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Increased vasodilator response to acetylcholine of renal blood vessels from diabetic rats

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Abstract—The vasodilator effect of acetylcholine (ACh) and nitroprusside and the vasoconstrictor effect of noradrenaline was assessed in the perfused kidney of streptozocin diabetic rats. Compared with control animals injected with acidified saline, the renal vasoconstrictor effect of noradrenaline was increased in diabetic rats both in terms of the dose required to produce 50% of the maximal effect (EC50) and in the maximal response achieved. The renal vasodilator effect of ACh (but not nitroprusside) was similarly enhanced in diabetic animals. The effect of ACh (but not nitroprusside) in the perfused kidney of both control and diabetic rats was reduced or abolished by mepacrine (10 μM), metyrapone (10 μM) or methylene blue (100 μM) suggesting that ACh exhibits vasodilator activity in the rat kidney by virtue of releasing endothelium derived relaxing factor (EDRF). These results are in contrast to previous published reports demonstrating reduced biosynthesis of EDRF in the aorta of diabetic rats. The mechanism which underlies the increased renal vascular response to ACh is not known. However, increased endothelial cell turnover or cholinoceptor number, elevated activity of enzyme(s) which synthesise EDRF or hyperresponsiveness of vascular smooth muscle to released EDRF should all be considered.

The vasodilator effect of acetylcholine (ACh) in preconstricted rings or bands of isolated arteries and on resistance blood vessels of perfused organs is mediated by a chemically unstable substance called endothelium derived relaxing factor (EDRF) (Furchgott 1983). The synthesis and release of EDRF from vascular endothelial cells, by regulating the calibre of resistance blood vessels, may play a part in controlling vascular perfusion within individual organs (Vanhoutte 1987). Conversely, impaired production of EDRF may contribute to the vasospasm of coronary arteries associated with angina pectoris (Ganz & Alexander 1985) and with the increased peripheral resistance in hypertension (Konishi & Su 1983; Lockette et al 1986). Recently, Oyama et al (1986) provided evidence that aortic EDRF biosynthesis was similarly reduced in rats made diabetic by streptozocin injection. This finding raises the possibility that a deficiency of vascular EDRF may contribute to the cardiovascular pathology of this condition. Since diabetes in man is associated with profound changes in the structure and function of the renal vasculature we have examined the response to an endothelium dependent (ACh) and an endothelium independent (nitroprusside) vasodilator drug of the rat perfused kidney taken from streptozocin diabetic rats.

Methods

Induction of experimental diabetes in rats. Male rats (Wistar, 250–300 g) were injected intraperitoneally with streptozocin (80 mg kg^{-1}) dissolved in saline (0·9% NaCl) acidified to pH 4·2 with HCl (0·1M). Control animals were injected with an equal volume of acidified saline. Blood samples (0·5 mL) were collected by cardiac puncture of ether anaesthetized animals, anticoagulated with heparin (2 u mL^{-1}) and centrifuged (1,000 g, 20 min, room temperature) to prepare plasma. Animals were killed either before (T = 0) or 12 days after streptozocin or acidified saline injection. Plasma glucose was assayed spectrophotometrically at a wavelength of 520 nm using a commercially available diagnostic kit (Sigma).

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Perfusion of rat kidney. Kidney perfusion was performed essentially as described by Armstrong et al (1976). Briefly, the left rat kidney cannulated via the descending abdominal aorta was removed to a heated, water-jacketed chamber. Preparations were perfused (8 mL min^{-1}) with warmed (37°C), oxygenated (95% O_2 : 5% CO_2) Krebs solution (composition, mM; NaCl 118, KCl 4·75, $MgSO_4$ 1·19, $NaHCO_3$ 25, K_2HPO_3 1·19, $CaCl_2$ 1·9, glucose 11·1, pH 7·2) containing indomethacin (8 μM) to prevent release of vasodilator prostanoids such as prostacyclin (PGI_2). Perfusion pressure was monitored continuously by means of a Bell & Howell pressure transducer connected to a Devices pen recorder. Drugs were injected in volumes of less than 20 μL to prevent vascular effects due to an injection artifact. After an equilibration period of 30 min, noradrenaline was injected using a dose cycle time of 3 min. Vasodilator responses to ACh and nitroprusside were assessed in so-called "high tone" preparations which were partially vasoconstricted by inclusion in the perfusing Krebs solution of a concentration of noradrenaline producing approximately 60–80% of the maximal response. A dose cycle time of 5 min was employed for both vasodilator drugs. Inhibitor drugs were added to the perfusing Krebs solution and allowed to perfuse the kidney for 20 min before injection of vasodilator drugs. Preparations were weighed before and after each experiment which typically had a duration of 3–4 h.

