ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Dec; 20 (12): 1093 - 1097
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical

Hyperpolarization caused by serotonin contributes to endothelium-dependent relaxations in the porcine coronary artery[1]

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KEY WORDS indomethacin; ketanserin; membrane potentials; methiothepin; nitric oxide; potassium channels; serotonin; vascular endothelium

ABSTRACT

AIM: The present study was designed to investigate the contribution of membrane hyperpolarization to endothelium-dependent relaxations induced by serotonin in the porcine coronary artery. **METHODS**: Rings with and without endothelium of porcine coronary arteries were suspended in conventional organ chambers for the measurement of isometric force. The cell membrane potential of the vascular smooth muscle cells was measured using glass microelectrodes, in the presence of indometacin, ketanserin, and/or N^{ω}-nitro-*L*-arginine. **RESULTS**: Serotonin induced a transient endothelium-, and concentration-dependent relaxation in rings contracted with prostaglandin $F_{2\alpha}$ in the presence of N^{ω}-nitro-*L*-arginine (maximal relaxation: 19 %). The N^{ω}-nitro-*L*-arginine resistant relaxation was abolished by high K^+ and tetrabutylammonium chloride. Serotonin also caused an endothelium-, concentration-dependent membrane hyperpolarizations with a maximal amplitude of -8.8 mV. The nitro-*L*-arginine resistant relaxations and hyperpolarizations were abolished by methiothepin, but not by glibenclamide. The time course of the endothelium-dependent relaxations and hyperpolarizations was similar. **CONCLUSION**: These results suggest a contribution of cell membrane hyperpolarization to the endothelium-dependent relaxations induced by serotonin in the porcine coronary artery.

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Received 1999-04-19 Accepted 1999-09-06

INTRODUCTION

Endothelium-dependent relaxations mediated by nonprostanoids can be divided into two components. The first one is inhibited by blokers of the *L*-arginine-nitric oxide pathway and likely represents the release of endothelium-derived nitric oxide (or a related compound)[1,2]. The other component most likely is due to endothelium-dependent hyperpolarization of the vascular smooth muscle cells[3,4]. This hyperpolarization is mediated by an unknown endothelium-derived substance (endothelium-derived hyperpolarizing factor), EDHF, which differs from nitric oxide. The contribution of EDHF is heterogenous among blood vessels[5]. A23187, bradykinin, acetylcholine, thrombin, and UK14304 cause endothelium-dependent, nitro-*L*-arginine resistant relaxations and hyperpolarizations of vascular smooth muscle in various tissues[6,7]. 5-Hydroxytryptamine (5-HT, serotonin) causes the release of endothelium-derived relaxing factor through activation of 5-HT_1-like serotonin receptor[8-10]. The present experiments were designed to investigate the contribution of membrane hyperpolarization to endothelium-dependent relaxations induced by serotonin in the porcine coronary artery.

MATERIALS AND METHODS

Organ chamber experiments Yorkshire pigs (♂, 20 - 25 kg, 8 - 10 wk) were anesthetized with telazol (100 mg intramuscularly) and sodium pentobarbital (12.5 mg/kg intravenously), and the hearts were removed. The left circumflex coronary artery (LCX) was dissected free, immersed in cold modified Krebs-

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Dec; 20 (12)
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Ringer bicarbonate solution [consisting of (mmol/L) NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.1, and calcium disodium edetate 0.026 at pH 7.4 (control solution)], and cleaned of connective tissue. They were then cut into rings (3 – 4 mm length). In some rings, the endothelium was removed mechanically by inserting the tip of a watchmaker's forceps into the lumen and gently rolling the preparation back and forth over a paper tissue wetted with cold control solution. The rings were suspended horizontally in organ chambers filled with 25 mL of control solution (37 ℃) gassed with 95 % O_2 + 5 % CO_2 (pH 7.4), and stretched to the optimal point of their length-active tension relation, as determined by the contraction to KCl (60 mmol/L) at progressive levels of stretch. The tissues were allowed to equilibrate for 60 min before beginning the experiments. Experiments were performed in the presence of indometacin (10 μmol/L; to prevent the formation of endogenous prostanoids); ketanserin (1 μmol/L; selective 5-HT_2 receptor blocker; to prevent direct activation of vascular smooth muscle), and/or N^{ω}-nitro-*L*-arginine (0.1 mmol/L; to block the production of nitric oxide). Some experiments were performed in the presence of methiothepin mesylate (1 μmol/L; non-selective 5-HT_1 and 5-HT_2 receptor blocker) or glibenclamide [3 μmol/L; to block ATP-sensitive K^+ channels]. Relaxations were expressed as a percentage of the active contractions induced by prostaglandin $F_{2\alpha}$, high K^+, or tetrabutylammonium chloride (TBA).

