Biphatic Relaxation Caused by Electrical Field Stimulation of the Mesenteric Arteries of Rats

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ABSTRACT—Electrical field stimulation (EFS) caused biphatic relaxation, first transient and then sustained, of rat mesenteric arteries precontracted by prostaglandin (PG) F2α. The transient relaxation was reduced about 45%, and the sustained relaxation was not observed after endothelium denudation of the arteries. Nω-Nitro-L-arginine (L-NOARG) inhibited the biphatic relaxation induced by EFS. At 1–100 μM, L-NOARG inhibited the transient relaxation more than the sustained relaxation. Methylene blue inhibited the biphatic relaxation and at 100 μM, L-NOARG abolished the transient relaxation. These results suggest that the transient relaxation mainly involves nitric oxide (NO), whereas the sustained relaxation involves both NO and some other factor(s).

Keywords: Endothelium-derived relaxing factor (EDRF), Mesenteric artery, Biphatic relaxation

Endothelial cells in blood vessels regulate the tone of vascular smooth muscle by releasing several compounds such as prostacyclin (1), substance P (SP) (2), and endothelium-derived relaxing factor (EDRF) (3). EDRF is now considered to be identical to nitric oxide (NO) (4) or to nitroso compounds (5). Recently, electro physiological studies have revealed that endothelium-derived hyperpolarization induces NO/prostanoid-independent relaxation by activation of endothelial cells in several blood vessels (6–8).

Neurotransmitters released from nerve endings in vascular tissues play an important role in regulating vascular tone. Kawasaki et al. (9) found that calcitonin gene-related peptide (CGRP) is a potent neurotransmitter in non-adrenergic, non-cholinergic (NANC) vasodilator nerves in the perfused mesenteric vascular bed of rats. Furthermore, we reported that SP caused a marked relaxation of porcine aorta via at least two EDRFs in the presence of Ca2+ through the NK-1 receptor subtype, which is coupled with a certain GTP-binding protein (10, 11). Mizuta et al. (12) showed that in rat mesenteric arteries, the tachykinin NK-3 agonist senktide causes vasodilation mediated by NO, although the detailed mechanism of its effect is not known.

In this study, we examined the mechanism of vasodilation induced by electrical field stimulation (EFS) in isolated mesenteric arteries of rats and obtained evidence that the EFS-induced biphatic vasodilation is mediated by NO released from the endothelium cells.

Experiments on relaxation responses were performed as described previously (12). Briefly, male Wistar rats (200–250 g) were killed by decapitation and their mesenteric arteries were removed. The arteries were placed in chilled Krebs buffer solution (composition: 118.7 mM NaCl, 4.7 mM KCl, 1.8 mM CaCl2, 1.2 mM KH2PO4, 1.2 mM MgSO4, 24.8 mM NaHCO3 and 10.1 mM glucose; pH 7.4). The arteries were then freed from adipose and connective tissues under a microscope and cut into rings (0.8 to 1.2 mm o.d., 2 mm length). Each ring was mounted on an L-shaped wire attached to a force-displacement transducer (T7-8-240; NEC Medicals, Tokyo) in an organ bath (US-5; UFER, Kyoto) which contained 5 ml of Krebs buffer solution. The organ bath was maintained at 37°C and gassed continuously with a mixture of 95% O2 and 5% CO2.

The arterial rings were adjusted to a resting tension of 1.0 g and allowed to stabilize for 2 hr, during which time the medium was replaced every 10 min. Before the start of experiments, equilibration was confirmed by measuring the contraction response to 50 mM K+. After the equilibration period, the relaxation responses of prostaglandin (PG) F2α (10 μM)-contracted rings to EFS were measured in Krebs buffer solution. To exclude the effect of the sympathetic nervous system, phenolamine (1 μM) (9) was administered 10 min before measuring PGF2α contraction.
For removal of the endothelium, the basilar aorta at a point near the branch to the mesenteric artery was connected with a syringe, and the other side was tied off. Then 5 ml of Krebs solution was injected rapidly 4–5 times. After each experiment, the absence of the endothelium was confirmed by the absence or marked suppression of relaxation induced by acetylcholine (ACh, 0.1 μM).

Two microelectrodes (10 mm long, 2.0 mm wide, 0.6 mm across; UFER) were inserted into our organ bath avoiding their contact with the segment and then fastened to a microelectrode holder. The segment was then stimulated with an electronic stimulator (SEN-3301; Nihon Kohden, Tokyo) via the microelectrodes.

Nω-Nitro-L-arginine (L-NOARG), methylene blue and tetrodotoxin were from Sigma (St. Louis, MO, USA). PGF2α was obtained from Funakoshi (Tokyo), ACh from Daiichi Pharmaceutical Co. (Tokyo), phenolamine from Ciba-Geigy (Basel, Switzerland), and atropine sulfate from Nacalai Tesque, Inc. (Kyoto).

