Flow- and agonist-mediated nitric oxide- and prostaglandin-dependent dilation in spinal arteries

Yasuaki Yashiro and Toshio Ohhashi. Flow- and agonist-mediated nitric oxide- and prostaglandin-dependent dilation in spinal arteries. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2217–H2223, 1997.—Isolated rabbit spinal resistance-sized arteries (100 µm in diameter and 3 mm long) were cannulated at both ends with glass micropipettes and perfused at constant pressure (60 mmHg). An increase of flow rate corresponding to a change of pressure gradient (ΔP) ranging from 0 to 20 mmHg produced a flow-dependent vasodilation. Treatment with 50 µM aspirin or 10 µM indomethacin produced a significant reduction of the flow-dependent vasodilation. Treatment with 50 µM aspirin or 10 µM indomethacin produced a significant reduction of the flow-dependent vasodilation only at 50 µM aspirin or 10 µM indomethacin. In contrast, treatment with N-nitro-L-arginine methyl ester (L-NAME, 30 µM) produced no significant change. In the presence of 10 µM indomethacin, however, 30 µM L-NAME caused a marked decrease in the arterial diameter at ΔP of 5 mmHg, which was completely reversed with additional administration of 1 mM L-arginine. Acetylcholine (ACh) produced a dose-dependent increase in the arterial diameter. The ACh-induced vasodilation was significantly reduced by 10 µM indomethacin or 50 µM aspirin and partially suppressed by 30 µM L-NAME. Pretreatment with both indomethacin and L-NAME completely reduced the ACh-induced vasodilation. In the presence of 10 µM indomethacin, additional treatment with 1 mM L-arginine significantly reversed the L-NAME-induced inhibition of the ACh-mediated vasodilation. Endothelial removal with Triton X-100 significantly reduced the ACh-induced vasodilation. Isosorbide dinitrate (a stable prostaglandin I₂ analogue), prostaglandin E₂, and arachidonic acid caused a dose-dependent dilation in the small arteries. These findings suggest that prostanooids play a major role in the flow- or ACh-induced vasodilation in the rabbit spinal resistance-sized small arteries.

The spinal blood vessels are not well defined. Although spinal blood vessels with specific resistance are not well defined. Although spinal blood vessels with specific resistance-sized small arteries have been limited. Previously, we studied the effects of vasoactive substances on isolated dog spinal branch of intercostal arteries and then demonstrated that ACh produced an endothelium-dependent and NO-mediated relaxation (29). No direct information, however, exists regarding the possible involvement of endogenous NO and prostaglandins on the regulation of vascular resistance in spinal circulation.

Thus we have attempted to examine mechanical activity in isolated rabbit spinal resistance-sized small arteries (~100 µm in diameter). In this study we focused on evaluating the relative importance of NO and vasodilator prostaglandins in 1) the flow-mediated regulation of the myogenic tone and 2) the response of the spinal small arteries to ACh with special reference to biological properties of the endothelium.

MATERIALS AND METHODS

Preparation and dissection. The techniques for dissection and cannulation of rabbit spinal resistance-sized small arteries were adopted from the methods originally described by Duling et al. (10). Japaneese white rabbits, weighing 1.5–3.0 kg, were anesthetized with pentobarbital sodium (40 mg/kg iv) and killed by exsanguination. Spinal cord (lumbar portion) was rapidly removed and placed in a cooled (4°C) dissection chamber filled with 3-(N-morpholino)propanesulfonic acid (MOPS)-buffered physiological salt solution containing 1% dialyzed bovine serum albumin (BSA). The chamber was placed on a recessed Plexiglas well, and chilled fluid was circulated through the well jacket from an Eyela cooling thermopump (model CTP 100, Tokyo Rikakikai, Tokyo, Japan). A posterior spinal resistance-sized small artery without a branch, ~100 µm in luminal diameter and 3 mm in length, was carefully dissected from the subarachnoidal space. The isolated small artery was carefully transferred from the dissection chamber to a temperature-controlled cannulation chamber (volume, 1 ml) mounted on a stage of an inverted microscope (model IMT-2, Olympus, Tokyo, Japan). Cannulation. The isolated small artery was cannulated at both ends by using a system of concentric glass pipettes (a perfusion pipette within a holding pipette, White Instruments, Bradbury Park, MD) mounted on a Narishige micromanipulator MJ-1 (Tokyo, Japan) that was equipped on the stage of the inverted microscope (Fig. 1). With vacuum applied to the lumen of the holding pipette, one end of the small artery was gently pulled into the pipette. The perfusion pipette was inserted into the lumen of the small artery. When one end of the small artery was thus cannulated, it was perfused at a hydrostatic pressure of 20 mmHg to remove red blood cells. Then the other end of the small artery was cannulated in the same manner.