Statistics. Results show mean ± s.e. mean with the number of observations shown in parentheses. Statistically significant differences between groups were determined using Student's unpaired t test. A probability (P) value of 0·05 or less was taken to indicate statistical significance.

Materials. Acetylcholine chloride, histamine dihydrochloride, indomethacin, mepacrine dihydrochloride, methylene blue, metyrapone, sodium nitroprusside, streptozocin and (−)-noradrenaline bitartrate were obtained from Sigma Ltd. Indomethacin was dissolved in Na_2CO_3 (0·5% w/v). All other drugs were dissolved in NaCl (0·9% w/v).

Results

Animals injected with streptozocin exhibited polydipsia and polyuria thereby providing indirect evidence for the production of experimental diabetes in these animals. Furthermore, streptozocin injection increased plasma glucose concentration. Thus, within 12 days of streptozocin administration plasma glucose concentration was increased approximately 4 fold ($16{\cdot}7 \pm 1{\cdot}1$ mmol L^{-1}, n = 17, c.f. $4{\cdot}0 \pm 0{\cdot}6$ mmol L^{-1} n = 15, in control animals injected with acidified saline, $P < 0{\cdot}001$). In control non-injected (T = 0) animals, plasma glucose concentration was $4{\cdot}1 \pm 0{\cdot}6$ mmol L^{-1} (n = 8). In addition, diabetic rats gained less weight ($36{\cdot}5 \pm 5{\cdot}5$ g, n = 17) than control animals ($87{\cdot}2 \pm 11{\cdot}1$ g, n = 15) although kidney weight was not significantly different between the two groups ($2{\cdot}3 \pm 0{\cdot}6$ g, n = 15, in control and $2{\cdot}4 \pm 0{\cdot}7$ g, n = 17, in diabetic rats). Kidneys from control and diabetic rats did not change in weight ($2{\cdot}4 \pm 0{\cdot}8$ g, n = 15, and $2{\cdot}5 \pm 0{\cdot}6$ g, n = 17, respectively, both $P > 0{\cdot}1$) following perfusion for up to 4 h suggesting that preparations did not become oedematous in the course of the experiment.

Basal perfusion pressure of kidneys from diabetic rats ($172{\cdot}0 \pm 14{\cdot}8$ mm Hg, n = 17) was not significantly different from perfusion pressure of kidneys from control (T = 0) animals ($167{\cdot}4 \pm 7{\cdot}4$ mm Hg, n = 15) or rats injected with acidified saline ($168{\cdot}5 \pm 11{\cdot}2$ mm Hg, n = 15). Bolus injections of increasing doses of noradrenaline resulted in transient and dose related increases in perfusion pressure of the rat kidney from control (T = 0), streptozocin and acidified saline-injected animals (Fig. 1). In

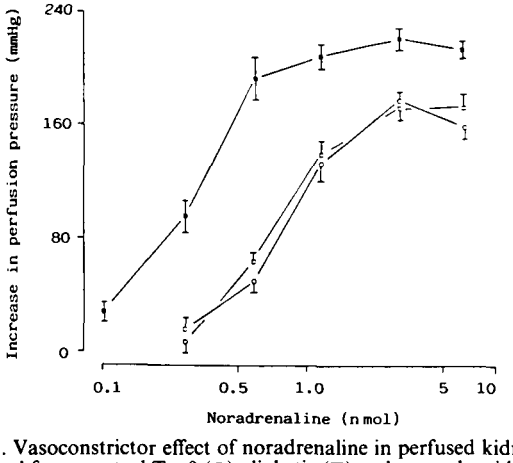

FIG. 1. Vasoconstrictor effect of noradrenaline in perfused kidneys obtained from control T = 0 (○), diabetic (■) and control, acidified saline-injected (□) rats. Results show increase in renal perfusion pressure in mm Hg and are mean ± s.e. mean, n = 10–17.

