Acetylcholine-induced vasodilation in the perfused kidney of the streptozotocin-induced diabetic rat: role of prostacyclin

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Abstract

Using the perfused kidneys of age-matched controls and streptozotocin (STZ)-induced diabetic rats, we previously demonstrated that endothelial dysfunction is present in STZ-induced diabetic rats and that acetylcholine (ACh) increases the level of 6-keto-prostaglandin F1α (a metabolite of prostacyclin) in the effluent from such perfused kidneys. Here, we investigated whether the ACh-induced relaxation in the perfused kidney is modulated by prostacyclin and/or thromboxane A2 (TXA2) in the STZ-induced diabetic state. ACh-induced renal vasodilation was significantly weaker in STZ-induced diabetic rats than in age-matched controls, and it was not affected by treatment with 10 µM furegrelate (TXA2-synthase inhibitor) or 1 µM SQ29548 (TXA2-receptor antagonist) in either group. However, it was attenuated by 10 µM tranylcypromine (prostacyclin-synthesis inhibitor), but only in the diabetic group. These results suggest that the endothelium-dependent relaxation induced by ACh in the renal vascular bed of STZ-induced diabetic rats is regulated by prostacyclin, not by TXA2. Increased prostacyclin-signaling may occur to help compensate for the impaired endothelial function seen in the kidney in long-term diabetic states.

Key words: acetylcholine, endothelium, kidney, perfusion pressure, prostacyclin, rat

Introduction

Acetylcholine (ACh) induces vasodilation by stimulating the release of endothelium-derived relaxing factors (EDRFs) in a variety of vascular beds (Furchgott and Zawadzki, 1980; Cohen, 1995; Pieper, 1998). Although nitric oxide (NO) appears to account for the actions of ACh in many settings, elimination of NO (e.g., by hemoglobin or NO-synthase inhibitors) does not always prevent endothelium-dependent vasodilation. Thus, evidence has accrued to suggest
that EDRFs other than NO may contribute to the vascular actions of ACh (Triggle et al., 2003; Matsumoto et al., 2004a; Tanaka et al., 2004). It is well appreciated that ACh stimulates the endothelial production of vasodilatory prostaglandins (Salom et al., 1991; Majid and Navar, 1992; Vizioli et al., 2005), and current evidence suggests that an endothelium-derived hyperpolarizing factor (EDHF) may also contribute to the vasodilator actions of ACh (Busse et al., 2002; Chen et al., 1988; Matsumoto et al., 2003a, 2003b, 2005, 2006a, 2006b; Takano et al., 2005; Yamamoto and Suzuki, 2005). Thus, EDRFs distinct from NO may mediate part of the ACh-induced vasodilation. Although ACh is known to be a potent vasodilator of the renal vasculature (Ito et al., 1991; Hayashi et al., 1992; Lerman and Rodriguez-Porcel, 2001), the mechanisms mediating this response have not been clearly defined.

The pathophysiological changes seen in diabetic nephropathy are histologically different from those seen in other types of renal disease (Sowers and Epstein, 1995; Thomson et al., 2004; Wolf et al., 2005). In fact, renal hemodynamic changes may make an important contribution to diabetic nephropathy, and it is well known that vascular disease is one of the complicating features of diabetes mellitus in humans (Anderson and Vora, 1995; Sowers and Epstein, 1995). The reactivity of vascular smooth muscle and the endothelium to vasoactive agents has been extensively studied in diabetes in both experimental animals and humans (Poston and Taylor, 1995; De Vriese et al., 2000b; Fitzgerald et al., 2005; Kobayashi et al., 2004, 2005; Matsumoto et al., 2004b). Concerning the macrovasculature, an accumulating body of evidence suggests that the relaxation responses induced in the aorta by endothelium-dependent agents are weaker in streptozotocin (STZ)-induced diabetic animals than in non-diabetic control animals (Kamata et al., 1989, 1999; De Vriese et al., 2000b; Kobayashi et al., 2000). Moreover, several reports have indicated that diabetic animals show an impairment of ACh-induced, endothelium-dependent hyperpolarization/relaxation in the microvasculature (Fukao et al., 1997; Makino et al., 2000; Fitzgerald et al., 2005). On the other hand, an overproduction of endothelium-derived contracting factor (EDCF), most likely prostanooids, has been implicated in the pathophysiology of endothelial dysfunction (De Vriese et al., 2000b; Vanhoutte et al., 2005). These EDCFs are thought to be released together with EDRFs and to oppose their effects on the smooth muscle cells. Although the balance between EDRFs and EDCFs may play an important role in setting vascular tone in physiological and pathophysiological states, little information is available concerning any changes that may occur during experimental diabetes in the endothelium-dependent relaxation induced by vasoactive agents in the isolated perfused kidney.