Instrumentation. After the cannulation, both micropipettes were connected to water manometers used to adjust intraluminal pressure via independent reservoirs. Another fluid reservoir pressurized by the manometer could be inserted.
into the fluid path between the small artery and the upstream reservoir by means of a three-way liquid switch.

The image of the cannulated small artery was recorded using a video camera (model C2400, Hamamatsu Photonics, Hamamatsu, Japan) and displayed on a television monitor. Arterial luminal diameters were measured manually using a video caliper incorporated with MacLab Chart v3.2 (AD Instrument, Castle Hill, Australia) data acquisition system.

Experimental protocols. The resistance-sized small artery was set to its in situ length, and intraluminal pressure was set at 60 mmHg. A constant pressure gradient (ΔP) of 5 mmHg between the upstream and downstream pipettes was established to maintain a steady flow through the lumen. The organ bath temperature was raised slowly and kept at 37.0 ± 0.5°C by using both Haake FE-2 and a bipolar temperature controller (model TC-202, Medical Systems, Tokyo Japan). The solution in the organ bath was then changed to MOPS solution without albumin and perfused at a constant rate of flow (0.9 ml/min) using a Perista pump (model SJ-1211, Atto, Tokyo, Japan). The vessels were equilibrated for at least 60 min, during which time they developed spontaneous myogenic tone. Small arteries whose initial diameters were not shortened more than 20% were discarded for further studies.

Seven sets of experiments were performed. In the first series of experiments, luminal flow rate of the spinal resistance-sized small arteries was changed by equal and opposite movements of the two reservoirs after spontaneous myogenic tone developed at the ΔP of 5 mmHg. The arterial diameter was measured at each level of flow rate corresponding to the ΔP of 5 (control state), 0 (zero flow), 5, 10, and 20 mmHg. At each step, ΔP was maintained for 2–5 min until a stable diameter was observed. The same procedure was repeated in each small artery after a 30-min incubation period of a series of experiments, another cyclooxygenase inhibitor indo- methacin (10 µM) or a NO synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME, 30 µM) was administered extraluminally to the organ bath. The luminal diameter at the ΔP of 5 mmHg was measured immediately before and during 30 min of the application of those agents. In the third series, both 30 µM L-NAME and 1 mM L-arginine were cumulatively applied to the organ chamber in the presence of 10 µM indomethacin and/or 30 µM L-NAME. The arterial preparation was treated with each inhibitor for 30 min before we constructed dose-response curves for ACh. In control experiments, time-dependent responses to ACh were examined.

In the fourth series, changes in the diameter of the small arteries at the ΔP of 5 mmHg were measured before and after cumulative application of ACh (10^{-8} to 10^{-5} M, using 10-fold concentration increments), and then a dose-response curve for ACh was constructed. ACh was also applied extraluminally. The arteres were allowed to stabilize at each concentration of ACh for 7 min. After a 60-min recovery period, the cumulative application of ACh was repeated in the presence of 10 µM indomethacin and/or 30 µM L-NAME. The arterial preparation was treated with each inhibitor for 30 min before we constructed dose-response curves for ACh. In control experiments, time-dependent responses to ACh were examined.