Electrophysiological studies Rings of coronary arteries were cut open along the longitudinal axis, and pinned down on the bottom of an organ chamber (1.5 mL) with the endothelial side upward. The arteries were superfused continuously with warm control solution (37 ℃) at constant flow rate (3 mL/min), and were allowed to equilibrate for at least 60 min before starting the recordings. A glass microelectrode filled with KCl 3 mol/L (tip resistance 50 – 80 MΩ) was inserted into the smooth muscle cell from the intimal side of the vessel[11]. The electrical signal was amplified by means of a recording amplifier (World Precision Instruments, New Haven, CT). The membrane potential was monitored continuously on an oscilloscope (Textronics 5223, Beaverton, OR, USA) and recorded on a pen recorder (Gould TA550, Cleveland, OH, USA). All experiments were performed in the presence of indometacin, ketanserin, and/or N^{ω}-nitro-*L*-arginine. Some experiments were performed in the presence of methiothepin or glibenclamide.

Drugs The following drugs were used: bradykinin, 5-hydroxytryptamine creatinine sulfate (serotonin), indometacin, potassium chloride, prostaglandin $F_{2\alpha}$, tetrabutylammonium chloride, and glibenclamide (all from Sigma Chemical Company, St, Louis, Mo); ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium); methiothepin mesylate (Research Biomedicals Inc Natick, MA); and N^{ω}-nitro-*L*-arginine (Aldrich, Milwaukee, WIS). All drugs were prepared with distilled water on the day of the study. Stock solutions of indometacin (10 μmol/L) were prepared in water and Na_2CO_3, and sonicated. Concentrations are expressed as final molar (mol/L) concentration in the bath solution.

Statistical analysis The results are expressed as means ± SEM. Unless otherwise specified, n refers to the number of animals studied. In rings contracted with prostaglandin $F_{2\alpha}$, responses are expressed as percent changes from the contraction level. Statistical evaluation of the data was performed with Student's t-test for either paired or unpaired observations (two tailed). Values were considered to be statistically different when P was less than 0.05.

RESULTS

Increasing concentrations of serotonin (10 nmol/L to 10 μmol/L) induced concentration-dependent relaxations in rings with endothelium contracted with prostaglandin $F_{2\alpha}$ (2 μmol/L) in the absence or presence of N^{ω}-nitro-*L*-arginine. The relaxation to serotonin 1 μmol/L was the most pronounced. N^{ω}-nitro-*L*-arginine (0.1 mmol/L) significantly inhibited the endothelium-dependent relaxations to serotonin (75.5 % ± 3.8 % inhibition of the maximal relaxation to serotonin 1 μmol/L). The N^{ω}-nitro-*L*-arginine resistant relaxations to serotonin were transient (maximal relaxation at 30 s) (Fig 1; $n = 5$).

A high concentration of KCl (40 mmol/L) evoked sustained contractions, whereas TBA (5 mmol/L) caused transient contractions of the porcine coronary artery. Serotonin (1 μmol/L) induced statistically