each group of animals responses to noradrenaline were unchanged throughout the time course of the experiment. Animals injected with acidified saline showed unchanged renal vascular response to noradrenaline (EC50 $0{\cdot}8 \pm 0{\cdot}2$ nmol, maximal response $176{\cdot}0 \pm 16{\cdot}5$ mm Hg, n = 15) compared with T = 0 animals (EC50 $0{\cdot}9 \pm 0{\cdot}1$ nmol, maximal response $181{\cdot}6 \pm 11{\cdot}1$ mm Hg, n = 15). In contrast, noradrenaline exhibited more potent vasoconstrictor activity in kidneys from diabetic animals (EC50 $0{\cdot}3 \pm 0{\cdot}1$ nmol, maximal response $231{\cdot}0 \pm 9{\cdot}8$ mm Hg, n = 17, both $P < 0{\cdot}05$).

Streptozocin (50 μM) added to the perfusing Krebs solution did not affect the vasoconstrictor effect of noradrenaline in acidified saline injected (EC50 $1{\cdot}0 \pm 0{\cdot}2$ nmol, maximal response $179{\cdot}6 \pm 12{\cdot}2$ mm Hg, n = 5) or diabetic (EC50 $1{\cdot}2 \pm 0{\cdot}3$ nmol, maximal response $167{\cdot}9 \pm 21{\cdot}1$ mm Hg, n = 5) animals.

Both ACh and nitroprusside exhibited dose-related and transient falls in perfusion pressure indicating vasodilatation of the noradrenaline-preconstricted ("high tone") rat kidney (Fig. 2). Responses to both vasodilators were unchanged

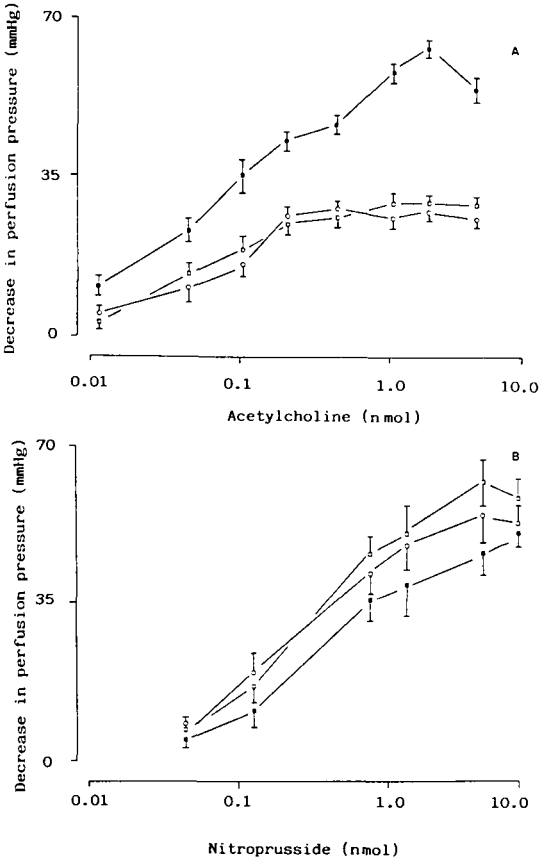

FIG. 2. Vasodilator effect of acetylcholine (A) and nitroprusside (B) in perfused kidneys obtained from control T = 0 (○), diabetic (■) and acidified saline-injected (□) rats. Results show decrease in renal perfusion pressure in mm Hg and are mean ± s.e. mean, n = 10–17.

throughout the period of the experiment. The vasodilator effect of ACh (but not nitroprusside) was enhanced in diabetic animals when compared with the effect in kidneys of control rats as evidenced by a shift to the left in the log dose-response curve and an increase in the maximal response of approximately 30 mm Hg.

Addition to the perfusing Krebs solution of mepacrine (10 μM), methylene blue (10 μM) or metyrapone (30 μM) substantially reduced or abolished the vasodilator response to acetylcholine in both control and diabetic rat kidney. The response to nitroprusside was not influenced by these drugs. Addition of streptozocin (50 μM) to the Krebs solution perfusing control or diabetic rat kidneys did not influence the vasodilator effect of either acetylcholine or nitroprusside. These results are summarized in Table 1.

