Tension-Induced Release of Endothelium-Derived Relaxing Factor; Possible Role in Establishment of Desensitization of Norepinephrine-Induced Contraction in Rat Aorta

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Abstract - In order to clarify the mechanisms involved in the endothelium-dependent development of desensitization of norepinephrine-induced contraction in rat aorta, we have tested the effect of repeated generation of tension without receptor stimulation. Even when tension alone, with a magnitude almost equal to that generated by norepinephrine, was applied to the endothelium-intact ring without norepinephrine, the ring became desensitized. In the absence of endothelium, the development of desensitization did not occur. Furthermore, L-NG-monomethyl arginine, which is an inhibitor of endothelium-dependent relaxing factor (EDRF) synthesis, prevented the occurrence of desensitization. It was even able to reestablish contractile force when added after the desensitization had developed, suggesting that an increased release of EDRF is necessary to produce the desensitization. Therefore, these results indicate that endothelium-dependent desensitization does not require adrenergic receptor stimulation, but rather that tension generation alone is sufficient to establish desensitization.

It has been suggested that EDRF plays important roles in maintaining vascular smooth muscle and blood pressure homeostases (1-3). It has been proposed that EDRF is nitric oxide (4) or an S-nitroso-containing molecule (5). Recently, we have found that with repeated application of norepinephrine and washes between each application, in the presence of endothelium, the strength of norepinephrine contraction gradually diminishes (6). On the contrary, in the absence of endothelium, repeated addition of norepinephrine by the same experimental procedure causes contraction without any appreciable desensitization. Inhibitors of EDRF such as L-NG-monomethyl arginine (L-NMMA) (7, 8) and hemoglobin, but not cyclooxygenase inhibitor like indomethacin, can prevent the occurrence of desensitization. These results suggest that endothelium contributes to the establishment of desensitization to norepinephrine-induced contraction in the rat aorta and that an increase in EDRF release is necessary for the development of this desensitization. However, it was unclear whether repeated stimulation of adrenergic receptors is necessary for the development of desensitization. We report here that endothelium-dependent desensitization does not require repeated
adrenergic receptor stimulation. Tension generation alone is sufficient for the establishment of desensitization.

Male Sprague Dawley rats (8-10 weeks old) were killed by decapitation. Rings of thoracic aorta were prepared as described previously (9). They were mounted at 37°C in an organ bath (8 ml) containing a Krebs-Ringer bicarbonate buffer of the following composition (mM): NaCl, 118; KCl, 4.7; CaCl₂, 2.5; NaH₂PO₄, 1.2; MgCl₂, 1.2; NaHCO₃, 25; and glucose 11. In some rings, the endothelium was mechanically removed by gentle rubbing. A mixture of 95% O₂/5% CO₂ was continuously bubbled through the buffer. Contractions were monitored with an isometric transducer and recorded on an ink-writing oscillograph (Nihonkoden). An initial resting force of 1.0 g was applied to the aortic ring for 1 hr. The presence of intact endothelium was verified by relaxation in response to 0.1 mM acetylcholine. The resting tension was readjusted before addition of norepinephrine. L-NMMA was provided by Dr. I. Sakuma, Department of Internal Medicine, Hokkaido University, Japan. Norepinephrine bitartrate and L-arginine were purchased from Sigma Chemicals Company, St. Louis, MO.

In order to clarify the mechanisms involved in the endothelium-dependent desensitization mentioned in the introductory statement, we have separated repeated tension generation from adrenergic receptor stimulation.

![Figure 1](image-url)

**Fig. 1** The effects of the presence of endothelium on the development of desensitization of norepinephrine-induced contraction. Norepinephrine (1 µM) was added to the organ bath (●). Acetylcholine (Ach) (0.1 mM) was added to assess endothelium-dependent relaxation. Organ bath was washed out (○) three times at intervals of 5 min. Then tension, with a magnitude nearly equal to that generated by the initial norepinephrine application, was applied to the aortic ring for 30 min without norepinephrine. This tension loading procedure was repeated six times. The first and the sixth tension loadings are shown. Endothelium was intact in (a) and absent in (b). Representative traces from four rings are shown.
Fig. 2 Inhibition by L-NMMA of the desensitized norepinephrine-induced contraction of endothelium-intact aorta and the antagonistic effect of L-arginine. Experimental procedure is the same as that in Fig. 1 except that L-NMMA (0.5 mM) was added at the indicated point. L-arginine (L-arg) (0.6 mM) was also added as indicated. Representative traces from eight rings are shown.

Even when tension, with a magnitude was nearly equal to that generated by norepinephrine, was applied to the endothelium-intact ring without norepinephrine, the ring became desensitized (Fig. 1a). In the absence of endothelium, the development of desensitization did not occur (Fig. 1b). Furthermore, L-NMMA, which is an inhibitor of nitric oxide synthesis, the most probable candidate for EDRF, inhibited the development of desensitization (Fig. 2a). When applied after desensitization had developed, L-NMMA reestablished contractile force (Fig. 2b). The effect of L-NMMA was reversed by L-arginine. The prompt reversal by L-NMMA suggests that the norepinephrine-induced contraction is inhibited by released EDRF. It is, however, unknown whether basal release of EDRF is increased or norepinephrine can stimulate more EDRF release after the repeated generation of tension.

Therefore, these results indicate that endothelium-dependent desensitization does not require repeated adrenergic receptor stimulation, but rather that tension generation alone is sufficient.

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References