In the fifth series, the responses to a single dose of ACh (10 µM) in the presence of 10 µM indomethacin were examined at the ΔP of 5 mmHg before and after the incubation of 30 µM L-NAME or 30 µM L-NAME + 1 mM L-arginine.

In the sixth series, a dose-response curve for ACh was obtained before and after endothelial denudation. The technique for endothelial denudation using nonionic detergent was followed by previous studies (15, 31). Thus, Triton X-100 (0.1%), a nonionic detergent, was perfused intraluminally at 60 mmHg intraluminal mean pressure with the ΔP of 5 mmHg for 30-60 s to remove endothelial cells. The procedure, however, often produced subsequent loss of spontaneous myogenic tone of the arterial preparation, although contractile response to high-potassium solution remained. In this series all preparations, therefore, were precontracted with a solution containing a high concentration of potassium (40 mM). Isotonic high-potassium MOPS solution was prepared by substituting NaCl with an equimolar amount of KCl. The denudation of the endothelium was confirmed histologically.

In the seventh series, isocarbacyclin (a stable prostacyclin analogue), prostaglandin E_2, or arachidonic acid was cumulatively applied extraluminally, and then dose-response curves for these agents were constructed with the spinal small arteries at the ΔP of 5 mmHg. In some small arteries responses to the agents were also examined after the treatment with 10 µM indomethacin.

At the end of each experiment, all arteries were relaxed completely with 10 µM nifedipine to obtain a maximum diameter at 60 mmHg intraluminal mean pressure with the ΔP of 5 mmHg. The extent of the diameter changes induced by ACh, isocarbacyclin, prostaglandin E_2, or arachidonic acid were expressed as a percentage of the nifedipine-induced maximum dilation. The nifedipine-induced dilation was sig-
nificantly larger than that produced by 10 µM sodium nitroprusside in these spinal small arteries.

Drugs and solutions. The composition of the MOPS solution (in mM) was as follows: 145 NaCl, 4.7 KCl, 2.0 CaCl2, 1.17 MgSO4, 2.0 pyruvate, 5.0 glucose, 0.02 EDTA, and 2.0 MOPS. The pH was adjusted to 7.4 ± 0.02 at 37°C. Solutions used for dissection, cannulation, and perfusion contained 1% BSA. ACh chloride and isosorbicarbacyc in were obtained from Daiichi Seiyaku (Tokyo, Japan) and Teijin (Tokyo, Japan), respectively. Sodium nitroprusside was obtained from Merck (Darmstadt, Germany). Aspirin, indomethacin, prostaglan- din E2 methyl ester, L-NAME hydrochloride, arachidonic acid sodium salt, and Triton X-100 were obtained from Sigma Chemical (St. Louis, MO). Aspirin, indomethacin, and isocarbacyc in were dissolved in ethanol and diluted with MOPS solution just before use. The concentration of the solvent was confirmed to produce no significant effect on spontaneous myogenic tone of small arterial preparations. The other drugs were directly dissolved in the MOPS solution.

Statistics. Experimental data in the text, figures, and table were expressed as means ± SE. One or two arteries were studied in each animal. The n value represents the number of vessels used. Comparisons of dose-response curves under different treatment were made using two-way analysis of variance and tested with Fisher’s protected least significant differences multiple-range test. Differences in the mean diameters before and after the treatment of inhibitors were compared by paired- or unpaired t-tests. A value of P < 0.05 was considered significant.

