol. Furthermore, breylum (2×10^{-5} M) abolished the relaxation ($N=5$). Treatment with 10^{-7} M phentolamine ($N=5$), 10^{-5} M hexamethonium ($N=6$), or 10^{-7} M atropine ($N=3$) did not significantly affect the relaxant response to 10^{-6} and 5×10^{-6} M tyramine. Under resting conditions, the contractile response to 5×10^{-6} M tyramine seen in the arteries treated with 10^{-5} M sotalol was significantly inhibited by 3×10^{-6} M cocaine ($42.0 \pm 5.1\%$ inhibition, $N=4$) and 10^{-7} M phentolamine ($68.0 \pm 7.5\%$ inhibition, $N=3$).

Under resting conditions, the addition of tyramine (2×10^{-5} to 2×10^{-3} M) produced a dose-dependent contraction in coronary

Fig. 1. Dose-response curves of tyramine in coronary arteries isolated from normal and reserpine-pretreated dogs. Preparations were contracted with prostaglandin $F_{2\alpha}$. Relaxations induced by 10^{-4} M papaverine were taken as 100%; mean absolute values in preparations from normal and reserpine-pretreated dogs were 695 ± 68 mg ($N=33$) and 659 ± 135 mg ($N=13$). Corresponding values in the control and reserpine-pretreated preparations are significantly different ($P < 0.001$). Figures in parentheses indicate the number of preparations used. Vertical bars represent the S.E.M.

Fig. 2. Dose-response curves of tyramine in coronary arteries obtained during repeated trials of experiments. Preparations were contracted with prostaglandin $F_{2\alpha}$ in the left figure. Maximum relaxations induced by tyramine at the first trial were taken as 100%; the mean value of the relaxation relative to papaverine (10^{-4} M)-induced relaxation was $47.7 \pm 4.4\%$ ($N=22$). In the right figure, experiments were carried out under resting conditions. Maximum contractions induced by tyramine at the first trial were taken as 100%; the mean value was 707 ± 177 mg ($N=7$). a, significantly different from the first trial, $P < 0.001$; b, $P < 0.01$; c, $P < 0.02$; d, $P < 0.05$.

arterial strips. The mean value of the maximum contractions induced by tyramine relative to those induced by 30 mM K^+ was $46.6 \pm 5.8\%$ ($N=17$). Contractile responses to tyramine were significantly potentiated by treatment with propranolol (10^{-6} M) and cocaine (3×10^{-6} M) (Fig. 4). Contractions induced by tyramine were dose-dependently inhibited by 10^{-7} and 10^{-6} M phentolamine (Fig. 5). In the presence of 10^{-6} M propranolol, the contractions were also inhibited by 10^{-6} M phentolamine; inhibitions of the response to 2×10^{-5} and 2×10^{-3} M tyramine were 82.2 ± 3.6 and $35.6 \pm 5.9\%$ ($N=5$), respectively. Cinanserin (10^{-7} M), aspirin (5×10^{-5} M) and chlorpheniramine (10^{-6} M) did not inhibit the tyramine-induced contraction.

Responses of coronary arteries to norepinephrine: The addition of norepinephrine (5×10^{-9} to 10^{-5} M) caused a dose-dependent relaxation when coronary arterial

strips had been contracted with prostaglandin $F_{2\alpha}$ (10^{-7} to 2×10^{-6} M). The maximum relaxation relative to that induced by papaverine (10^{-4} M) and the median effective concentration (ED50) of norepinephrine were $92.9 \pm 2.6\%$ and $[4.0 \pm 0.6] \times 10^{-8}$ M ($N=9$), respectively. In coronary arteries isolated from reserpine-pretreated dogs, the response to norepinephrine was not significantly potentiated: mean values of the maximum relaxation and the ED50 of norepinephrine were $95.1 \pm 1.4\%$ and $[3.3 \pm 0.8] \times 10^{-8}$ M ($N=8$), respectively. The dose-relaxation curve of norepinephrine was not significantly modified by cocaine (3×10^{-6} M) and phentolamine (10^{-7} M): mean values of the ED50 of norepinephrine were $[6.4 \pm 0.8] \times 10^{-8}$ M ($N=6$) in control and $[7.8 \pm 1.6] \times 10^{-8}$ M in the presence of cocaine, and $[5.8 \pm 0.8] \times 10^{-8}$ M ($N=6$) in control and $[4.4 \pm 0.4] \times 10^{-8}$ M in the presence of phentolamine. Treatment with sotalol (10^{-5}

Fig. 3. Modification by cocaine and propranolol of the relaxant response to tyramine in coronary arteries contracted with prostaglandin F_{2a} . Relaxations induced by 10^{-4} M papaverine were taken as 100%; mean absolute values for left and right figures were 736 ± 106 mg ($N=9$) and 706 ± 114 mg ($N=7$), respectively. a, significantly different from controls, $P < 0.001$; b, $P < 0.01$; c, $P < 0.05$.

