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Attenuation of Depressor Response Induced by Platelet Activating Factor and Acetylcholine in Streptozotocin-Induced Diabetic Rats

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The in vivo and in vitro effects of platelet-activating factor (PAF, 1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine) and acetylcholine (ACh) on vascular relaxation responses were examined in streptozotocin-induced diabetic rats. Intravenous injection of PAF and ACh (0.03 to 10 μg/kg) decreased the mean blood pressure in both control and diabetic rats in a dose-dependent fashion. Initial blood pressure in diabetic rats did not significantly differ from that in control rats. However, depressor responses induced by PAF and ACh in diabetic rats were attenuated more than those in control rats. In perfused mesenteric arterial bed preconstricted with methoxamine (10^-3 – 10^-4 M), PAF (10^-11 – 3 x 10^-10 M) produced a concentration-dependent relaxation. However, this relaxation was significantly attenuated in the diabetic preparation compared with the control preparation. ACh also produced a concentration-dependent vasodilation in perfused mesenteric arterial bed. The concentration–response curve for the relaxation of the mesenteric arterial bed to ACh in diabetic preparation was shifted to the right compared with that in control preparation. A pretreatment with oxymetholone (10^-6 M) also shifted the concentration–response curves for relaxation to ACh in both control and diabetic preparation to the right. There was no difference in relaxation induced by sodium nitroprusside between the diabetic and the control preparation. From the present results and previous work, which PAF showed an endothelium-dependent vasodilation in the perfused mesenteric arterial bed, it is suggested that impairment of endothelium-dependent relaxation in the perfused mesenteric arterial bed of diabetic rats may account, in part, for the attenuation of PAF- and ACh-induced depressor effect observed in diabetic rats in vivo.

Keywords — diabetes; platelet activating factor; acetylcholine; mesenteric arterial bed; endothelium-dependent relaxation; blood pressure

Introduction

Platelet-activating factor (PAF) has been shown to produce strong and long-lasting hypotension in various animal species.1) It has been reported that the endogenous PAF participates in blood pressure (BP) regulation in various hypertensive models.2,3) Recently, extremely low concentrations of PAF have been shown to produce an endothelium-dependent vasodilator effect in perfused rat mesenteric arterial bed4) and rat mesenteric arterial strip.5) This vasodilator action of PAF at low concentrations is thought to be the cause of its hypotensive action in vivo. On the other hand, previous works disclosed functional changes in endothelium accompanying diabetes mellitus.6,7) We have also reported functional changes in endothelial cells which induce an attenuation of release of endothelium-derived relaxing factor (EDRF) in aortic strips of streptozotocin (STZ)-induced diabetic rats8) and alloxan-induced diabetic rabbits.9,10) In diabetic rats, effects of vasoactive agents on vasodilatation have been studied mainly in isolated large vessels, i.e. the aorta.6–10) Although attenuated vasodilation response in diabetic vessels is consistently observed in such preparations, the relevance of these findings could be questioned as large vessels are unlikely to contribute to peripheral vascular resistance and BP regulation. It is therefore of interest, due to the importance of small resistance vessels in the regulation of BP, to evaluate the effect of vasodilating agents, PAF and acetylcholine (ACh), on such vessels in diabetic rats. In the present study, the effect of the diabetic state on

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vasodilator action of PAF and ACh was examined in STZ-induced diabetic rats by measuring the systemic BP in vivo and vascular relaxation in the isolated mesenteric arterial bed.

Materials and Methods

Drugs — PAF (1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine) was purchased from Nova Biochem, Switzerland. STZ, methoxamine hydrochloride, sodium nitroprusside (SNP), haemoglobin, and bovine serum albumin (fraction V, BSA) were purchased from Sigma Chemical Co., St. Louis, U.S.A. Acetylcholine chloride was purchased from Daiichi Pharmaceutical Co., Tokyo, Japan. Heparin was purchased from Novo Industry A/S., Denmark. Urethane was purchased from Junsei Chemical Co., Ltd., Tokyo, Japan. Alpha-chloralose was purchased from Tokyo Kasei Industry Co., Ltd., Tokyo, Japan. PAF was dissolved in ethanol and stored at −80 °C. On the day of use, the ethanol was dried under a stream of nitrogen gas and PAF was dissolved in distilled water containing 0.25% BSA. Sonication for 30 s ensured that PAF was completely dissolved. All other drugs were freshly dissolved in 0.9% saline for each experiment. Levels of plasma glucose were measured with commercially available assay kits (Uni-Kit, Chugai Pharmaceutical Co., Tokyo, Japan).