RESULTS

Effects of aspirin, indomethacin, or L-NAME on flow-dependent diameter of spinal small arteries. The isolated rabbit spinal resistance-sized small arteries developed spontaneous myogenic tone at 60 mmHg intraluminal mean pressure with the ΔP of 5 mmHg and then constricted to 74.0 ± 10.0% of their maximum diameters. The flow-diameter relationships of the spinal small arteries are summarized in Fig. 2. An increase of flow rate corresponding to a change of ΔP ranging from 0 to 20 mmHg caused a significant flow-dependent increase in the small arterial diameter (ΔP = 0 mmHg, 86.6 ± 2.4 µm vs. ΔP = 20 mmHg, 94.4 ± 2.9 µm; P < 0.01). Mean intraluminal pressure of the arterial preparation was kept at 60 mmHg throughout all experiments. Treatment with 50 µM aspirin significantly reduced the flow-dependent vasodilation only at the ΔP of 5 mmHg but unchanged the vasodilations at the ΔP of >10 mmHg. Extraluminal treatment with 10 µM indometha- cin, another cyclooxygenase inhibitor, also produced a significant decrease in the flow-dependent diameter of the spinal small arteries at the ΔP of 5 mmHg (8.4 ± 1.8% decrease in diameter, 100.6 ± 4.8 µm without indomethacin vs. 91.9 ± 3.8 µm with indomethacin, P < 0.05). On the other hand, pretreatment with 30 µM L-NAME produced no significant change in the diameter (104.9 ± 5.2 µm without L-NAME vs. 104.4 ± 5.1 µm with L-NAME). Such experimental data are summarized in Fig. 3. In the presence of 10 µM indomethacin, however, the pretreatment with 30 µM L-NAME caused a marked decrease in the arterial diameter at the ΔP of 5 mmHg (Fig. 4; 14.9 ± 4.4% decrease in diameter, 87.0 ± 4.5 µm without L-NAME vs. 73.2 ± 2.1 µm with
L-NAME, P < 0.05). The inhibitory effect of L-NAME was significantly reversed with the addition of 1 mM L-arginine into the organ bath. Single administration of 1 mM L-arginine, on the other hand, caused no significant effect on the diameter of spinal small arteries at the ΔP of 5 mmHg.

Effects of ACh on diameter of spinal small arteries. ACh produced a dose-dependent increase in the arterial diameter at the ΔP of 5 mmHg. The dose-response curves for ACh are summarized in Fig. 5. The vasodilative effect of ACh was suppressed by 10 µM indomethacin (P < 0.01), although a higher concentration of ACh (10⁻⁵ M) still caused a slight increase in the diameter (Fig. 5A). A similar reduction of the ACh-induced vasodilation was also observed with 50 µM aspirin. Pretreatment with 30 µM L-NAME also significantly attenuated the ACh-induced vasodilation (P < 0.05), but a marked vasodilation remained at higher concentrations of ACh (10⁻⁶–10⁻⁵ M) (Fig. 5B). Pretreatment with both indomethacin and L-NAME completely eliminated the ACh-induced vasodilation (Fig. 5C). The effects of 30 µM L-NAME and/or 1 mM L-arginine on the 10 µM ACh-induced vasodilation in the presence of 10 µM indomethacin were also studied. The 10 µM ACh-induced vasodilation in the presence of 10 µM indomethacin was completely suppressed by pretreatment with 30 µM L-NAME (31.9 ± 3.7% in the control without L-NAME vs. -1.6 ± 3.4% of maximum dilation with L-NAME, P < 0.01). On the other hand, in the presence of 10 µM indomethacin, additional treatment with 1 mM L-arginine significantly reversed the L-NAME-induced inhibition of the ACh-mediated vasodilation (23.1 ± 5.1% in the control without L-NAME vs. 23.5 ± 5.3% of maximum dilation with L-NAME and L-arginine).

Effects of endothelium on ACh-induced dilation of spinal small arteries. Figure 6 summarizes the effects of endothelial removal on the ACh-induced vasodilations. In these experiments, the small arteries were precontracted with the perfusion of a high-potassium (40 mM) MOPS solution, to about 50.3% of the maximum diameter. ACh produced a dose-dependent increase in the diameter of the precontracted spinal small arteries at 60 mmHg intraluminal mean pressure with the ΔP of 5 mmHg. Denudation of the endothelium with perfusion of Triton X-100 did not significantly alter the inner diameter (58.5 ± 6.6 µm, before the denudation vs. 62.8 ± 9.4 µm, after denudation). On the other hand, ACh-induced vasodilation was significantly attenuated by the denudation.

