Persistence of Cyclopiazonic Acid-Induced Endothelium-Dependent Vasodilatation in Spontaneously Hypertensive and Streptozotocin-Induced Diabetic Rats

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ABSTRACT—We recently reported that cyclopiazonic acid (CPA) releases a novel endothelium-derived relaxing factor that is not prostacyclin, nitric oxide or endothelium-derived hyperpolarizing factor in rat mesenteric arterial bed. The acetylcholine-induced vasodilatation of the isolated mesenteric bed in spontaneously hypertensive rats (SHR) was not different from that in Wistar Kyoto rats (WKY), but it was significantly smaller in streptozotocin (STZ)-induced diabetic rats than in age-matched controls. The CPA-induced vasodilatation was not affected in SHR or in STZ-induced diabetic rats. These results suggest that the CPA-induced endothelium-dependent vasodilatation is resistant to the effects of diabetes.

Keywords: Cyclopiazonic acid, Diabetes, Endothelium-derived relaxing factor

The importance of the endothelium in the regulation of vascular tone has become increasingly evident. Endothelial cells release a variety of vasoactive factors including the prostaglandins I2 and E2 (1), nitric oxide (NO), which accounts for the biological properties of endothelium-derived relaxing factor (EDRF) (2), and endothelium-derived hyperpolarizing factor (EDHF) (3, 4). We recently found evidence for a novel EDRF that is neither NO nor EDHF (5). Cyclopiazonic acid (CPA), a specific inhibitor of Ca\(^{2+}\)-ATPase activity in the sarcoplasmic reticulum in striated muscle (6, 7), is able to relax the perfused mesenteric arterial bed, and this vasodilatation is abolished by the removal of the endothelium. Such CPA-induced vasodilatation is not affected by N\(^{O}\)-nitro-L-arginine (L-NNA) plus methylene blue (MB) in an isotonic high K\(^+\) (60 mM) medium containing indomethacin, indicating that CPA releases an EDRF that is neither the prostacyclin, NO nor EDHF. It is known that CPA increases the production of cyclic AMP, but not cyclic GMP, in the mesenteric arterial bed in a concentration-dependent manner and that the CPA-induced increase in cyclic AMP is abolished by removal of the endothelium (5). What role, if any, is played by CPA-induced EDRF in regulating resistance vascular tone in disease states, such as diabetes and hypertension, is as yet unknown. In the present study, we examined the CPA-induced vasodilatation of the mesenteric arterial beds in streptozotocin (STZ)-induced diabetic rats, spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY).

Male SHR (22- to 26-weeks-old) and age-matched WKY were purchased from Tokyo Laboratory Animals Co., Ltd. (Tokyo). Blood pressure in unanesthetized animals was measured by the tail-cuff method before the experiment began. A separate group of 8- to 10-week-old male Wistar rats (Tokyo Laboratory Animals Co., Ltd.) received a single injection of STZ (60 mg/kg) in the tail vein in order to induce diabetes. Age-matched controls were injected with the same volume of buffer solution. Food and water were available to all rats ad libitum. The experiments were performed ten weeks after the injection.