Animals and the Induction of Diabetes — Male Wistar rats, 8 weeks old, received a single injection of STZ of 60 mg/kg, dissolved in citrate buffer solution (pH 4.5) into the tail vein to induce diabetes in rats (diabetic rats). Age-matched control rats were injected with a similar volume of buffer solution alone (control rats). Food and water were given freely. Eight weeks after the treatment, rats were used for in vivo and in vitro experiments.

BP in Anesthetized Rats — Control and diabetic rats were anesthetized by an intravenous injection of urethane (500 mg/kg) and alpha-chloralose (40 mg/kg). Mean blood pressure (MBP) was measured with a pressure transducer (Nihon Kohden, TP-200T) through a polyethylene cannula inserted into the left carotid artery. Heart rate (HR) was monitored with a cardio- 

tachometer (Nihon Kohden, AT-601G) which triggered by the arterial pressure pulse. Both variables were recorded continuously on a pen-writing oscillograph (Nihon Kohden, WT-645G). A femoral vein was cannulated with a polyethylene cannula for drug administration. Increasing doses of PAF and ACh, from 0.03 to 10 μg/kg, were administered intravenously to both groups of rats.

Vascular Responses in Perfused Mesenteric Arterial Bed — After anesthesia with ether, control and diabetic rats were injected with 1000 units/kg of heparin intravenously. After a midline incision, blood was obtained from the abdominal aorta for estimation of the blood glucose level. The blood was centrifuged at 3000 rpm for 10 min and the plasma was stored at −80 °C until analyzed. After the bleeding, the mesenteric vascular bed was rapidly dissected and perfused according to the method described by McGregor,11 with various modifications.4) Briefly, the entire mesenteric arterial bed and associated intestinal tract was removed from the rat. The mesenteric artery and vein were tied close to the caecum, and the remaining intestine was separated from the vascular bed along the intestinal wall. A cannula of PE-50 tubing was inserted approximately 1.5 cm into the superior mesenteric artery from its junction with the aorta and tied firmly. The isolated preparation was placed in an organ bath and perfused through the cannula with Krebs–Henseleit solution (KHS) by a peristaltic pump (ATTO, SJ-1220) at a rate of 5 ml/min. The KHS was composed (in mM) of NaCl 118.0, KCl 4.7, NaHCO₃ 25.0, CaCl₂ 1.8, NaH₂PO₄ 1.2, MgSO₄ 1.2, dextrose 11.0. The perfusate was maintained at 37 °C and aerated constantly with a gas containing 95% O₂ and 5% CO₂. Changes in perfusion pressure were monitored with a pressure transducer (Nihon Kohden, AP-2001) and recorded on a pen recorder (TOA, FBR-252-A). After an equilibration period of 60 min, the perfusion circuit was changed to a closed system, i.e., the perfusion solution was collected in a second bath and recirculated into the mesenteric arterial bed. The total volume of the closed system was 50 ml, and agents were administered via the second bath. The mesenteric arterial bed was precontracted
with $10^{-5} - 10^{-4}$ M methoxamine. PAF was infused in single doses to avoid tachyphylaxis. ACh and SNP were administered cumulatively. Each preparation received one drug for determining the relaxation response.

Statistical Analysis — Relaxations induced by a drug were expressed as a percentage of methoxamine-induced increase in perfusion pressure. Each data was expressed as the mean ± S.E. Significance of differences were identified by Student's $t$-test for unpaired observations.

Results

Levels of Plasma Glucose and Body Weight of Rats

Eight weeks after an injection of STZ, the plasma glucose level and body weight of diabetic rats were significantly elevated and decreased compared with those in control rats, respectively (glucose; control 183 ± 6 mg/dl, diabetic 550 ± 12 mg/dl, body weight; control 379 ± 5 g, diabetic 258 ± 4 g, $p<0.05$, $n=44$).