Effects of prostacyclin analogue or arachidonic acid. The effects of isocarbacyclin, prostaglandin E₂, and arachidonic acid on mechanical activity of the spinal small arteries are shown in Table 1. All agents caused dose-dependent increases in the arterial diameter at the ΔP of 5 mmHg. The arachidonic acid-induced vasodilation was completely eliminated by the pretreatment with 10 µM indomethacin (Fig. 5A). On the other hand, additional treatment with 1 mM L-arginine significantly reversed the L-NAME-induced inhibition of the ACh-mediated vasodilation (23.1 ± 5.1% in the control without L-NAME vs. 23.5 ± 5.3% of maximum dilation with L-NAME and L-arginine).

Fig. 5. Dose-response curves for ACh in rabbit spinal small arteries at ΔP of 5 mmHg in presence or absence of agents. A: in presence (●) or absence (○) of 10 µM indomethacin (n = 4). B: in presence (●) or absence (○) of 30 µM L-NAME (n = 5). C: in presence (●) or absence (○) of 10 µM indomethacin and 30 µM L-NAME (n = 5). Values are means ± SE. *P < 0.05 vs. absence of agents. **P < 0.01 vs. absence of agents.

Fig. 6. Effect of endothelial denudation on ACh-induced vasodilation in rabbit spinal small arteries precontracted with high potassium (40 mM). ○, Control response to ACh (endothelium intact, n = 4); ●, response to ACh after endothelial denudation (n = 4). Values are means ± SE (n = 4). *P < 0.05 vs. control response. **P < 0.01 vs. control response.
μM indomethacin, whereas the isocarbacyclin-induced vasodilation was not affected by indomethacin (10 μM).

DISCUSSION

Our major findings in this study are summarized as follows: 1) rabbit spinal resistance-sized small arteries develop flow-dependent dilation, 2) endogenous vasodilator prostanoids play a major role in the flow-dependent dilation and the ACh-induced dilation in the spinal small arteries at 60 mmHg intraluminal mean pressure with the ΔP of 5 mmHg, and 3) the ACh-induced vasodilation is endothelium dependent. Thus this is a first demonstration of flow- and ACh-induced vasodilation in isolated, cannulated, and perfused spinal resistance-sized small arteries.

Role of NO and prostaglandins on flow-dependent vasodilation. Pretreatment with 10 μM indomethacin, the concentration of which is well known to inhibit cyclooxygenase activity in tissues (16, 18), caused a significant reduction in arterial diameter, suggesting that there is a flow-dependent release of endogenous vasodilator prostaglandins (Fig. 3A). In rat intracerebral arterioles, vasodilator prostaglandins are not involved in the flow-dependent vasodilation (25). On the other hand, 10 μM indomethacin completely inhibits the flow-induced dilation of rat cremaster muscle arterioles (16).

It is well known that high concentrations of indomethacin have a nonspecific vasoconstrictive effect. The nonspecific pharmacological action of indomethacin may be ruled out by the present experimental evidence that another cyclooxygenase inhibitor, aspirin, also caused a significant reduction of the flow-dependent vasodilation only at the ΔP of 5 mmHg. At the ΔP of >10 mmHg, however, 50 μM aspirin caused no significant reduction of the flow-dependent vasodilation (Fig. 2). The mechanisms involved in such findings remain unclear. Further investigation will be needed to evaluate the effect of aspirin on the flow-dependent vasodilation at the ΔP of >10 mmHg.

