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Short communication

Ca^{2+}, HISTAMINE ANTAGONISTS AND RELAXATION TO ELECTRICAL IMPULSES IN DOG CORONARY ARTERY *

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Isolated dog coronary arteries relax in response to electrical stimulation (0.1-8.0 Hz, 9 V, 1.0 ms) following contraction induced by serotonin. Cimetidine, metiamide and ranitidine inhibited this relaxation. The relaxation was not blocked by pyrilamine. Reducing the concentration of Ca^{+} (0.1 mM) decreased the rate of relaxation whereas relaxation was more rapid when the Ca^{2+} concentration was increased (3.2 mM). These results suggest that relaxation to electrical stimulation is modulated by Ca^{2+} and by the H_2-subclass of histamine receptors.

Coronary smooth muscle Cimetidine Metiamide Ranitidine Histamine Pyrilamine

1. Introduction

Transmural electrical stimulation elicits a relaxation response in isolated dog coronary arteries made to contract in response to several vasoactive agents (Rooke et al., 1982). Rooke et al. (1982) demonstrated that the amplitude of this relaxation depended on the intensity, pulse duration and frequency of the electrical stimulus. The goal of the present study was to characterize the influence of histamine antagonists and Ca^{2+} on the relaxation response to electrical stimulation.

2. Materials and methods

Adult male and female mongrel dogs (15-35 kg) were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and the left anterior descending coronary artery excised. The arteries were stored in physiological salt solution (PSS) and cut helically into strips (1.5 × 10 mm) under a dissecting microscope. The strips were mounted vertically on a glass or plastic holder in a tissue bath containing PSS. The upper ends of the strips were connected to force transducers (Grass FT.03) and the resting tension of each strip was adjusted to 1.0 g. Before the start of experiments, the strips were allowed to equilibrate in PSS for 3 h. The bathing medium was maintained at 37°C and aerated with 95% O_2-5% CO_2. The pH of the PSS was 7.4 and the composition (mmol/l) was as follows: NaCl 130; KCl 4.7; KH_2PO_4 1.18; $MgSO_4 \cdot 7H_2O$ 1.17; $CaCl_2 \cdot H_2O$ 1.6; $NaHCO_3$ 14.9; dextrose 5.5; $CaNa_2EDTA$ 0.03. The Ca^{2+} concentration of the PSS was varied without compensating for changes in tonicity.

Strips of the coronary arteries were electrically stimulated by the use of two platinum wire elec-

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trodes placed parallel to the preparations. Electrical impulses consisted of square waves (9 V, 1.0 ms) provided by a direct current power suply and switching transistor triggered by a stimulator (Grass Model S4E). At each frequency of stimulation, the strips were stimulated for 8 min.

The following pharmacological agents were used: serotonin (5-hydroxytryptamine creatinine sulfate, Sigma Chemical Co.), pyrilamine (Sigma Chemical Co.), cimetidine (Smith, Kline and French Pharmaceuticals), metiamide (Smith, Kline and French Pharmaceuticals), ranitidine (Glaxo Pharmaceuticals), histamine HCl (Sigma Chemical Co.), nitroprusside (Roche Laboratories) and isoproterenol HCl (Sigma Chemical Co.).

The results of these experiments were analyzed by several statistical procedures. Frequency-response and dose-response curves were calculated as geometrical means. Paired and unpaired t-tests and curve fitting analyses (logit transformation) were performed. A P value less than 0.05 was considered to be statistically significant.

3. Results

Coronary artery strips, made to contract in response to serotonin (1.3×10^{-6} M), developed 488 ± 64 mg force ($n = 6$). Electrical stimulation (0.1-8.0 Hz, 1.0 ms, 9 V) applied during these contractions caused relaxation. Half-maximal relaxation occurred at 1.1 ± 0.2 Hz ($n = 6$; computed by logic transformation of frequency-response curves). Maximal relaxation was a $95 \pm 2\%$ reduction of the contractile response to serotonin. These relaxation responses were reproducible.