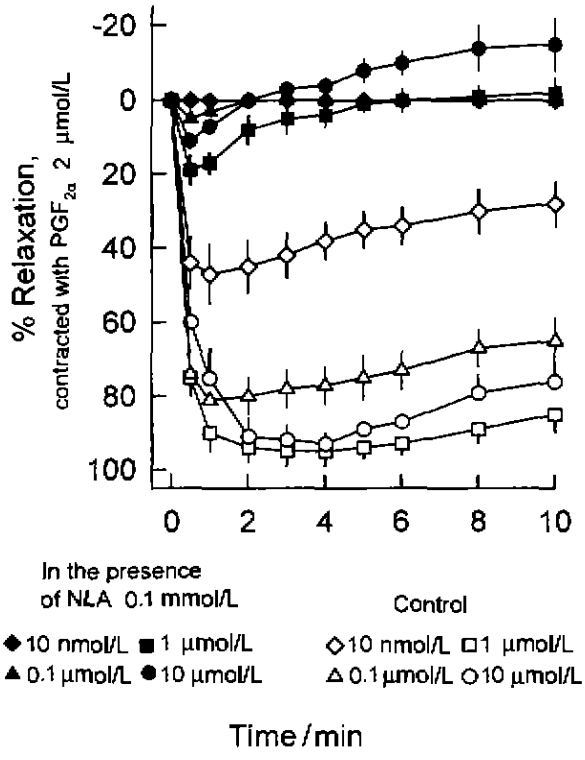

Fig 1. Endothelium-dependent relaxations induced by different concentrations of serotonin in porcine coronary arteries with endothelium ($n = 5$) in the absence (open symbols) or presence (closed symbols) of N^{ω}-nitro-*L*-arginine (0.1 mmol/L). The experiments were performed in the presence of ketanserin (1 μmol/L) and indometacin (10 μmol/L). The responses are expressed as percentage of the contraction evoked by prostaglandin $F_{2\alpha}$ (2 μmol/L). Data are shown as means ± SEM. The differences in relaxations to the different concentration of serotonin were statistically significant in the absence and presence of N^{ω}-nitro-*L*-arginine ($P < 0.05$).

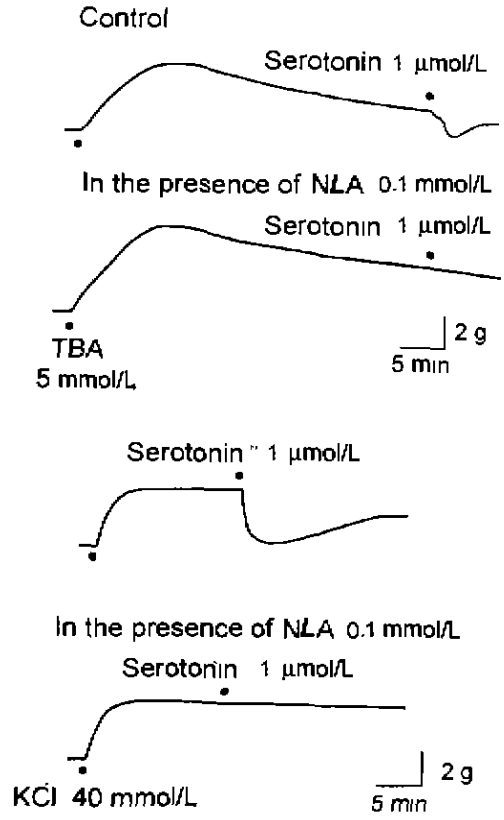

Fig 2. Effect of tetrabutylammonium (TBA, 5 mmol/L, upper panel) and high KCl (40 mmol/L, lower panel) on the relaxations induced by serotonin in porcine coronary arteries with endothelium. The rings were contracted with high TBA and KCl, respectively. The experiments were performed in the presence of ketanserin (1 μmol/L) and indometacin (10 μmol/L).

significant relaxations of rings contracted with either KCl or TBA. These relaxations were abolished by N^{ω}-nitro-*L*-arginine (0.1 mmol/L) (Fig 2, $n = 5$).

The nitro-*L*-arginine resistant relaxations to serotonin were abolished by methiothepin, but not by glibenclamide (data not shown). Relaxations to serotonin were not observed in rings without endothelium (data not shown).

The resting membrane potential of smooth muscle cells of the porcine coronary artery averaged (-49.8 ± 0.8) mV and (-48.7 ± 0.9) mV in tissues with and without endothelium, respectively (18 impalements in arteries from 4 animals). Serotonin (10 nmol/L – 10 μmol/L) induced endothelium-dependent, concentration-dependent membrane hyperpolarizations in the presence of N^{ω}-nitro-*L*-arginine, which were transient in nature with a duration of less than 2 min (Fig 3). The maximal amplitude of hyperpolarization was (-8.8 ± 2.2) mV at serotonin 10 μmol/L. The N^{ω}-nitro-*L*-arginine resistant hyperpolarizations to serotonin were abolished by methiothepin but not by glibenclamide (data not shown). The hyperpolarization was not observed in tissues without endothelium ($n = 4$, data not shown).