It is worth noting that 30 μM L-NAME alone had no effect on the diameter of the spinal resistance-sized small arteries, whereas it produced a significant decrease in the diameter in the presence of 10 μM indomethacin (Fig. 3B, Fig. 4). Because the inhibitory effect of L-NAME was completely reversed with additional administration of 1 mM L-arginine (Fig. 4), it is suggested that endogenous NO may also mediate the flow-dependent vasodilation. The mechanisms underlying such an interaction between NO and prostaglandins are not clear; however, several hypotheses can be suggested to explain the mechanisms. First, a slight decrease of the diameter produced by indomethacin may facilitate shear stress-dependent NO production from the endothelial cells. The shear-induced endothelium-dependent NO production has been shown in many studies (6).

Second, as suggested by recent studies (9, 23, 28), synthesis of NO and prostaglandins may, in part, be modulated by exogenous prostaglandins and NO, respectively. Doni et al. (9) reported that exogenous administration of NO exerts a dose-dependent inhibition on the bradykinin-stimulated release of prostacyclin from bovine endothelial cells. On the other hand, Marotta et al. (23) reported that exogenous prostaglandin E2 or the prostacyclin analogue iloprost inhibits the induction of inducible NO synthase in endotoxin-activated murine macrophages. Thus these studies may be, in part, compatible with our conclusion that endogenous NO mediates the flow-dependent vasodilation in the presence of indomethacin.

It is also possible to speculate that cyclooxygenase inhibitors per se stimulate NO synthase activity. López-Farré et al. (21) suggested that aspirin significantly affects NO generation by neutrophils obtained from rabbits.

Third, an involvement of cyclooxygenase-dependent superoxide anion production could also be considered. Kontos et al. (17) suggested that oxygen radicals are generated in association with accelerated arachidonate metabolism via cyclooxygenase in cat cerebral arterioles. Thus indomethacin may cause reduced production of superoxide anion in tissues, which results in the reduction of NO degradation.

ACh-induced vasodilation in rabbit spinal resistance-sized small arteries. ACh produced a marked vasodilation in the rabbit spinal resistance-sized small arteries. In rat intracerebral arterioles, ACh has little effect when applied extraluminally (5, 24). Since the first description by Furchgott and Zawadski (12), ACh-

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Table 1. Effects of arachidonic acid, isocarbacyclin, and prostaglandin E2 on rabbit spinal small arteries at 60 mmHg intraluminal mean pressure with pressure gradient of 5 mmHg

<table>
<thead>
<tr>
<th>Agent Dose, -log mol/l</th>
<th>Arachidonic acid</th>
<th>Isocarbacyclin</th>
<th>Prostaglandin E2 + 10 μM indomethacin</th>
<th>Arachidonic acid + 10 μM indomethacin</th>
<th>Isocarbacyclin + 10 μM indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>17.0 ± 8.4</td>
<td>15.4 ± 4.4</td>
<td>0.0 ± 0.0</td>
<td>-0.8 ± 0.8</td>
<td>5.8 ± 2.4</td>
</tr>
<tr>
<td>8</td>
<td>31.8 ± 7.6</td>
<td>30.6 ± 11.0</td>
<td>1.9 ± 1.9</td>
<td>-0.8 ± 0.8*</td>
<td>281 ± 14.6</td>
</tr>
<tr>
<td>7</td>
<td>47.3 ± 10.7</td>
<td>91.6 ± 3.1</td>
<td>10.7 ± 4.1</td>
<td>-0.8 ± 0.8*</td>
<td>84.2 ± 7.0</td>
</tr>
<tr>
<td>6</td>
<td>69.0 ± 12.6</td>
<td>100.0 ± 0.0</td>
<td>20.0 ± 7.8</td>
<td>-0.8 ± 0.8*</td>
<td>94.3 ± 4.5</td>
</tr>
<tr>
<td>5</td>
<td>90.0 ± 4.8</td>
<td>100.0 ± 0.0</td>
<td>26.4 ± 6.9</td>
<td>0.1 ± 1.3†</td>
<td>95.1 ± 3.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of isolated small arteries. Agent doses refer to arachidonic acid, isocarbacyclin, or prostaglandin E2. Vasodilation is expressed as % of nifedipine-induced maximum dilation. * P < 0.05 vs. arachidonic acid before 10 μM indomethacin treatment. † P < 0.01 vs. arachidonic acid before 10 μM indomethacin treatment.