The rate and amplitude of relaxation in response to electrical stimulation (2 Hz) in arterial strips made to contract to serotonin was greater as the concentration of Ca^{2+} in the bathing medium was increased (left panel; fig. 1). When the concentration of Ca^{2+} was 0.1 mM, the contractile response to serotonin (1.3×10^{-6} M) was reduced (258 ± 57 mg; $n = 6$) compared to that in 1.6 mM Ca^{2+} (473 ± 58 mg: $n = 6$) or in 3.2 mM Ca^{2+} (745 ± 106 mg; $n = 6$). Relaxation in response to electrical stimulation in 0.1 mM Ca^{2+} was slow and incomplete compared to that in 1.6 mM or in 3.2 mM Ca^{2+}. To determine whether the magnitude of the serotonin response was responsible for the differences in relaxation when the Ca^{2+} concentration was altered, serotonin concentrations of 1.3×10^{-7} M to 1.3×10^{-5} M were used to produce variations in the magnitude of contraction (right panel, fig. 1). Contractile responses to 1.3×10^{-7} M serotonin (232 ± 32 mg; $n = 6$) were less than those to 1.3×10^{-6} M serotonin (473 ± 58 mg; $n = 6$) and to 1.3×10^{-5} M serotonin (678 ± 104 mg; $n = 6$), yet relaxation to electrical stimulation following contraction induced by 1.3×10^{-7} M serotonin was faster and more complete than that of strips made to contract with 1.3×10^{-6} M serotonin. Relaxation in response to electrical stimulation of arterial strips made to contract with 1.3×10^{-5} M serotonin was greatly reduced compared to that in strips contracted with 1.3×10^{-6} M serotonin.

To examine the relationship between relaxation and contractile magnitude, similar experiments to those described above were performed except that isoproterenol (10^{-7} M), nitroprusside (3.7×10^{-8} M) and histamine (9.0×10^{-5} M) were used to induce relaxation instead of electrical stimulation. When the concentration of Ca^{2+} in the bathing medium was altered, the magnitude of contraction in response to serotonin (1.3×10^{-6} M) varied as described above (0.1 mM Ca^{2+} 2.49 ± 22 mg; 1.6 mM Ca^{2+} 571 ± 53 mg; 3.2 mM Ca^{2+} 749 ± 52 mg; $n = 6$). Relaxation in response to all vasodilators was decreased when the concentration of Ca^{2+} was 3.2 mM (isoproterenol $70 \pm 4\%$; nitroprusside $58 \pm 4\%$; histamine $38 \pm 4\%$) and increased at 0.1 mM Ca^{2+} (isoproterenol $99 \pm 1\%$; nitroprusside $98 \pm 2\%$; histamine $79 \pm 6\%$); relaxation in response to all vasodilators was intermediate at 1.6 mM Ca^{2+} (isoproterenol $85 \pm 5\%$; nitroprusside $76 \pm 5\%$; histamine $54 \pm 5\%$). Changing the contractile magnitude by varying the concentration of serotonin (1.3×10^{-7} to 1.3×10^{-5} M) at 1.6 mM Ca^{2+} caused relaxation in response to these vasodilators to be decreased when the contractile magnitude was large and increased when the contractile response was small. Contractile responses to serotonin were: 1.3×10^{-7} M serotonin 262 ± 18 mg; 1.3×10^{-6} M 562 ± 47 mg; 1.3×10^{-5} M 701 ± 44 mg; $n = 6$. Relaxations in response to the