Statistical analyses of data were performed by the two-tailed Student’s t-test or Dunnett’s test.

EFS (20 V, 4 Hz, 5 sec) caused biphasic relaxation of rat mesenteric arteries precontracted by PGF2α (Fig. 1). The EFS-induced relaxation had two components, transient and sustained relaxation. The EFS-induced biphasic relaxation showed frequency-dependence, but sustained relaxation was not observed at less than 2 Hz (Fig. 1, Table 1). Thus we examined the biphasic relaxation further using stimuli of 0.5 to 8 Hz. The biphasic relaxation reached almost a maximum at 4 Hz. Therefore, the following experiments were done using a stimulus of 4 Hz, which was the best for obtaining reproducible biphasic relaxation. In arteries denuded of endothelium, the EFS-induced transient relaxation was reduced by 45.6%, and no sustained relaxation was observed (Fig. 2).

To clarify the mechanism of the biphasic relaxation, we examined the effects of the NO synthase inhibitor L-NOARG on EFS-induced relaxation in rat mesenteric arteries (Table 1). Pretreatment with the NO synthase inhibitor L-NOARG (1–100 μM) inhibited the biphasic relaxation induced by EFS concentration-dependently. The inhibitory potency of L-NOARG on transient relaxation was stronger than that on sustained relaxation: it inhibited the transient relaxation about 60% and the sustained relaxation about 30%. The soluble guanylate cyclase inhibitor methylene blue (1–100 μM) inhibited the biphasic relaxation concentration-dependently, and 100 μM methylene blue completely inhibited the EFS-induced transient relaxation (Table 1).

In the present study, we demonstrated that EFS-induced vasodilation of rat mesenteric arteries has two components, transient and sustained relaxation. Atropine (1 μM), phenolamine (1 μM), tetrodotoxin (1 μM) and diclofenac sodium (10 μM) had no effects on the biphasic relaxation induced by EFS (data not shown). These results suggest that it was not due to a neuromeric factor, such as non-adrenergic or non-cholinergic neurons, or the arachidonic cascade. Geogette and Ignarro (13) also reported that EFS-induced relaxation of bovine pulmonary arteries was not due to a neuromeric mechanism since it was not inhibited by tetrodotoxin, guanethidine, atropine, propranolol and indomethacin. However, they did not observe the EFS-induced biphasic relaxation. This

Table 1. The effect of frequency of EFS, L-NOARG and methylene blue on the EFS-induced biphasic vasodilation

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>n</th>
<th>Transient</th>
<th>Sustained</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>3</td>
<td>34.54±5.71</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>55.67±3.99</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>63.95±4.06</td>
<td>73.27±4.71</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>72.00±1.59</td>
<td>78.35±1.52</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>71.98±3.83</td>
<td>79.01±3.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>L-NOARG (μM)</th>
<th>n</th>
<th>Transient</th>
<th>Sustained</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>76.23±1.77*</td>
<td>80.85±1.41*</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>46.90±2.53**</td>
<td>72.50±2.29**</td>
</tr>
<tr>
<td>100</td>
<td>6</td>
<td>42.04±3.42**</td>
<td>72.31±2.83**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Methylene blue (μM)</th>
<th>n</th>
<th>Transient</th>
<th>Sustained</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>65.58±5.67*</td>
<td>50.08±2.87*</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>53.91±6.13**</td>
<td>45.55±10.23*</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
<td>0.00±0.00**</td>
<td>23.22±2.91**</td>
</tr>
</tbody>
</table>

Values are expressed as percent of the PGF2α contraction. *P<0.05 and **P<0.01 vs control.
difference shows that different types of blood vessels have different mechanisms.

The biphasic relaxation was dependent on endothelial cells. In addition, it was inhibited by the NO synthase inhibitor L-NOARG, which inhibited the transient relaxation strongly, but the sustained relaxation only weakly. These results indicate that the transient relaxation is mainly due to NO. In the present study, the biphasic relaxation was inhibited by the soluble guanylate cyclase inhibitor methylene blue, which inhibited the transient relaxation especially strongly. These results suggest that the transient relaxation is caused by activation of guanylate cyclase by NO since Hatake et al. (14) reported that NG-nitro-L-arginine inhibits increase of cyclic GMP. These observations indicate that EFS-elicited transient relaxation of rat mesenteric artery is mediated by neuronally independent, but endothelium-dependent, stimulation of NO and cGMP formation. On the other hand, the sustained relaxation may be regulated by other endothelium-derived substances besides NO because the sustained relaxation did not appear when the artery was stimulated at low frequency. In addition, the inhibition of the sustained relaxation by L-NOARG and methylene blue was weak compared with the transient relaxation.

The present study shows that EFS elicits biphasic relaxation of rat mesenteric arteries. The transient EFS-induced relaxation was mainly caused by NO, whereas the sustained relaxation was caused by NO and other factors.

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