Endothelium-Mediated Spontaneous Response in Aortic Rings of Deoxycorticosterone Acetate–Hypertensive Rats

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Aortic rings isolated from normotensive Sprague-Dawley rats (CONT) exhibited spontaneous tone when the preparations were stretched. After administering deoxycorticosterone acetate (DOCA), the rats became hypertensive, and this spontaneous tone increased remarkably. The spontaneous tone was dependent on the extracellular calcium concentration. Incubation with the calcium entry blocker D-600 attenuated the spontaneous response to a greater degree in rings from DOCA rats than in rings from CONT rats. Nifedipine relaxed the already developed spontaneous tone. Removal of the endothelium greatly depressed spontaneous tone, but did not diminish the contraction caused by norepinephrine. On the basis of our findings, we conclude that 1) spontaneous tone depends on calcium influx, presumably through specific stretch-operated membrane channels, 2) these stretch-dependent channels are blocked by D-600 and nifedipine, 3) spontaneous tone is enhanced in DOCA hypertension, and 4) the endothelium appears to act as a receptor for stretch, mediating—at least in part—the spontaneous contractile response by releasing a constrictor agent. (Hypertension 1989;13:256–261)

The spontaneous response of blood vessels was first observed in 1902 by Bayliss,1 who demonstrated that the vascular smooth muscle contracted when exposed to a stretching force. When the muscle was in a tonic-contracted state, it reacted to a diminution in stretch by relaxing. Bayliss observed these phenomena in denervated vascular beds and excised arteries. Although the effect that bears his name is considered myogenic in nature, its precise mechanism of production is still unclear.2,3

In recent years, investigators have demonstrated the "Bayliss effect" in isolated vascular rings and strips.4-11 All of these studies concluded that extracellular calcium must be present for the spontaneous tone to develop. Their underlying assumption is that calcium enters the cell through a specific channel that is activated by stretch.7,12 In addition, the stretch channel seems to be uniquely sensitive to temperature7 and resistant to blockade by certain calcium channel blockers.6,11

Some investigators have observed spontaneous tone or vasomotion in vessels isolated from spontaneously hypertensive rats (SHR), but it was absent or reduced in those from Wistar-Kyoto (WKY) rats,5,8,9 which suggests that calcium influx through stretch channels could be abnormally high in hypertension. Harder13,14 has recently hypothesized that, in cat cerebral arteries, the endothelium is involved in transducing the distending pressure to membrane depolarization of the vascular smooth muscle. In our experiments, we observed the development of spontaneous tone in aortic rings isolated from Sprague-Dawley rats. The present study was designed to determine 1) whether the spontaneous response was accentuated after the rats became hypertensive from deoxycorticosterone acetate (DOCA), and 2) whether the endothelium played a significant role in this response.

Materials and Methods

Thirty Sprague-Dawley rats were used in this study. Blood pressure was monitored by an indirect tail-cuff method. After measuring control level blood pressure, all of the rats were anesthetized with ether and the left kidney was removed. In 14 of the rats, no other intervention was performed (CONT rats). In the remaining 16 rats, a silastic pellet containing DOCA (200 mg/kg body wt) was implanted subcutaneously (DOCA rats). Postoperatively, the rats were fed normal chow, and the drinking water contained 1% NaCl and 0.2% KCl. Blood pressure was monitored weekly. Hypertension took 3–4 weeks to develop in the DOCA rats. The rats were 3 months old when nephrectomized and 4 months old when studied.

For the isolated smooth muscle experiments, the rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and the thoracic aorta was carefully
removed and placed in physiological salt solution (PSS) of the following composition (mM): NaCl 130, KCl 4.7, NaHCO₃ 14.9, NaH₂PO₄ 1.17, CaCl₂ 1.6, MgSO₄ 1.16, EDTA 0.03, and dextrose 5.5. The arteries were dissected free of fat and connective tissue and were cut in rings 4 mm in length. Two L-shaped wires were passed through the lumen with special care not to damage the endothelium, and the rings were suspended between a fixed post and a force transducer. The preparations were immersed in organ baths kept at 40° C and bubbled with 5% CO₂-95% O₂. Although spontaneous tone did develop at 37° C, the higher temperature was employed because we observed that maximal spontaneous tone occurred between 40° and 42° C. These findings confirm those of Winquist and Baskin, who reported that the maximal spontaneous response was obtained at 43° C. We chose to use 40° C because it is closer to the physiological temperature, and the added tone obtained by increasing the temperature to 43° C was not great. Rings from CONT and DOCA rats were incubated in the same bath.

The rings were placed in calcium-free PSS (same composition as PSS, but CaCl₂ was omitted and EGTA was added to achieve a concentration of 2 mM). The preparations were then stretched to obtain a passive force of 3 g. After 1 hour of stabilization, the preparations were shifted to PSS with various CaCl₂ concentrations (0.2, 0.4, 0.8, 1.6, and 3.2 mM), and the spontaneous tone was recorded. Preparations were incubated with either phentolamine (1 µM) or the calcium entry blocker D-600 (1 or 100 nM) during the final 20 minutes in calcium-free PSS and were then transferred to PSS with CaCl₂, still in the presence of phentolamine or D-600. In other experiments, nifedipine was prepared in a stock solution and added to the bath to obtain the desired concentration.