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FIG. 3

Indomethacin (Fig. 3, Fig. 4). Because the inhibitory decrease in the diameter in the presence of 10 μM indomethacin (Fig. 3B, Fig. 4). The inhibitory decrease in the diameter in the presence of 10 μM indomethacin (Fig. 3B, Fig. 4). Because the inhibitory decrease in the diameter in the presence of 10 μM indomethacin (Fig. 3B, Fig. 4). Because the inhibitory decrease in the diameter in the presence of 10 μM indomethacin (Fig. 3B, Fig. 4). Because the inhibitory decrease in the diameter in the presence of 10 μM indomethacin (Fig. 3B, Fig. 4). Because the inhibitory decrease in the diameter in the presence of 10 μM indomethacin (Fig. 3B, Fig. 4). Because the inhibitory decrease in the diameter in the presence of 10 μM indomethacin (Fig. 3B, Fig. 4). Because the inhibitory decrease in the diameter in the presence of 10 μM indomethacin (Fig. 3B, Fig. 4). Because the inhibitory decrease in the diameter in the presence of 10 μM indomethacin (Fig. 3B, Fig. 4).
induced vasodilation has been known to be mediated by endogenous NO. ACh has also been shown to release vasodilator prostaglandins from several vascular beds (1, 7). In contrast, Förstermann et al. (13) demonstrated in the strips of rabbit extrapulmonary, celiac, and mesenteric arteries that the ACh-dependent formation of vasodilative prostaglandins makes only a limited contribution to the ACh-induced vasodilation. Copeland et al. (3) also showed in rabbit pial arterioles with the use of a cranial window technique that ACh-induced release of prostacyclin does not play a major role in the ACh-induced vasodilation. In the present study, the ACh-induced vasodilation was significantly reduced by 10 µM indomethacin, the concentration of which is known to inhibit significantly the cyclooxygenase activity in tissues (16, 18). The finding suggests that in the rabbit spinal resistance-sized small arteries ACh produces an endogenous prostaglandin-mediated vasodilation. The experimental evidence that rabbit spinal small arteries are very sensitive to exogenous vasodilator prostaglandins such as prostacyclin and prostaglandin E2 and to production of endogenous vasodilator prostaglandins through activation of arachidonic acid cascade strongly supports the conclusion.

The ACh-induced vasodilation was completely suppressed by the pretreatment with both indomethacin and L-NAME (Fig. 5), and the inhibitory effect of L-NAME was reversed by additional treatment with L-arginine in the presence of indomethacin. These findings suggest that ACh may corelease vasodilator prostaglandins and NO, which result in a marked vasodilation of spinal resistance-sized small arteries. This is consistent with the studies indicating that NO and vasodilator prostaglandins contribute to the ACh-induced vasodilation in the hamster cremaster microcirculation in vivo (8). Boczkowski et al. (2) also showed that NO and vasodilator prostaglandins act in concert to regulate rat diaphragmatic arteriolar response to ACh.

The ACh-induced vasodilation of spinal small arteries is mainly dependent on the function of the endothelium. The endothelial removal with Triton X-100 perfusion (0.1%, 30 to 60 s) significantly reduced the ACh-induced vasodilation in the spinal small arteries. The same Triton X-100 perfusion had no significant effect on the isocarbacycin-induced vasodilation in the spinal small arteries, suggesting that the ability of the vascular smooth muscles to relax is not impaired by this procedure. Among the spinal small arteries studied, some preparations were still reactive to the high concentration of ACh after the Triton X-100 perfusion. This may be, in part, related to the possibility that some endothelial cells remained even after the Triton X-100 treatment. Another possibility, i.e., that ACh-induced vasodilation is not totally dependent on the endothelial cells, should also be considered (11, 30).

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REFERENCES