M) or propranolol (10^{-6} M) reversed the relaxation induced by low concentrations (5×10^{-9} to 5×10^{-7} M) of norepinephrine to a contraction, and these compounds attenuated the relaxant response to the amine in high concentrations (2×10^{-6} to 5×10^{-5} M).

In the presence of 10^{-6} M propranolol, norepinephrine caused a dose-dependent contraction under a resting condition. The mean value of maximum contractions induced by norepinephrine relative to those induced by 30 mM K^+ was $43.5 \pm 6.4\%$ ($N=13$), which was less than the maximum contraction induced by tyramine ($63.0 \pm 5.2\%$, $N=12$) in

the presence of propranolol. Norepinephrine-induced contraction was attenuated by 10^{-7} M phentolamine and abolished at 10^{-6} M (Fig. 5).

DISCUSSION

In an earlier report (3), it has been demonstrated that tyramine produces a contraction both in mesenteric and cerebral arteries isolated from dogs; the functional role of endogenous norepinephrine liberated from adrenergic nerves by tyramine is much greater in mesenteric arteries than in cerebral arteries. In contrast, tyramine in concentrations of 10^{-6} and 5×10^{-6} M elicited a

relaxation of dog coronary arteries contracted with prostaglandin $F_{2\alpha}$. Coronary arteries isolated from reserpine-pretreated dogs failed to relax in response to tyramine. Tachy-

Fig. 4. Modification of the contractile response to tyramine by propranolol and cocaine. Maximum contractions induced by tyramine in the control solution were taken as 100%; mean absolute values for left and right figures were 822 ± 183 mg ($N=7$) and 867 ± 252 mg ($N=5$), respectively. a, significantly different from controls, $P < 0.001$; b, $P < 0.01$; c, $P < 0.02$; d, $P < 0.05$.

Fig. 5. Modification by phentolamine of contractile responses to tyramine and norpinephrine. In the right figure, all preparations were treated with propranolol (10^{-6} M). Maximum contractions induced by tyramine (left) and norpinephrine (right) in the control solution were taken as 100%; mean absolute values for left and right figures were 581 ± 108 mg ($N=12$) and 1508 ± 257 mg ($N=10$), respectively. a, significantly different from controls, $P < 0.001$; b, $P < 0.01$; c, $P < 0.02$; d, $P < 0.05$.

phylaxis of the relaxation developed by repeated trials of tyramine, and the relaxation was abolished by cocaine, bretylium, sotalol, and propranolol. Norepinephrine in concentrations up to 10^{-5} M produced a marked relaxation of coronary arteries. These findings suggest that norepinephrine liberated from adrenergic nerves innervating the coronary arterial wall stimulates beta-adrenoceptors, resulting in relaxations. In our previous report using metoprolol, a selective beta₁-adrenoceptor antagonist, the beta-adrenoceptors in dog coronary artery are postulated to be beta₁ (15).

In contrast to the present findings, responses of the coronary vascular bed to sympathetic nerve stimulation *in vivo* are indirect vasodilatation associated with increased myocardial metabolism (16) and direct vasoconstriction mediated by the stimulation of vascular alpha-adrenoceptors (17–21). In most of these *in vivo* reports, however, such a vasoconstriction induced by the stimulation was obtained after the treatment with practolol or propranolol in order to eliminate the myocardial factor. In isolated coronary arteries, tyramine in a low concentration of 5×10^{-6} M also produced contractions, possibly *via* the release of norepinephrine in the presence of beta-blockade with sotalol, since the contractions were inhibited by cocaine and phentolamine. However, it is unlikely that the contractile response to tyramine of coronary arteries isolated from reserpine-pretreated dogs is mediated by liberated norepinephrine because of a marked depletion of endogenous norepinephrine (approx. 97%, unpublished data) by the pretreatment with reserpine.

The release of norepinephrine as a mechanism of contractions induced by high concentrations (2×10^{-5} M or higher) of tyramine may be excluded since treatment with reserpine *in vivo* or with cocaine *in vitro* did not reduce the contraction. Potentiation of

the contraction induced by cocaine and propranolol seems to be associated with an inhibition of relaxations. This inhibition by these antagonists seems to be induced by an interference with the neuronal uptake of tyramine and by a beta-adrenoceptor blockade, respectively. Phentolamine attenuated contractions induced by high concentrations (2×10^{-5} M or higher) of tyramine. Norepinephrine-induced contractions which were unmasked by treatment with beta antagonists were also attenuated by phentolamine. These findings may indicate that high concentrations of tyramine contract coronary arterial strips due to a direct stimulatory action on alpha-adrenoceptors. However, non-specific vasoconstrictor actions may also be involved, since coronary arterial contractions induced by high concentrations of tyramine in the presence of beta-blockade were refractory to phentolamine at high concentrations which were sufficient to suppress the response to norepinephrine. Non-specific stimulatory action of tyramine is postulated in isolated intestinal preparations (22). Because treatment with cinanserin, chlorpheniramine, or aspirin failed to alter the tyramine-induced contraction, the involvement of serotonergic, histaminergic H₁, or prostaglandin-related mechanisms may be excluded.

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