The endothelium was removed from some preparations by gently rubbing the endothelial surface. In some rats the same ring was studied before and after rubbing the endothelium. The failure of acetylcholine (1 µM) to relax a contraction induced with norepinephrine (1 µM) demonstrates the effectiveness of endothelium removal.

At the end of the experiments the rings were blotted and weighed, and the force generated was expressed as milligrams force per milligrams tissue. Statistical comparisons were performed with Student's t test. Significance was accepted with values of p<0.05.

**Results**

Before the final (terminal) anesthetization, the blood pressure of the CONT group (n=9) was 118±4 mm Hg; that of the DOCA group (n=11) was 163±6 mm Hg. Body weights were 407±27 g (CONT) and 432±20 g (DOCA).

**Characteristics of Spontaneous Tone**

To quantify the spontaneous tone, the aortic rings were first incubated in calcium-free PSS. They developed no contraction. When calcium was added to the bath all of the rings developed tone, and the magnitude of this tonic contraction in CONT and DOCA rats was compared. The left panels of Figure 1 show typical responses after adding calcium (1.6 mM) to the bath. Contractures developed more rapidly and with greater force in rings from DOCA rats than those from the CONT rats. Figure 2 depicts concentration-response curves after the addition of calcium. Maximum force was reached with 1.6 mM calcium. Aortic rings from DOCA rats generated a force approximately four times as great as that generated by those from CONT rats. The failure of phentolamine (1 µM) to reduce the
responses demonstrates that these contractions are not dependent on norepinephrine release from nerve terminals in the ring.

Calcium Entry Blockers on Spontaneous Tone

The failure of a tonic contraction to occur in a calcium-free PSS confirms that calcium from an extracellular source is necessary for a contraction to develop. The right panel of Figure 1 illustrates studies that were undertaken to determine whether the calcium involved in this contraction entered the cell through channels that could be blocked by calcium entry blockers. The upper two tracings demonstrate that when the rings were pretreated with D-600 (1 nM), the magnitude of the tonic contraction diminished dramatically. In the lower panel, representative tracings illustrate the effect of adding the calcium entry blocker nifedipine to the PSS after the rings developed the spontaneous contraction. Whereas a concentration of 1 nM caused a partial relaxation, a concentration of 10 μM completely abolished the contraction. Figure 3 depicts the effect of two concentrations of D-600 on the magnitude of spontaneous contractions produced by different concentrations of calcium. This calcium entry blocker produced a much greater relaxation in DOCA rat rings than in CONT rat rings.

Endothelium and Spontaneous Tone

We evaluated possible involvement of the endothelium in the spontaneous contraction by observing the contraction after wiping the endothelium from the inside of the rings. We then evaluated the effectiveness of this procedure for removing the endothelium by determining whether the rings relaxed in response to treatment with acetylcholine, which is known to produce relaxation through the action of the endothelium-derived relaxing factor (EDRF). Figure 4 illustrates that our procedure for wiping off the endothelium completely eliminated the relaxing effect of acetylcholine. The tracings in Figure 4 also show that endothelium removal significantly reduced the magnitude of the spontaneous tone in rings from both DOCA and CONT rats. Panel A of Figure 5 summarizes these observations. Since the magnitude of the contractile response to norepinephrine was not reduced (Panel B), these data also demonstrate that the reduction in spontaneous contractions caused by our wiping procedure does not merely reflect a weakening of the muscle.

Discussion

When aortic rings isolated from Sprague-Dawley rats were stretched, the preparations showed spontaneous tone development. This tone was not dependent on the release of norepinephrine from the
nerve terminals induced by stretch, because the tone was still present after blocking the action of norepinephrine in the preparations with phenolamine. This finding is consistent with those of other authors, and with Bayliss' original description when he worked with denervated preparations.

The rat aorta possesses intracellular calcium stores released by several agonists, which can produce contraction even in the absence of extracellular calcium. However, when the rings were stretched to the same degree in a calcium-free PSS the spontaneous tone did not appear, thus indicating that stretching the arteries does not release calcium from intracellular stores, as accomplished by other agonists.

The addition of calcium to the medium caused a concentration-dependent tonic contraction that maximized at 1.6 mM calcium. The necessary presence of calcium influx has been a constant finding in other studies, and researchers have assumed that calcium influx occurs through specific channels of the vascular smooth muscle physically activated by the stretch.

The spontaneous tone was notably enhanced when the rats became hypertensive after DOCA treatment, and was three to four times greater in the rings from DOCA rats than in the rings from CONT rats. Other researchers have described spontaneous tone in cerebral arteries and thoracic aortae in SHR, but it was not present or reduced in those same vessels in WKY rats.