We therefore set out to remedy this situation. We previously suggested that (a) the endothelium-dependent vasodilation induced by ACh in the renal vascular bed of age-matched control rats is due to the release of NO and EDHF, (b) whereas the vasodilation induced by ACh in the STZ-diabetic kidney is mediated by prostacyclin as well as by NO and EDHF on the ground that ACh increased the level of 6-keto-prostaglandin F$_1\alpha$ (a metabolite of prostacyclin) in the effluent from the perfused diabetic kidneys (Kamata and Hosokawa, 1997b). Therefore, in the present we investigated the effect of a suppression of prostanooid signaling on ACh-induced endothelium-dependent relaxation in the isolated perfused kidney as a way of clarifying the involvement of endothelium-derived prostanooids.
**Methods**

**General**

The experimental design was approved by the Hoshi University Animal Care and Use Committee, and all studies were conducted in accordance with “Guide for the Care and Use of Laboratory Animals” published by the US National Institute of Health, and “Guide for the Care and Use of Laboratory Animals” adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University (accredited by the Ministry of Education, Culture, Sports, Science, and Technology, Japan).

**Materials**

Bovine serum albumin (fraction V) (BSA), heparin, methoxamine hydrochloride, papaverine, and streptozotocin (STZ) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Sodium furegrelate and SQ29548 were from Cayman Chemical (Ann Arbor, MI, U.S.A.). Tranlycypromine hydrochloride was from Biomol International, L.P. (Plymouth Meeting, PA, U.S.A.). Acetylcholine chloride (ACh) was from Daiichi Pharmaceuticals (Tokyo, Japan). All concentrations are expressed as the final molar concentration of the base in the organ bath.

**Animal model of diabetes**

Male Wistar rats (8 wk old and 200–220 g body weight) received a single injection via the tail vein of STZ 60 mg/kg dissolved in a citrate buffer. Age-matched control rats were injected with the buffer alone. Food and water were given ad libitum. The experiments described here were performed ten weeks after the injection. Ten weeks after the administration of STZ (diabetic group) or buffer (control group), plasma glucose was determined using a commercially available enzyme kit (Wako Chemical Company, Osaka, Japan).

**Preparation of the perfused kidneys**

Perfusion pressure was recorded from the rat kidney as in our previous papers (Kamata and Hosokawa, 1997a, 1997b; Kamata and Hayashi, 1999; Kamata and Mizutani, 1999; Kamata and Yamashita, 1999). Rats were anesthetized with pentobarbital (50 mg/kg intraperitoneally) and then given an intravenous injection of 1,000 units/kg of heparin. The rats were killed by decapitation, the abdomen was opened by midline incision, and the left renal artery was cannulated via an incision made in the aorta. An incision was also made in the left renal vein for the insertion of a cannula made from PE90 tubing. Starting 10 min after decapitation, the kidney was perfused with warm (37°C), oxygenated (95% O₂, 5% CO₂) Krebs-Henseleit solution (KHS). This solution consisted of (in mM) 118.0 NaCl, 4.7 KCl, 25.0 NaHCO₃, 1.8 CaCl₂, 1.2 NaH₂PO₄, 1.2 MgSO₄, 11.1 dextrose, and 0.25% BSA. A constant-flow perfusion pump (Model MP-3; Tokyo Rikakikai, Tokyo, Japan) was used for the perfusion, which proceeded at a rate of 4 ml/min through a cannula inserted into the aorta. The venous effluent was re-circulated (except during the first 60 min; see below), and renal perfusion pressure was 65 ± 5.8 mmHg when the renal vascular bed was perfused at the rate mentioned above. Under these conditions, ACh-induced
relaxation was maximal (data not shown). Vascular responses were detected as changes in perfusion pressure, which was monitored continuously via a pressure transducer (Model AP-2001; Nihon Kohden, Tokyo, Japan) and recorded on a pen recorder (Model 3021; Yokogawa, Tokyo, Japan). Following the 60-min equilibration period, the perfusion circuit was transformed into a closed system (i.e., the perfusion solution from the vein was collected in a second bath and re-circulated through the kidney).

