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Short communication

Endothelium-dependent contraction induced by acetylcholine in isolated rat renal arteries

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Abstract

We investigated whether or not acetylcholine elicited an endothelium-dependent contraction and whether an arachidonic acid metabolite was involved in the acetylcholine-induced contraction in ring preparations of rat renal arteries. Acetylcholine (0.1–100 μM) caused a transient contraction in endothelium-intact arteries in a concentration-dependent manner. The contraction induced by acetylcholine (10 μM) was enhanced by pretreatment with N^G-nitro-L-arginine (100 μM), a nitric oxide synthase inhibitor, and was abolished by mechanical removal of the endothelium. Atropine (0.1 μM), quinacrine (1 and 3 μM), manoalide (0.1 and 1 μM), aspirin (1 and 10 μM), indomethacin (30 and 300 nM), ONO-3708 (9,11-dimethyl-methane-11,12-methano-13,14-dihydro-13-aza-14-oxo-15(β)-cyclophenyl-ω-pentenor-thromboxane A_2 L-arginine salt) (10 nM), S-1452 (calcium (5*Z*)-1*R*,2*S*,3*S*,4*S*-7-[3-phenylsulphonyl-aminobicyclo[2.2.1]hept-2yl]-5-heptenoate hydrate) (3 nM) and SQ29,548 ([1*S*-[1α,2β(5*Z*),3β,4α]]-7-[3-[[2-[(phenylamino) carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid) (3 and 10 nM), but not hexamethonium (1 μM), OKY-046 (sodium (*E*)-3-[4-(1-imidazolylmethyl)phenyl]-2-propenoic acid hydrochloride monohydrate) (100 μM) and CS-518 (sodium 2-(1-imidazolylmethyl)-4,5-dihydrobenzo[*b*]thiophene-6-carboxylate) (10 μM) significantly attenuated the acetylcholine (10 μM)-induced endothelium-dependent contractions in renal arteries pretreated with N^G-nitro-L-arginine. These findings suggest that acetylcholine causes endothelium-dependent contraction by stimulation of muscarinic receptors in rat renal arteries, and that an arachidonic acid metabolite(s) of the cyclooxygenase pathway is involved in this endothelium-dependent contraction.

Keywords: Acetylcholine; Renal artery, rat; Endothelium-dependent contraction; Arachidonic acid metabolite

1. Introduction

Since Furchgott and Zawadzki (1980) found that acetylcholine evoked endothelium-dependent relaxation in the rabbit aorta, endothelial cells have been demonstrated to modulate vascular smooth muscle tone by releasing endothelium-derived relaxing factor (EDRF) and endothelium-dependent contracting factor (EDCF). One EDRF is now recognized to be nitric oxide (NO) (Palmer et al., 1987). However, it has been demonstrated that, in canine basilar arteries, acetylcholine causes a contraction that is attenuated by cyclooxygenase inhibitors and thromboxane A_2 synthetase inhibitors (Usui et al., 1983). Later, the contraction induced by acetylcholine was proved to be an endothelium-dependent contraction, and the EDCF involved is probably thromboxane A_2 (Shirahase et al., 1987; Usui et al., 1987).

In most peripheral arteries, acetylcholine causes endothelium-dependent relaxation. However, acetylcholine has been reported to cause endothelium-dependent contractions in the pulmonary artery of rabbits (Altiere et al., 1986) and in the aorta of spontaneously hypertensive rats (Auch-Schwelk et al., 1990; Kato et al., 1990). Recently, we observed that, in rat renal arteries, acetylcholine caused an endothelium-dependent contraction. In the present study, we examined whether arachidonic acid metabolites contribute to acetylcholine-induced contraction in rat renal arteries.