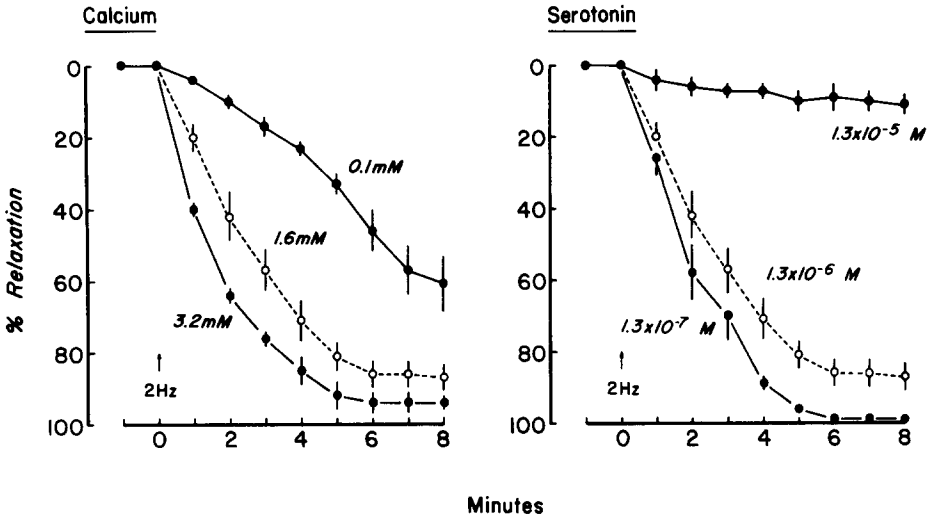

Fig. 1. Ca^{2+}, serotonin and relaxation to electrical stimulation. Alterations in the concentrations of Ca^{2+} (*left panel*) and serotonin (*right panel*) affected the rate and amplitude of relaxation in response to 2 Hz electrical stimulation. The amplitude of relaxation is normalized to the magnitude of contraction in response to serotonin which existed just prior to electrical stimulation (indicated by arrow). Values are the mean ± S.E.M. for 6 coronary arteries (6 dogs).

three vasodilators at respective contractile responses to serotonin were: 10^{-7} M isoproterenol 99 ± 1%, 85 ± 4%, 61 ± 4%; 3.7×10^{-8} M nitroprusside 99 ± 1%, 76 ± 4%, 57 ± 3%; 9.0×10^{-5} M histamine 81 ± 4%, 53 ± 5%, 22 ± 2%.

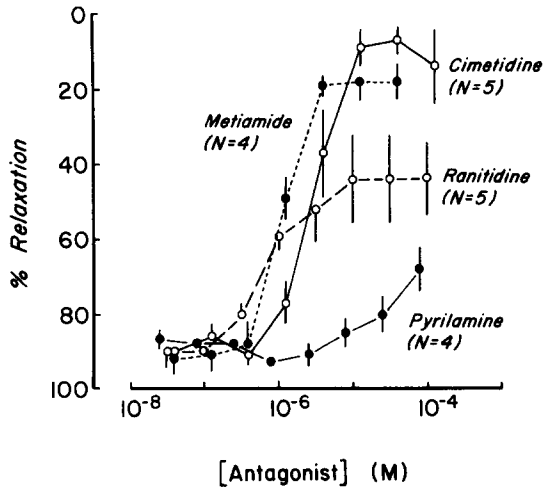

Fig. 2. Histamine antagonists. Relaxation to 2 Hz electrical stimulation was inhibited by cimetidine, metiamide and ranitidine, but not by pyrilamine. The amplitude of relaxation is expressed as a percentage of the contractile response to serotonin (1.3×10^{-6} M). Values are the mean ± S.E.M. Values in parentheses are the number of coronary arteries.

Relaxation in response to electrical stimulation (Hz) following contraction induced by serotonin (1.3×10^{-6} M; force developed 502 ± 23 mg; n = 18) was inhibited by antagonists of the H_2-subclass of histamine receptors (cimetidine, metiamide, ranitidine; fig. 2). The relaxation was not blocked by pyrilamine except at high concentrations of this H_1-receptor antagonist. The concentrations of the drugs which produced half-maximal inhibitory responses were (computed by logit transformation): (1) cimetidine 1.75 (± 0.33) $\times 10^{-6}$ M (n = 5); (2) metiamide 1.04 (± 0.19) $\times 10^{-6}$ M (n = 4); (3) ranitidine 0.88 (± 0.18) $\times 10^{-6}$ M (n = 5).

Arterial strips made to contract to serotonin (1.3×10^{-6} M; force developed 494 ± 26 mg; n = 15) relaxed in response to the cumulative addition of histamine (9.0×10^{-9} to 9.0×10^{-5} M) to the muscle bath. Half-maximal relaxation occurred at 2.97 (± 0.54) $\times 10^{-6}$ M histamine and the maximal relaxation was a 58 ± 6% reduction of the contractile response to serotonin. Treatment of the strips with cimetidine (4.0×10^{-5} M, n = 5), metiamide (1.2×10^{-5} M, n = 5) and ranitidine

(3.2×10^{-5} M, n = 5) blocked the relaxation to histamine completely. Pyrilamine (2.5×10^{-5} M, n = 5) reduced the maximal relaxation to histamine to a small extent (50 ± 7%). Relaxation in response to isoproterenol (10^{-7} M) and nitroprusside (3.7×10^{-8} M) was not altered by the histamine antagonists.