After equilibration, the perfused-kidney preparation was constricted by perfusion with $5 \times 10^{-7}$ to $3 \times 10^{-6}$ M methoxamine, which resulted in a perfusion pressure of 150–160 mmHg. It was then relaxed by perfusion with $10^{-6}$ M ACh, to confirm the integrity of the endothelium. For the relaxation studies, the perfused kidneys were preconstricted with an equieffective concentration of methoxamine ($5 \times 10^{-7}$ to $3 \times 10^{-6}$ M). This concentration produced 85–90% of the maximal response. When the methoxamine-induced response had reached a plateau, ACh ($10^{-10}$ to $3 \times 10^{-6}$ M) was added cumulatively. To standardize the vasodilator responses obtained with ACh, papaverine ($10^{-4}$ M) was injected into each kidney, and the resulting vasodilator response was expressed as 100%. To investigate the effects of furegrelate (thromboxane A$_2$ (TXA$_2$)-synthase inhibitor; $10^{-5}$ M), SQ29548 (TXA$_2$-receptor antagonist; $10^{-6}$ M) and tranylcypromine (prostacyclin synthesis inhibitor; $10^{-5}$ M) on the ACh-induced response, the perfused kidney was incubated in the appropriate solution for 20 min before the addition of methoxamine. None of the inhibitors used in this study altered the basal tension in the perfused kidneys from the all groups.

**Statistical analysis**

Data are expressed as the mean ± standard error of mean (S.E.M.). Multiple comparisons between treatment groups were performed using an analysis of variance (ANOVA) followed by a Bonferroni test.

**Results**

**General characteristics of the experimental animals**

Ten weeks after treatment with STZ, the body weight was significantly lower, and the plasma glucose concentration significantly higher, in diabetic rats than in the age-matched controls (data not shown).

**Relaxation response to ACh and effects of various agent on this response**

In the perfused methoxamine-preconstricted kidney, cumulative application of ACh ($10^{-10}$ to $3 \times 10^{-6}$ M) caused a concentration-dependent vasodilation that reached its maximum at $3 \times 10^{-6}$ M. The ACh-induced vasodilation was significantly weaker in diabetic rats (Fig. 1). To examine the part played by TXA$_2$ or prostacyclin in the present ACh-induced relaxation, the perfused kidneys were incubated with furegrelate (thromboxane A$_2$ (TXA$_2$)-synthase inhibitor; $10^{-5}$ M) (Vila et al., 2001; Bolla et al., 2004), SQ29548 (TXA$_2$-receptor antagonist; $10^{-6}$ M) (Hernanz et al., 2003; Bolla et al., 2004) or tranylcypromine (prostacyclin synthesis inhibitor; $10^{-5}$ M) (Blanco-Rivero et al., 2005). Pretreatment of the kidney with furegrelate ($10^{-6}$ M) (Fig. 2) or
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with SQ29548 (10⁻⁶ M) (Fig. 3) had no effect on the ACh-induced vasodilation in either the age-matched controls or the STZ-induced diabetic group. Interestingly, pretreatment of the kidney with tranylcypromine (10⁻⁵ M) led to a significant attenuation of the ACh-induced vasodilation in the diabetic group but not in control group (Fig. 4).
In the present study, on the perfused kidneys of STZ-induced diabetic rats, we (a) confirmed that ACh-induced relaxation is impaired (vs. age-matched control rats) and (b) found evidence...
that this relaxation is mediated not only by NO and EDHF, but also by prostacyclin, whereas endothelium-derived TXA₂ makes little or no contribution. The above conclusions are supported by the following observations made either previously (1, 2 and 5) or in the present study (3 and 4): 1) ACh-induced vasodilation in the perfused kidneys was significantly weaker in STZ-induced diabetic rats than in age-matched controls, and while it was completely abolished by treatment with 60 mM K⁺ plus L-NNA plus methylene blue in the control rats, it was significantly (but not completely) inhibited by such treatment in the diabetic rats (Kamata and Hosokawa, 1997b). 2) ACh-induced vasodilation in the perfused kidneys was not affected by indomethacin in control rats, but it was attenuated by indomethacin in STZ-diabetic rats (Kamata and Hosokawa, 1997b), 3) ACh-induced renal vasodilation was not affected by SQ29548 and/or furegrelate in either of the present groups (Figs. 2 and 3), 4) ACh-induced renal vasodilation was inhibited by tranylcypromine in diabetic rats, but not in control rats (Fig. 4), and 5) ACh increased the level of 6-keto-prostaglandin F₁α in the effluent from the perfused kidneys of diabetic rats (Kamata and Hosokawa, 1997b).