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2. Materials and methods

Male Wistar-Kyoto rats weighing 350–400 g were used. Under sodium pentobarbital anesthesia (50 mg/kg i.p.), the rats were exsanguinated from the abdominal aorta. The right and left main renal arteries were quickly isolated and placed in physiological salt solution (PSS). The composition of the PSS was as follows (mM): NaCl, 120; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; and glucose, 10 (pH 7.4). Under a dissecting microscope, arteries were cleaned of fat and connective tissue, and then cut into rings 2 mm in length. In some rings, the endothelium was removed mechanically by gentle rubbing of the intimal surface with a stainless steel rod. Each ring was suspended between two stainless steel wire hooks and mounted in an organ bath chamber filled with 10 ml of PSS maintained at 37°C and bubbled with a mixture of 95% O_2 and 5% CO_2 (pO_2: 609 ± 6 mm Hg, $n = 3$). Isometric tension was measured with a force-displacement transducer and recorded on a polygraph. The rings were maintained at a resting tension of 1 g and were allowed to equilibrate for at least 90 min.

After the equilibration period, the rings were exposed 2 or 3 times to 60 mM KCl. The responsiveness to acetylcholine (0.1–100 μM) in quiescent rings or rings precontracted with EC_{80} (the concentration necessary to produce 80% of the maximum response) phenylephrine (0.3–3 μM) was then studied in the absence or presence of 100 μM N^G-nitro-L-arginine applied 30 min before the addition of acetylcholine.

The effects of various blocking agents on acetylcholine (10 μM)-induced contraction were assessed in endothelium-intact arteries pretreated with N^G-nitro-L-arginine (100 μM) under basal conditions. After two successive contractile responses to acetylcholine were elicited, blocking agent or vehicle was added to the organ bath 30 min prior to the third application of acetylcholine.

Aspirin, indomethacin, catalase, superoxide dismutase, hexamethonium chloride, phenylephrine hydrochloride, physostigmine salicylate and papaverine hydrochloride were purchased from Sigma Chemical Co., St. Louis, MO, USA; quinacrine dihydrochloride hydrate, manoalide and SQ29,548 ([1*S*-[1α,2β(5*Z*),3β,4α]]-7-[3-[[2-[(phenylamino)carbonyl]-hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-hep-tenoic acid) from Research Biochemicals, Natick, MA, USA; acetylcholine hydrochloride from Daiichi Pharmaceutical Co., Tokyo, Japan; atropine sulfate from Tanabe Pharmaceutical Co., Osaka, Japan; N^G-nitro-L-arginine from Peptide Institute, Osaka, Japan. OKY-046 (sodium (*E*)-3-[4-(1-imidazolylmethyl)phenyl]-2-propenoic acid hydrochloride monohydrate) and ONO-3708 (9,11-dimethyl-methane-11,12-methano-13,14-dihydro-13-aza-14-oxo-15(β)-cyclophenyl-ω-pentenor-thromboxane A_2 L-arginine salt) were kindly provided by Ono Pharmaceutical Co., Osaka, Japan; S-1452 (calcium (5*Z*)-1*R*,2*S*,3*S*,4*S*-7-[3-phenylsulphonyl-aminobicyclo[2.2.1]-hept-2yl]-5-heptenoate hydrate) by Shionogi and Co., Osaka, Japan; CS-518 (sodium 2-(1-imidazolylmethyl)-4,5-dihydrobenzo[*b*]thiophene-6-carboxylate) by Sankyo Co., Tokyo, Japan.

The drugs were dissolved in distilled water with the following exceptions: manoalide, indomethacin and SQ29,548 were dissolved in absolute ethanol; aspirin was dissolved in dimethyl sulfoxide. Dilutions were prepared in distilled water. The concentrations of drugs are expressed as final molar bath concentrations.

The results are expressed as means ± S.E.M. and n indicates the number of rats. Significant differences were assessed by either paired or unpaired Student's t-test or one-way analysis of variance followed by the Newman-Keuls' test. Differences with P values less than 0.05 were considered statistically significant.

3. Results

3.1. Endothelium-dependent contraction induced by acetylcholine in rat renal arteries

When a single concentration of acetylcholine (0.1–100 μM) was applied to renal arteries with endothelium under basal conditions, acetylcholine evoked a transient contractile response in a concentration-dependent manner. The contraction produced by acetylcholine at 0.1, 0.3, 1, 10 and 100 μM resulted in an absolute tension of 0.02 ± 0.01 g ($n = 6$), 0.09 ± 0.02 g ($n = 6$), 0.24 ± 0.03 g ($n = 6$), 0.30 ± 0.03 g ($n = 14$) and 0.32 ± 0.02 g ($n = 4$), respectively. The acetylcholine-induced contraction (0.3 μM) was significantly augmented by pretreatment with an acetylcholinesterase inhibitor, physostigmine (3 μM) (the absolute tension of physostigimine-treated arteries was 0.19 ± 0.01 g, $n = 6$) ($P < 0.01$).