The appearance of spontaneous tone characterized the hypertensive vessels and was interpreted as an indication of a greater leakiness to calcium in the smooth muscle cell membranes of these tissues. In our study, however, spontaneous tone was present even in normotensive rats and was enhanced by the hypertensive process. If this phenomenon were also present in resistance vessels, the increase in
spontaneous tone could play a role in the pathogenesis of hypertension in these rats, and could contribute to the increased vascular resistance present in this disease.

To assess the quantitative importance of the spontaneous tone with respect to that obtained with other forms of stimulation, we compared the spontaneous tone with the tone developed by exposure to norepinephrine (1 μM). The spontaneous response produced a contraction that was 50–60% of the maximum response to norepinephrine in DOCA rats. This finding is consistent with the value found in the experiments of Noon et al.16 In the normotensive rings, the spontaneous tone comprised approximately 15–20% of the response to norepinephrine. These observations indicate that the force generated by the spontaneous response is remarkable, and can influence the response of vascular smooth muscle in physiological and pathological processes.

Stretch channels were found resistant to the action of various calcium entry blockers in several studies.6,7,11 The authors interpreted this resistance as evidence that the calcium necessary for spontaneous tone enters the cell through pathways different from those normally sensitive to these drugs (i.e., mainly the voltage-operated or receptor-operated channels). In our experiments, however, the spontaneous tone was modulated by two calcium entry blockers, D-600 and nifedipine. Previous incubation with D-600 decreased the spontaneous tone, which nifedipine also depressed after the tone had fully developed and stabilized. Other studies have established the sensitivity of spontaneous tone to some calcium entry blockers, but with higher concentrations of the drugs.11

This spontaneous response could have important implications regarding the action of calcium blockers in normotensive and hypertensive animals. An increased sensitivity of isolated arteries to the relaxant effect of calcium blockers has been described in preparations contracted with high concentrations of norepinephrine or potassium chloride.17–20 However, a greater vasodilator response to calcium blockers has been described in hypertensive humans as compared with their normotensive controls.21,22 In this setting, the extreme stimulatory conditions referred to before (i.e., high norepinephrine or potassium chloride concentrations) are not present, and the calcium blockers are still active. It would seem that in this setting the calcium blockers are interfering with a calcium influx not driven primarily through voltage-dependent or agonist-dependent channels. The action of calcium blockers on stretch-dependent channels, if also present in resistance vessels, could constitute an alternative explanation for the increased relaxant action of calcium blockers in hypertension.

It is not clear which structure within the vascular wall is sensitive to stretching. This role has generally been ascribed to myogenically active smooth muscle cells that increase their firing rate when subjected to mechanical deformation.2 More recent studies suggest that endothelium plays a role in this response. Harder13,14 noted that the pressure-induced contraction in cat cerebral arteries was abolished after endothelium removal. Recently, stretch-activated membrane channels have been described in endothelial cells23 with the hypothesis that they could act as mechanotransducers.

In the present experiments, however, the removal of the endothelium significantly attenuated—but did not totally prevent—the spontaneous response. The endothelial wiping alone did not alter the reactivity of smooth muscle, since the responses to norepinephrine were similar before and after the endothelial disruption.

Since the endothelium removal did not totally suppress the spontaneous tone development, we must consider two alternatives: 1) the endothelium was not totally wiped out, or 2) the endothelium was completely removed, but there is some component of the spontaneous tone that is not endothelium-mediated. Because the wiping procedure abolished the acetylcholine-induced relaxation while preserving the norepinephrine response, we assume that the endothelium had been effectively removed. The spontaneous contraction observed after endothelium removal would therefore represent a specific myogenic response, which is enhanced when the endothelium is present.

It is possible that the endothelium modulates the spontaneous response through some of its well-known vasoactive agents: the EDRF or the endothelium-derived contracting factor (EDCF). If the endothelium releases an EDRF, as the experiments with acetylcholine suggest, our results would seem to be paradoxical in that suppression of a relaxant factor should enhance rather than attenuate a contractile response. There is the possibility that the endothelium was also releasing an EDCF, a phenomenon that has been substantiated.24–27 Yanagisawa et al.28 have recently reported a potent vasoconstrictor peptide produced by vascular endothelial cells. They have named this peptide endothelin. In addition, certain forms of hypertension in the rat have been associated with a decrease in EDRF29 or with EDCF production.30 In our experiments, the elimination of a contractile factor would explain the decrease in spontaneous tone after endothelium removal.

An alternative explanation is that mechanical deformation can directly promote calcium entry to the endothelial cell.23 By releasing EDCF or any other nonidentified contractile agent, this calcium would in turn enhance the spontaneous tone.

In summary, we provide experimental evidence indicating that 1) spontaneous tone is present in CONT rats and increases significantly when the animals become hypertensive from DOCA, which reflects an increased leakiness of the stretch-activated channels in hypertension; 2) the endothelium seems to mediate, at least in part, the response.
of the vascular smooth muscle to stretch; and 3) the spontaneous response is quantitatively important to explanations of challenges in the overall reactivity of the vessel. If these changes in conductance vessels like those used in this study are present in resistance arteries, they could contribute to the enhanced vascular reactivity that is found in hypertension.

References


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