In the present study, an impairment of ACh-induced vasodilation was seen in the perfused kidneys of STZ-induced diabetic rats, as previously reported for other vessels in this diabetic model (Kamata et al., 1989; Kamata and Kobayashi, 1996; De Vriese et al., 2000b; Kobayashi et al., 2000; Matsumoto et al., 2003a, 2004b; Kamata et al., 2005). This is in agreement with previous reports both by us (Kamata and Hosokawa, 1997b; Kamata and Hayashi, 1999; Kamata and Yamashita, 1999) and by others (Dai et al., 1993; Yousif, 2003), but in contrast to reports by Bhardwaj and Moore (1988), Gebremedhin et al. (1989) and Alabadi et al. (2001) who reported enhanced endothelium-dependent vasodilation of the renal vascular bed to ACh in experimentally induced diabetes. Our results also differs from those published by Beenen et al. (1996) and Garcia et al. (1999) who reported that endothelium-dependent relaxation in the isolated perfused kidney was not altered in experimentally induced diabetes. The reason for these discrepancies concerning the effect of diabetes on endothelium-dependent relaxation remains unclear. It does not appear to be related to differences in animal species or vessel size.

Impaired endothelium-dependent vasodilation may arise as a result of several mechanisms: decreased production of one of the EDRFs, enhanced inactivation of EDRF, impaired diffusion of EDRF to the underlying smooth muscle cells, decreased responsiveness of the smooth muscle to EDRF, and/or enhanced generation of EDCF s (Poston and Taylor, 1995; De Vriese et al., 2000b; Fitzgerald et al., 2005; Kobayashi et al., 2004, 2005; Matsumoto et al., 2004b). In vascular tissues in diabetes, ACh-induced relaxation appears to be blunted by the synthesis of an endothelium-derived constrictor prostanoid(s) (Tesfamariam et al., 1989). In fact, the impaired response to ACh seen in the diabetic rat aorta (Shimizu et al., 1993), diabetic rabbit aorta (Tesfamariam et al., 1989), and diabetic rat brain pial artery (Mayhan et al., 1991) can be normalized by treatment with a COX inhibitor and/or a PGH₂-TXA₂ receptor antagonist. In the present study, preincubation with SQ29548 did not affect the ACh-induced relaxation in either group. Moreover, the thromboxane-synthase inhibitor furegrelate did not modify this response in either group. These results suggest that TXA₂ is not an important candidate for the modulation of endothelium-dependent relaxation in the present perfused kidney. In our previous (Kamata and Hosokawa, 1997b) and present studies, we found that in age-matched
control rats, the ACh-induced vasodilation in perfused kidneys was not affected by indomethacin (Kamata and Hosokawa, 1997b) or by tranylcyromine (Fig. 4), effectively ruling out the involvement of vasodilator prostanoids (e.g., prostacyclin) in non-diabetic rats. In sharp contrast, the same response in the diabetic rats was significantly inhibited by indomethacin (Kamata and Hosokawa, 1997b) and by tranylcyromine (Fig. 4), indicating that prostacyclin, as well as NO and EDHF, may be involved in ACh-induced renal vasodilation in the STZ-induced diabetic rat. This conclusion is supported by previous findings that (a) ACh-induced increment in 6-keto-prostaglandin F$_{1a}$ (a metabolite of prostacyclin) is seen in the renal perfusate in STZ-induced diabetic rats (Kamata and Hosokawa, 1997b), (b) prostacyclin, a powerful vasodilator, is synthesized by endothelial cells (Moncada et al., 1977), and (c) renal prostacyclin synthesis is increased at various stages of diabetes (Schambelan et al., 1985; Kopecky and Schroeder, 1988; Chang et al., 1991).

Although we are unclear as to the mechanism underlying the increase in prostacyclin signaling in perfused kidneys from STZ-induced diabetic rats, we think that important factors may be the presence of substantial compensatory interactions among the three EDRFs (viz. NO, prostacyclin, EDHF) within a given blood vessel. Indeed, in our previous studies the impairment of ACh-induced endothelium-dependent vasodilation in the diabetic perfused kidney was attributed to a decrease in NO or EDHF signaling (Kamata and Hosokawa, 1997b; Kamata and Yamashita, 1999). Moreover, there are reports of impaired NO signaling (Nangle et al., 2006) and EDHF signaling (De Vriese et al., 2000a; Nangle et al., 2006) in diabetic renal arteries. Thus, prostacyclin signaling may be enhanced as a way of partially or completely restoring endothelial vasodilator function in diabetes. Indeed, there are several pieces of evidence to suggest that such compensatory interactions occur in various pathophysiological states (Triggle et al., 2003; Bryan et al., 2005).

In conclusion, we have found evidence that the endothelium-dependent relaxation induced by ACh in the renal vascular bed is at least partly regulated by prostacyclin (but not by TXA$_2$) in STZ-induced diabetic rats, but not in control rats. Possibly, an increased in prostacyclin signaling may occur as a compensatory mechanism when endothelial function is impaired in the kidney during long-term diabetic states.

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**References**


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