A typical recording of the acetylcholine (10 μM)-induced contraction is depicted in Fig. 1A. The acetylcholine (10 μM)-induced contraction was significantly enhanced by pretreatment with N^G-nitro-L-arginine, a NO synthase inhibitor (Fig. 1A). The absolute tension developed with acetylcholine before and after treatment with N^G-nitro-L-arginine was 0.26 ± 0.04 g and 0.54 ± 0.06 g ($n = 8$) ($P < 0.01$). Acetylcholine (10 μM) did not produce contractions in renal arteries without endothelium in the absence or presence of N^G-nitro-L-arginine ($n = 6$) (Fig. 1A). There was no significant difference in the contractile response to 60 mM KCl between endothelium-intact (0.81 ± 0.1 g, $n = 8$) and endothelium-denuded renal arteries (0.76 ± 0.09 g, $n = 6$), indicating that damage to vascular smooth muscle

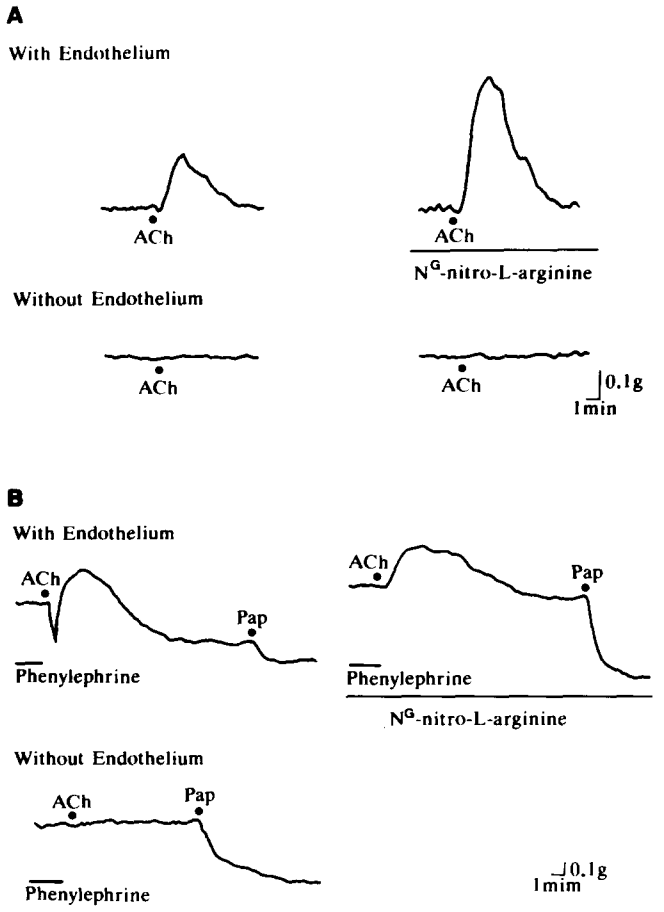

Fig. 1. Representative recordings showing the responses to acetylcholine in quiescent (A) or precontracted (B) rat renal arteries with and without endothelium. The responsiveness to acetylcholine (ACh) (10 μM) of quiescent rings or rings precontracted with EC_{80} phenylephrine (0.3–3 μM) was examined in the absence or presence of N^G-nitro-L-arginine (100 μM) applied 30 min before the addition of acetylcholine. Pap indicates the administration of 100 μM papaverine.

during mechanical removal of the endothelium is not responsible for the abolition of contraction caused by acetylcholine.