Rats were anesthetized with diethyl ether and then given an i.v. injection of 1000 units/kg of heparin. They were killed by decapitation and the mesenteric arterial bed rapidly dissected out and placed in oxygenated, modified Krebs-Henseleit solution (KHS) of the following composition: 118.0 mM NaCl, 4.7 mM KCl, 25.0 mM NaHCO\(_3\), 1.8 mM CaCl\(_2\), 1.2 mM NaH\(_2\)PO\(_4\), 1.2 mM MgSO\(_4\) and 11.0 mM dextrose. The mesenteric artery and vein were tied off near the caecum and the remaining intestine separated from the arterial bed along the intestinal wall. The mesenteric arterial bed was then perfused by our previously described method (8). Briefly, warm (37°C), oxygenated (95% O\(_2\) – 5% CO\(_2\)) KHS was
pumped into the mesenteric arterial bed, by using a peristaltic pump operating at a rate of 5 ml/min, through a cannula inserted into the superior mesenteric artery. Vascular responses were detected as changes in perfusion pressure, which were monitored continuously with a pressure transducer and recorded on a pen recorder. Following a 60-min equilibration period, the perfusion circuit was transformed into a closed system: i.e., the perfusion solution was collected in a second bath, from which it was re-circulated through the mesenteric arterial bed. The total volume of the closed system was 50 ml, and agents were administered via the bath. After equilibration, the mesentery preparation was constricted by perfusion with 5 x 10^{-6} to 4 x 10^{-5} M methoxamine, the actual concentration being chosen to produce a perfusion pressure of 115 to 130 mmHg. It was then relaxed by perfusion with 10^{-6} M acetylcholine (ACh) to confirm the integrity of the endothelium. For the relaxation studies, the mesenteric arterial bed was preconstricted with the concentration of methoxamine (5 x 10^{-6} to 4 x 10^{-5} M) found to produce 70–80% of the maximal response in a given animal (i.e., each rat received an equieffective concentration). After the methoxamine-induced constriction had reached a plateau, ACh, CPA, sodium nitroprusside (SNP) or forskolin was added (each in a cumulative fashion). Indomethacin (10^{-5} M) was present in the perfusate throughout the experiments. Drug-induced relaxation was expressed as a percentage of the increase in perfusion pressure induced by the equieffective concentration of methoxamine (i.e., a "100% relaxation" is a complete abolition of the effect of the methoxamine).

Indomethacin, forskolin, sodium nitroprusside, methoxamine hydrochloride, cyclopiazonic acid and streptozotocin were purchased from Sigma Co. (St. Louis, MO, USA). Acetylcholine chloride was purchased from Daiichi Pharmaceutical Co., Ltd. (Tokyo).

Results are each expressed as a mean±S.E.M. Statistical differences were assessed by Student’s t-test for unpaired observations, following a one-way analysis of variance, and P<0.05 was considered to be significant.

As previously reported (9), ACh (10^{-10} to 10^{-6} M) induced a concentration-dependent vasodilatation of mesenteric arterial beds preconstricted with methoxamine, and this effect did not differ significantly between SHR and WKY (Fig. 1). However, the vasodilatation caused by ACh was significantly attenuated in STZ-induced diabetic rats (Fig. 1). The EC_{50} values for ACh were 2.4±0.5 x 10^{-9} M and 8.4±0.8 x 10^{-8} M (P<0.001, n=12) in age-matched control and diabetic rats, respectively. In marked contrast, the CPA (10^{-8} to 10^{-4} M)-induced vasodilatations of SHR and STZ-induced diabetic rats were not significantly different from those of their respective controls (Fig. 1). The EC_{50} values for CPA were 7.9±0.7 x 10^{-6} M (age-matched controls, n=6) and 2.0±0.07 x 10^{-5} M (diabetic rats, n=6).

In contrast to the results with ACh, the concentration-dependent vasodilatations produced by SNP (10^{-9} to 10^{-5} M) and forskolin (10^{-9} to 10^{-6} M) in diabetic rats were similar to those in age-matched controls (data not shown).

The main conclusions from the present study are i) that CPA-induced vasodilatation of the mesenteric arterial bed is not different among preparations from WKY, SHR, age-matched control and STZ-induced diabetic rats, whereas ii) ACh-induced vasodilatation is markedly attenuated in STZ-induced diabetic rats, but not in SHR.

