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Neutrophils increase histamine contractions in pig coronary artery: a role for lipoxygenase products

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The possible role of neutrophils and the endothelium in the induction of hyperreactivity of the pig coronary artery to histamine was studied. In pig isolated coronary arteries histamine caused a concentration-dependent contraction; maximal contraction to histamine, however, was reduced in endothelium-denuded arteries. Pre-incubation of intact coronary arteries with isolated neutrophils did not affect the contractile response to histamine, while pre-incubation of endothelium-denuded arteries induced a hyperreactivity to histamine. This induction of hyperreactivity to histamine by neutrophils in pig coronary arteries seems to be mediated by lipoxygenase products, since it could be prevented by pre-incubation of the preparations with lipoxygenase inhibitors, but not with inhibitors of cyclo-oxygenase or with scavengers.

Keywords neutrophils histamine lipoxygenase products

Introduction

The contractile activity of vascular smooth muscle can be modulated by several endogenous substances. Hyperreactivity of vascular smooth muscle has been implicated in the genesis of coronary artery spasm (Maseri, 1984). Coronary occlusion is followed by endothelial damage, infiltration of neutrophils and an increased reactivity to vasoconstrictive stimuli (Van Benthuysen *et al.*, 1987). These phenomena may lead to reocclusion. Histamine is one of the endogenous candidates for the genesis of coronary spasm because it constricted coronary arteries of animals and men (Toda, 1987). Furthermore, coronary arteries of cardiac patients contained more histamine and were hyperreactive to histamine (Kalsner & Richards, 1984). In the present study we investigated the possible role of neutrophils and the endothelium in the induction of hyperreactivity of the coronary arteries to histamine.

Methods

Pig hearts were obtained from the local slaughterhouse. The right coronary artery was dissected free. When necessary the endothelium was removed by rubbing the intimate surface of the artery with a cotton swab. Coronary artery segments were suspended between two stainless steel wire hooks and mounted in an organ bath filled with Krebs-solution at 37° C, continuously aerated with a mixture of 95% O_2 and 5% CO_2. The vascular segments were gradually stretched to their optimal resting tension of 7.5 g. Tension was measured isometrically with a Harvard transducer. After equilibration, histamine was added to the organ bath using a cumulative concentration schedule. With each preparation only one concentration effect curve was obtained.

Venous blood was obtained from healthy pigs and collected in heparinized tubes. Neutrophils were isolated by means of differential dense centrifugation on Percoll and H_2PO_4 lysis of

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contaminating erythrocytes. Neutrophils were suspended in Krebs-solution at a final concentration of 10^8 ml^{-1}. Neutrophils were incubated with arterial segments at 37° C for 30 min. Afterwards, the arterial segments were mounted in an organ bath and the reactivity to histamine was measured.

Results and Discussion

Histamine caused a concentration-dependent contraction of pig isolated coronary artery at doses ranging from 10^{-6} to 3.10^{-4} M with a maximal response of 13 ± 2 g and with a pD_2 value of 5.01 ± 0.20 (mean ± s.e. mean results). The maximal contraction to histamine was reduced by 57 ± 18% in preparations from which the endothelium had been removed. The pD_2 values were not different (4.78 ± 0.08 and 5.01 ± 0.20 in endothelium-denuded and intact preparations, respectively). Pre-incubation of intact coronary arteries with isolated neutrophils had no influence on the contractile response to histamine (maximal contraction of 13 ± 2 g and 12 ± 1 g, respectively, and pD_2 values of 5.01 ± 0.20 and 4.69 ± 0.19, respectively). But when the same experiments were performed with coronary arteries without endothelium, the maximal contraction to histamine was increased (Figure 1). Neutrophils can release mediators like enzymes, chemotactic factors, oxygen-derived free radicals, lipoxygenase products or cyclo-oxygenase products which could be responsible for this hyperreactive response to histamine (Claeys *et al.*, 1985; Weiss, 1989). To examine the contribution of free radicals, neutrophils were preincubated with superoxide dismutase (SOD; 300 iu ml^{-1}) and catalase (5000 iu ml^{-1}), before the coronary artery segment was added and the reactivity to histamine was measured. The results are summarized in Figure 1. As can be seen, SOD and catalase do not prevent the induction of hyperreactivity to histamine. Therefore, it does not seem likely that hyperreactivity of the coronary artery to histamine is caused by the release of free radicals from the neutrophils. One of the other possibilities that was explored was the contribution of lipoxygenase or cyclo-oxygenase products. Neutrophils were preincubated for 30 min with inhibitors of lipoxygenase such as nordihydroguaiaretic acid (NDGA; 10 μM), AA861 (10 μM) or an inhibitor of the cyclo-oxygenase such as indomethacin (indo; 10 μM) or inhibitor of both enzymes like BW755C (30 μM).

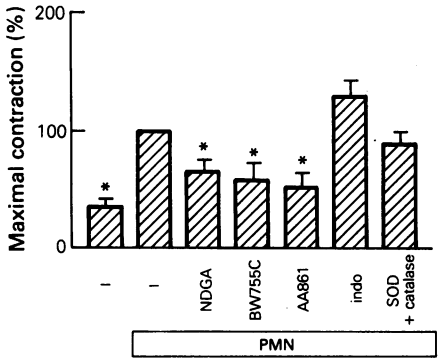

Figure 1 Maximal contraction to histamine of pig isolated coronary arteries from which the endothelium has been removed. Data are presented as mean ± s.e. mean and expressed as percentage of the maximal contraction obtained in the denuded coronary artery incubated with neutrophils. * Statistically significant ($P < 0.05$) from maximal contraction obtained in the denuded coronary artery incubated with neutrophils. The pD_2 values of the dose-response curves were 4.86 ± 0.11 for contractions in endothelium-denuded preparations ($n = 17$), 4.98 ± 0.09 in endothelium-denuded preparations preincubated with neutrophils ($n = 17$), 4.98 ± 0.25 in the NDGA-treated group ($n = 3$), 4.86 ± 0.12 in the BW755C-treated group ($n = 4$), 5.31 ± 0.05 in the AA861-treated group ($n = 3$), 5.07 ± 0.08 in the indomethacin-treated group ($n = 4$) and 5.14 ± 0.09 in the group treated with SOD and catalase ($n = 3$).

Then the coronary artery was added to the neutrophil suspension and again an incubation time of 30 min was allowed before the coronary artery segment was mounted in the organ bath and the reactivity to histamine was measured. It was found that preincubation with NDGA, AA861 or BW755C antagonized the induction of hyperreactivity by neutrophils (Figure 1). Preincubation with indomethacin had no effect and even slightly enhanced the contractile response to histamine (Figure 1). From these results it was concluded that lipoxygenase products mediate the hyperreactivity to histamine in isolated pig coronary arteries from which the endothelium had been removed.

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