The application of acetylcholine (10 μM) produced a transient relaxation followed by a contraction and then a continuous relaxation in rat renal arteries that had been precontracted at submaximal contraction with phenylephrine (Fig. 1B). In the same arteries, the acetylcholine (10 μM)-induced transient relaxation and continuous relaxation were significantly attenuated by the pretreatment with 100 μM N^G-nitro-L-arginine for 30 min (Fig. 1B). The acetylcholine-induced continuous relaxation, expressed as a percentage of the relaxation induced by 100 μM papaverine in intact arteries and N^G-nitro-L-arginine-treated arteries, was 82 ± 6% (n = 4) and 11 ± 4% (n = 4) ($P < 0.01$), respectively. Furthermore, contractions and relaxations induced by acetylcholine (10 μM) were nearly abolished by mechanical removal of the endothelium (n = 4) (Fig. 1B).

3.2. Effects of various agents on endothelium-dependent contraction induced by acetylcholine in rat renal arteries

The effects of various blocking agents on acetylcholine (10 μM)-induced contraction in the presence of N^G-nitro-L-arginine (100 μM) in quiescent arteries were examined. Table 1 summarizes the results of the experiments. The results are expressed as percentages of the acetylcholine-induced contraction obtained be-

Table 1
Effects of various agents on acetylcholine-induced endothelium-dependent contraction in rat renal arteries

Agents	Concentration	Contraction (%)	
Muscarinic receptor antagonist			
Control		89 ± 6	(4)
Atropine	0.1 μM	0 ± 0 [b]	(4)
Nicotinic receptor antagonist			
Control		94 ± 4	(4)
Hexamethonium	1 μM	90 ± 4	(4)
Phospholipase A_2 inhibitors			
Control		92 ± 4	(6)
Quinacrine	1 μM	63 ± 12 [a]	(3)
	3 μM	45 ± 2 [b]	(3)
Control		93 ± 3	(6)
Manoalide	0.1 μM	57 ± 12 [b]	(3)
	1 μM	0 ± 0 [b]	(3)
Cyclooxygenase inhibitors			
Control		94 ± 3	(7)
Aspirin	1 μM	64 ± 3 [b]	(3)
	10 μM	3 ± 2 [b]	(4)
Control		96 ± 4	(6)
Indomethacin	30 nM	18 ± 3 [b]	(3)
	300 nM	0 ± 0 [b]	(3)
Thromboxane A_2 synthetase inhibitors			
Control		90 ± 3	(4)
OKY-046	100 μM	94 ± 8	(4)
Control		87 ± 5	(4)
CS-518	10 μM	86 ± 3	(4)
Thromboxane A_2 receptor antagonists			
Control		96 ± 4	(4)
ONO-3708	10 nM	44 ± 5 [b]	(4)
Control		91 ± 2	(4)
S-1452	3 nM	38 ± 6 [b]	(4)
Control		96 ± 2	(7)
SQ29,548	3 nM	64 ± 7 [b]	(4)
	10 nM	30 ± 2 [b]	(3)
Radical scavengers			
Control		93 ± 4	(4)
Superoxide dismutase		97 ± 5	(4)
	150 units/ml		
+ catalase	1000 units/ml		

The contraction induced by acetylcholine (10 μM) applied for a second time was taken as 100%. The value of the contraction elicited by the third application of acetylcholine under pretreatment with agents was compared with that elicited by the third application of acetylcholine in the absence of agents (control). The numbers in parentheses indicate the number of rats. [a] $P < 0.05$ and [b] $P < 0.01$, compared with the respective control.

fore the treatment with agent or vehicle. Values in the presence of agent were compared with those in the presence of vehicle alone (control). A muscarinic receptor antagonist, atropine (0.1 μM), abolished acetylcholine-induced contractions, while a nicotinic receptor antagonist, hexamethonium (1 μM), did not affect the contractions. The phospholipase A_2 inhibitors quinacrine (1 and 3 μM) and manoalide (0.1 and 1 μM), and the cyclooxygenase inhibitors aspirin (1 and 10 μM) and indomethacin (30 and 300 nM) significantly attenuated the contractions produced by acetylcholine in a concentration-dependent manner; 1 μM manoalide and 300 nM indomethacin nearly abolished the contractile response. The thromboxane A_2 receptor antagonists ONO-3708 (10 nM) (Fujioka et al., 1986), S-1452 (3 nM) (Narisada et al., 1988) and SQ29,548 (3 and 10 nM) (Ogletree et al., 1985) also significantly attenuated the acetylcholine-induced contractions. The thromboxane A_2 synthetase inhibitors OKY-046 (100 μM) (Iizuka et al., 1981) and CS-518 (10 μM) (Ushiyama et al., 1988) did not significantly affect the contraction produced by acetylcholine. In addition, combined treatment with superoxide dismutase (150 units/ml) and catalase (1000 units/ml) had no effect on the acetylcholine-induced contraction. Atropine, phospholipase A_2 inhibitors, cyclooxygenase inhibitors and thromboxane A_2 receptor antagonists at the concentrations used in the present study did not affect the contractile responses elicited by noradrenaline (3 μM) and KCl (60 mM).