DISCUSSION

The present experiments demonstrate that, in the porcine coronary artery, serotonin causes transient

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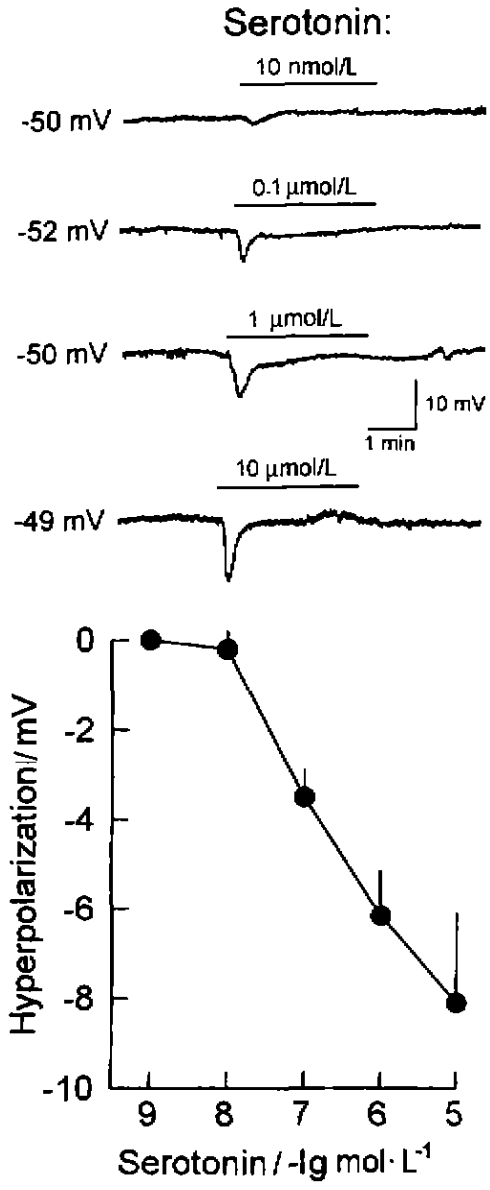

Fig 3. Membrane hyperpolarizations induced by different concentrations of serotonin in smooth muscle cells of porcine coronary arteries with endothelium, in the presence of N^{ω}-nitro-*L*-arginine (0.1 mmol/L), ketanserin (1 μmol/L) and indometacin (10 μmol/L). Inset: original recordings. Graph: data are shown as means ± SEM values ($n = 5$). The average resting membrane potential of the smooth muscle cells was −49.8 ± 0.8 mV.

endothelium-dependent, concentration-dependent relaxations and hyperpolarizations in the presence of an inhibitor of NO synthase. Endothelium-dependent hyperpolarizations caused by various agonists are transient in nature in different blood vessels from various species, but contribute to endothelium-dependent relaxations[6,7]. Serotonin causes the release of EDRF through activation of 5-HT_1-like receptors[8-10]. The possibility that the relaxation in the presence of N^{ω}-nitro-*L*-arginine is caused by residual nitric oxide, the production of which was not blocked by N^{ω}-nitro-*L*-arginine, is unlikely, because the relaxations in tissues contracted with high K^+ and TBA were abolished by treatment with the inhibitor of nitric oxide synthase. Moreover, the time course of the hyperpolarizations and nitro-*L*-arginine resistant relaxations was similar, and the same threshold concentration of serotonin induced both phenomena. Thus, this endothelium-dependent nitro-*L*-arginine resistant relaxation to serotonin is probably mediated by membrane hyperpolarization through the release of EDHF, which is resistant to inhibitors of the *L*-arginine-nitric oxide pathway[3,4]. EDHF is believed to cause hyperpolarization, and the resulting relaxation, by opening K^+ channels[4]. In the present study, these nitro-*L*-arginine resistant relaxations and hyperpolarizations to serotonin were abolished by methiothepin (non-selective 5-HT_1 and 5-HT_2 serotonin receptors blocker) and TBA (non-selective K^+ channel inhibitor), but not by glibenclamide (ATP-sensitive K^+ channel inhibitor). These findings suggest that, in the porcine coronary artery, serotonin causes the release of EDHF through the activation of 5-HT_1 serotonin receptors, which may activate a Ca^{2+} dependent K^+ channel distinct from ATP-sensitive K^+ channels[4].

In conclusion, the present study demonstrates that serotonin causes endothelium-dependent, nitro-*L*-arginine resistant membrane hyperpolarizations, which contribute to the endothelium-dependent relaxations of the porcine coronary artery. The endothelial receptors involved appear to belong to the 5-HT_1 family of serotonin receptors.

ACKNOWLEDGMENT To Mr Barnabas DESTA, Mr Dewayne O CONEY for outstanding technical help, and Ms Marie PALUMBO for the preparation of the manuscript.

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