Changes in BP in Anesthetized Rats

Initial values of the MBP and HR in control rats were not significantly different from those in diabetic rats (MBP; control 123 ± 5 mmHg, diabetic 108 ± 7 mmHg, HR; control 334 ± 18 bpm, diabetic 307 ± 13 bpm, $n=7$). Intravenous injection of PAF, at a dose-range of 0.03—10 μg/kg, decreased the MBP of both control and diabetic rats in a dose-dependent fashion. Intravenous injection of ACh, at a dose-range of 0.03—10 μg/kg, also produced a dose-dependent decrease in MBP in both groups of rats. Typical responses to PAF and ACh tested are shown in Figs. 1 and 2, respectively. Changes in MBP produced by PAF and ACh in diabetic rats were significantly less than those in control rats in all doses tested (Fig. 3). The duration of hypotensive effect induced by PAF was dose-dependent. The duration of hypotensive effect induced by PAF in diabetic rats was significantly shorter than that in the control at doses of 1 μg/kg (data not shown), 3 μg/kg (control 18 ± 3 min, diabetic 6 ± 1 min, $n=7$, $p<0.01$) and 10 μg/kg (control 29 ± 2 min, diabetic 18 ± 2 min, $n=7$, $p<0.01$). HR was negligibly affected by the injection of PAF at doses tested both in control and diabetic rats (data not shown). Intravenous
Fig. 2. A Typical Recording of Changes in BP Produced by Intravenous Injection of ACh (0.03 — 10 μg/kg) in Anesthetized Control and Diabetic Rats

Fig. 3. Concentration-Response Curves for Changes in BP Produced by PAF and ACh in Anesthetized Control and Diabetic Rats

●, control; ○, diabetic. Each point represents the mean ± S.E. of 7 animals. a, b) Significantly different from control at p<0.05, p<0.01.
injection of ACh, at doses of 3 and 10 μg/kg, produced a marked decrease in HR of both groups of rats. However, there was no significant difference in ACh-induced changes of HR between control and diabetic rats (data not shown).

**Perfused Mesenteric Arterial Bed of Rat**

The basal perfusion pressure of the control and diabetic rat mesenteric arterial bed was 40 ± 1 mmHg and 32 ± 1 mmHg (n = 33), respectively. Perfusion with methoxamine (10⁻⁵—10⁻⁴ M) increased the perfusion pressure to 97 ± 3 mmHg (n = 33) in the control group and to 80 ± 3 mmHg (n = 27) in the diabetic group, respectively.

**Relaxation in Response to PAF**

In the perfused mesenteric arterial bed precontracted with methoxamine (10⁻⁵—10⁻⁴ M), and PAF (3 × 10⁻¹²—3 × 10⁻¹⁰ M) produced a concentration-dependent relaxation. As shown in Fig. 4, the relaxation response induced by PAF in the diabetic group was significantly attenuated compared with that in the control group at 10⁻¹¹ M or more.

**Relaxation in Response to ACh**

Increasing doses of ACh produced a concentration-dependent vasodilation both in control and diabetic preparations (Fig. 5). However, the concentration-response curve of the diabetic group was significantly shifted to the right compared with that of the control group.
A pretreatment of the preparation with $10^{-6}$ M oxyhaemoglobin shifted the curve of the control group to the right further. The concentration–response curve of the diabetic group was also shifted to the right by the pretreatment.

**Relaxation in Response to SNP**

Increasing doses of SNP produced a concentration-dependent vasodilation both in control and diabetic preparations (Fig. 6). However, there was no significant difference in vasodilator response to SNP between the control and diabetic groups.