4. Discussion

Previous studies (Rooke et al., 1982) have demonstrated that the conditions of electrical stimulation (9 V, 1.0 ms) used in these experiments causes relaxation of dog coronary arteries. These relaxation responses are not altered by tetrodotoxin, adrenergic denervation by cold storage, α- and β-adrenergic blockade, atropine, indomethacin nor removal of the endothelium (Rooke et al., 1982). We have confirmed these observations (data not shown). Recent observations by Toda and Hayashi (1982) and by Cohen et al. (1983) suggest that electrical stimulation with a shorter pulse duration (0.2 ms) produces relaxation of dog coronary arteries which is blocked by β-adrenergic antagonists. The current experiments demonstrate that higher pulse durations of electrical stimulation produce relaxation in dog coronary arteries which is: (1) dependent on the concentration of Ca^{2+} in the bathing medium; and (2) blocked by antagonists of the H_2-subclass of histamine receptors. Both of these observations suggest that this relaxation is a response to an endogenous vasodilator liberated by the electrical stimulation.

4.1. Ca^{2+} dependence

Relaxation of dog coronary arteries in response to histamine, isoproterenol and nitroprusside is inversely related to contractile magnitude (large relaxation when contraction is small and vice versa). This relationship is evident when the contractile magnitude is altered by changing Ca^{2+} concentration (serotonin concentration constant) or when the contractile magnitude is altered by changing the serotonin concentration (Ca^{2+} concentration constant). In contrast, at high Ca^{2+} when contractile magnitude is large, the rate and extent of relaxation in response to electrical stimulation are greater than that predicted for a relationship based on contractile magnitude. Conversely, at low ca^{2+} concentration, the rate and extent of relaxation in response to electrical stimulation are less than that predicted for a small contractile response. Relaxation in response to electrical stimulation at a constant Ca^{2+} concentration (1.6 mM) does vary in the predicted manner when the magnitude of contraction is altered with various concentrations of serotonin. These results suggest that Ca^{2+} influences the release of an endogenous vasodilator. When the concentration of Ca^{2+} is low, relaxation is slow and incomplete because less endogenous vasodilator is released; when the concentration of Ca^{2+} is high, relaxation is fast and complete because the amount of endogenous vasodilator released is greater. Thus, it is likely that the release mechanism for an endogenous vasodilator in dog coronary arteries is similar to that in other secretary cells in that Ca^{2+} is required for stimulus-secretion coupling (Berridge, 1975).

4.2. Histamine antagonists

Based on the evidence presented in this study, it is possible that histamine is the transmitter responsible for relaxation in response to electrical stimulation. Antagonists of the H_2-subclass of histamine receptors blocked relaxation to electrical stimulation in a dose-dependent manner. Furthermore, exogenous histamine caused relaxation; and this relaxation was inhibited by H_2-receptor antagonists. Relaxation in response to electrical stimulation and to histamine were little affected by the H_1-antagonist, pyrilamine. Relaxation in response to isoproterenol and nitroprusside were not altered by the histamine antagonists. It has been demonstrated that the vascular wall contains high concentrations of histamine (El-Ackad and Brody, 1975; Garland and Keatinge, 1982). It may be that electrical stimulation of isolated dog coronary arteries releases histamine from cellular stores in the wall of the blood vessel; the released histamine produces relaxation through activation of H_2-receptors on the smooth muscle cell membrane. Interestingly, the release of histamine from cellular

storage sites is known to be a Ca^{2+} dependent response (Berridge, 1975).

Two observations suggest that histamine is not the endogeneous vasodilator released in response to electrical stimulation: (1) relaxation in response to exogenous histamine was less than that in response to electrical stimulation (maximal response to histamine $58 \pm 6\%$ change from serotonin contraction; maximal response to electrical stimulation $95 \pm 2\%$); and (2) relaxation in response to histamine was totally blocked by ranitidine (3.2×10^{-5} M) whereas relaxation to electrical stimulation was inhibited by only 50% in the presence of this H_2-receptor antagonist. It is possible that the release of the endogenous vasodilator is merely modulated by H_2-receptor antagonists. Alternately, histamine may be only one of two or more endogenous vasodilators released in response to electrical stimulation.

Regardless of the precise molecular mechanism the results of the current study are important in that they demonstrate that the relaxation is due to the release of an endogenous vasodilator rather than to a direct effect on the coronary vascular smooth muscle cells (Rooke et al., 1982).

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