4. Discussion

The present study clearly demonstrated that in rat renal arteries acetylcholine produces not only endothelium-dependent relaxation, but also endothelium-dependent contraction. NO released from endothelial cells may be involved in the endothelium-dependent relaxation induced by acetylcholine, since an NO synthase inhibitor markedly attenuated the acetylcholine-induced relaxation. By contrast, the results showing that acetylcholine-induced endothelium-dependent contractions were abolished or markedly reduced by the inhibition of phospholipase A_2 and cyclooxygenase indicate that an arachidonic acid metabolite(s) from the cyclooxygenase pathway in endothelial cells is involved in the endothelium-dependent contraction produced by acetylcholine. In addition, the acetylcholine-induced endothelium-dependent contraction is mediated by muscarinic receptors in endothelial cells, since acetylcholine-induced contractions were inhibited by atropine but not by hexamethonium.

The superoxide anion has been reported to be an EDCF in canine cerebral arteries (Katusic and Vanhoutte, 1989). Oxygen-derived free radicals are generated during the conversion of prostaglandin G_2 to prostaglandin H_2. However, acetylcholine-induced endothelium-dependent contraction in rat renal arteries was not affected by the pretreatment with superoxide dismutase plus catalase, suggesting that superoxide anion is not responsible for the endothelium-dependent contraction induced by acetylcholine in rat renal arteries.

In the canine basilar artery, endothelium-dependent contractions induced by various agents have been demonstrated to be attenuated by phospholipase A_2 inhibitors, cyclooxygenase inhibitors, thromboxane A_2 synthetase inhibitors and thromboxane A_2 receptor antagonists. Thus, thromboxane A_2 has been proposed to be EDCF (Usui et al., 1983, 1987; Shirahase et al., 1987). Thromboxane A_2 has also been suggested to be the EDCF in the rabbit pulmonary artery (Altiere et al., 1986). In the present study, acetylcholine-induced endothelium-dependent contraction in rat renal arteries was significantly attenuated by thromboxane A_2 receptor antagonists (ONO-3708, S-1452 and SQ29,548), suggesting that acetylcholine-induced contraction is mediated by a prostanoid(s) that contracts vascular smooth muscle via interaction with thromboxane A_2 receptors. However, thromboxane A_2 synthetase inhibitors (OKY-046 and CS-518) failed to inhibit acetylcholine-induced contraction in rat renal arteries. Prostaglandin H_2, a common precursor of prostaglandins and thromboxane A_2, has been suggested to be the EDCF produced by acetylcholine stimulation in the aorta of spontaneously hypertensive rats (Auch-Schwelk et al., 1990; Kato et al., 1990). Although prostaglandin H_2 is known to interact with thromboxane A_2 receptors (Halushka et al., 1989), it is not clear in the present study whether or not prostaglandin H_2 is involved in acetylcholine-induced endothelium-dependent contraction in rat renal arteries. Further experiments are needed to identify the nature of the putative cyclooxygenase metabolites contributing to the endothelium-dependent contraction in rat renal arteries.

Thus, acetylcholine causes endothelium-dependent contraction by stimulation of muscarinic receptors in rat renal arteries, and an arachidonic acid metabolite(s) of the cyclooxygenase pathway is involved in this response. In addition to EDRF (NO), EDCF (arachidonic acid metabolite(s)) may play an important role in renal circulation.

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