**Discussion**

The present study demonstrated that PAF- and ACh-induced depressor action in vivo were significantly attenuated in diabetic rats. It is now well established that vasoactive agents, such as ACh, histamine, and bradykinin produce an endothelium-dependent relaxation through the release of EDRF from endothelial cells.\(^{12}\) PAF has been shown to produce a strong and long-lasting hypotension in various animal species.\(^{1}\) Although this action of PAF was thought to be endothelium-dependent,\(^{13-16}\) this effect was exhibited in a rather high dose of PAF compared with a dose which produces hypotension in vivo. The vasodilator effect of PAF has been reported to be independent of the autonomic nervous system, purinergic mechanisms and arachidonic acid metabolites in the perfused dog hind limb.\(^{17}\) Other studies in the rat hindquarter have suggested that PAF-induced hypotension is not related to cholinceptors, histamine receptors, or β-adrenceptors but is an indirect vasodilation, mediated by unknown factor(s).\(^{18}\) Moreover, PAF did not induce platelet aggregation in rats.\(^{19}\) Recent studies have demonstrated that extremely low concentrations of PAF produced a long-lasting endothelium-dependent relaxation in the mesenteric arterial bed and this action was mediated by a vasodilator substance released from endothelial cells but which is not a prostaglandin.\(^{5,5}\) Moreover, the endothelium-dependent relaxation in resistance vessels may be the main mechanism which induces hypotension in vivo.\(^{4}\)

On the other hand, diabetes has been reported to produce a specific impairment of endothelium-dependent relaxation in the aorta of STZ-induced diabetic rats,\(^{6,8}\) spontaneously diabetic rats\(^{7}\) and alloxan-induced diabetic rabbits\(^{9,10}\) in vitro. From the present results and previous reports, it is possible that the attenuation of PAF- and ACh-induced depressor effect may be due to an impairment of endothelial function, which decreases production and/or release of EDRF, in the mesenteric artery. In the present study, the duration of PAF-induced hypotension was significantly shortened in diabetic rats, suggesting that the activity of a PAF degrading enzyme might be increased in diabetic rats.

To clarify the mechanism of the attenuation of the depressor effect in diabetic rats, the relaxation responses of PAF and ACh were investigated in the perfused mesenteric arterial bed. Because the aorta offers a resistance to flow, the contribution of vessels of smaller diameter to peripheral vascular resistance is much greater than that of large vessels. Thus, the regulation of this bed may make a significant contribution towards systemic BP and circulating blood volume. In a perfused mesenteric arterial bed, the significant attenuation of PAF-induced vasodilation was observed in the diabetic preparation. Furthermore, ACh-induced vasodilation was also attenuated in the diabetic preparation. Pretreatment with oxyhaemoglobin shifted the concentration–response curve of ACh to the right in both control and diabetic rats. Oxyhaemoglobin has been reported to inhibit the EDRF-induced vasodilation by binding EDRF.\(^{20}\) This data shows EDRF may also be involved in the vasodilation of the mesenteric arterial bed induced by ACh. In contrast, concentration–response curves for the relaxation effect of SNP, an endothelium-independent substance,\(^{21}\) showed no significant difference between the control and diabetic preparation of mesenteric arterial bed. Thus, these findings demonstrated that the state of diabetes mellitus causes the selective impairment of the endothelial cells but not vascular smooth muscle. Recently, nitric oxide production by vascular endothelial cells contributes to the maintenance of BP.\(^{22,23}\)
Taking these findings and present results into consideration, the BP in diabetic rats should be higher than that in control rats. In the present study, however, the resting BP in diabetic rats was not different from that in control rats. The reason for this result is not clear at present, but it is conceivable that changes in the tone of the autonomic nervous system, production of prostacyclin\textsuperscript{24} and/or changes in activities of calcitonin gene-related peptide neurons\textsuperscript{25} may be involved in the maintenance of BP in diabetic rats. Of particular interest in this connection is that previous reports showed conflicting results concerning the elevation of BP in STZ-induced diabetic rats, e.g., Kawashima et al.\textsuperscript{26} have shown the significant elevation of BP in STZ-induced rats, whereas, Kusaka et al.\textsuperscript{27} have concluded that STZ-induced hypertension does not exist. Moreover, in the present study, the function of the endothelial cells may not be completely impaired, since the maximal relaxation response of mesenteric arterial bed by ACh in diabetic rats was not different from that in control rats.

In conclusion, the impairment of the endothelial function in the resistance vessels may be responsible for the attenuation of PAF- and ACh-induced depressor effects in diabetic rats.

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References

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