The endothelium-dependent relaxation induced by ACh has been shown to be impaired in aortas from SHR (10). In marked contrast to that result, we earlier found no difference, in terms of the ACh-induced endothelium-dependent vasodilatation, between the mesenteric arterial beds of SHR and WKY (9). It has been shown that the relaxation responses of blood vessels to endothelium-dependent agents are attenuated in STZ-induced diabetic rats (11–14). The quantity of cyclic nucleotides released from the mesentery preparation by ACh is also less in diabetic rats (15). These findings strongly suggest that

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Body weight (g, n=20)</th>
<th>Blood pressure (mmHg, n=20)</th>
<th>Plasma glucose (mg/dl, n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-matched control</td>
<td>485.9±10.3</td>
<td>120.3±3.2</td>
<td>163.5±6.8</td>
</tr>
<tr>
<td>Diabetic</td>
<td>283.6±6.4**</td>
<td>117.3±6.1</td>
<td>573.6±24.3**</td>
</tr>
<tr>
<td>SHR</td>
<td>462.3±6.5</td>
<td>163.2±6.9**</td>
<td>N.D.</td>
</tr>
<tr>
<td>WKY</td>
<td>453.2±7.3</td>
<td>120.4±3.2</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

Values are means±S.E.M. n, number of animals. **P<0.01, vs control; *P<0.01, vs WKY. N.D., not determined.

The basal perfusion pressures in mesenteric arterial beds from STZ-induced diabetic, age-matched control, SHR and WKY rats were 64.1±6.2 mmHg (n=12), 69.1±7.4, 67.3±8.9 mmHg (n=12) and 65.9±8.7 mmHg (n=12), respectively. Perfusion with methoxamine (5 x 10^{-6} to 4 x 10^{-5} M) increased the perfusion pressures to 119.8±12.7 mmHg (n=12), 121.5±7.5 mmHg (n=12), 126.7±19.5 mmHg (n=12) and 123.6±15.3 mmHg (n=12), respectively.

The pre-experimental level of systolic blood pressure in STZ-induced diabetic rats, age-matched control rats, SHR and WKY are summarized in Table 1. Ten weeks after the injection of STZ, body weight was significantly decreased and plasma glucose concentration significantly increased in the diabetic rats (Table 1).
the diabetic state causes some functional impairment (decreased release or synthesis of NO or rapid destruction of NO) of the endothelium in the mesenteric arterial bed of rats.

In marked contrast to the ACh-induced vasodilatation, the CPA-induced vasodilatation of the mesentery was not affected in either SHR or STZ-induced diabetic rats. We recently reported that the vasodilatation of the mesenteric arterial bed induced by CPA is not due to the production of prostanoids, NO or EDHF and that the effect of CPA occurs through the production of cyclic AMP, but not of cyclic GMP (5). Thus, we proposed that there is a novel EDRF that is not prostaglandin I₂, NO or EDHF and which can be released by CPA from the endothelium of the mesenteric arterial bed (5). It now appears that the vasodilatation induced by the novel EDRF may be resistant to the diabetic state. CPA, a specific inhibitor of Ca²⁺-ATPase activity in the sarcoplasmic reticulum in striated muscle (6, 7), can deplete the rapidly exchanging intracellular Ca²⁺ stores by blocking the refilling of Ca²⁺ stores. Therefore, CPA may influx Ca²⁺ into endothelial cells through non-specific Ca²⁺ channels (capacitative Ca²⁺ channels) in the mesenteric arterial bed. Since CPA-induced vasodilatation was resistant to diabetes, it is tempting to speculate that capacitative Ca²⁺-channels may not be impaired in diabetic states.

The vasodilator response of the mesenteric arterial bed to SNP or forskolin did not differ significantly between age-matched controls and STZ-induced diabetic rats. Therefore, the decreased responsiveness to ACh of the mesentery from diabetic rats seen in the present study was not due to a general alteration in the relaxation mechanisms of the vascular smooth muscle.

In conclusion, although the STZ-induced diabetic state involves a decrease in NO synthesis or release through a specific impairment of the function of the endothelium in the mesenteric arterial bed, CPA-induced vasodilatation, supposed to be mediated by a novel EDRF-cyclic AMP pathway, is resistant both to the diabetic state and to spontaneous hypertension.

Acknowledgments

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REFERENCES