Discussion

The vasodilator effect of ACh on resistance blood vessels of the perfused rat lung (Cherry & Gillis 1987), rabbit heart (Stewart et al 1987) and rabbit ear (Griffiths et al 1987) is reportedly mediated by EDRF. In each of these studies, this conclusion is based largely upon the finding that a range of drugs which prevent the formation or activity of EDRF in isolated rings or bands of large arteries similarly inhibit the vasodilator activity of ACh in perfused organs. In the present study, the vasodilator effect of ACh, but not that of nitroprusside, was reduced or abolished by mepacrine, metyrapone or methylene blue all of which have been shown to inhibit the relaxant effect of ACh in large arteries (Forstermann & Neufang 1984; Martin et al 1985).

Table 1. Effect of drugs on the vasodilator response to ACh and nitroprusside (NP) in normal and diabetic rat kidney.

	Vasodilator response (mm Hg)			
	Control		Diabetic	
	ACh	NP	ACh	NP
No drug	32·2 ± 2·2	57·0 ± 5·4	62·3 ± 3·4	45·4 ± 3·9
Mepacrine	2·1 ± 0·9*	49·9 ± 8·7	0	41·1 ± 4·3
Metyrapone	3·4 ± 1·2*	49·7 ± 6·5	1·1 ± 0·9*	38·5 ± 7·4
Methylene blue	1·2 ± 0·9*	50·0 ± 3·4	0	47·6 ± 4·9
Streptozocin	34·5 ± 1·9	56·7 ± 4·5	65·5 ± 5·6	49·9 ± 7·6

Responses to ACh (1·0 nmol) and nitroprusside (NP, 5·0 nmol) in the perfused "high tone" control (acidified saline injected) and diabetic rat kidney are shown. Doses of vasodilator drugs were chosen which produced approximately 80% of the maximal response. Preparations were perfused with mepacrine (10 μM), metyrapone (10 μM), methylene blue (100 μM) or streptozocin (50 μM) for 20 min before challenge with vasodilator drug. For control experiments preparations were perfused with Krebs solution containing 0·9% w/v NaCl (0·5 mL added to 200 mL Krebs) as diluent. Results show mean ± s.e. mean, n = 8, * $P < 0·001$.

Thus, as in other perfused organs, it seems likely that the vasodilator effect of ACh on resistance blood vessels of the perfused kidneys from both control and diabetic rats is indeed mediated by EDRF.

We believe that the changes in renal vascular response to standard vasoconstrictor and vasodilator drugs in streptozocin-injected rats reported in this study reflect the production of experimental diabetes in these animals. We cannot exclude the possibility that rats treated in this way experienced renal failure secondary to diabetes which, in turn, influenced the vascular responses of the perfused kidney. However, this seems unlikely in that rats administered streptozocin exhibited marked polydipsia and polyuria and did not gain weight suggesting the absence of oedema which is an early clinical feature of renal failure in human diabetics. Additionally, the effects described in this paper are most probably not a direct action of streptozocin which has a reported serum half life in the rat of approximately 15 min (Rossini et al 1977) and thus little or no unchanged drug would be expected to occur in the bloodstream of animals 12 days after injection. Furthermore, streptozocin added to the Krebs solution perfusing control or diabetic rat kidneys, had no effect on the renal response to noradrenaline, ACh or nitroprusside at least at a concentration of 50 μM.

Of major interest in the present work is the finding of increased vasodilator responses to ACh in the kidney of streptozocin diabetic rats. The precise mechanism of this effect is not clear although several possible explanations exist. For example, an increase in the total number and the turnover of endothelial cells lining resistance blood vessels in these organs has been reported in rats with experimentally induced diabetes (Moore & Frew 1965). Such endothelial cell proliferation, if it occurred in the kidneys of diabetic animals in this study, may result in enhanced responses to ACh by virtue of an elevation in the total number of endothelial muscarinic cholinoceptors. This explanation is supported by the observation that the maximal response to ACh was increased in diabetic rat kidneys compared with kidneys from control animals. However, radioligand binding studies in atria from streptozocin diabetic rats have revealed a decrease not an increase in muscarinic cholinoceptor density (Carrier & Aronstam 1987). Similar measurements have not been performed in diabetic rat kidney and thus the possibility that changes in cholinoceptor number on renal endothelial cells underlies the increase in sensitivity to ACh observed in this study cannot be discounted.

An alternative explanation is that cholinoceptor activation in the diabetic rat kidney results in an increase in the biosynthesis or activity of released EDRF. This conclusion is supported by the observation that the response to nitroprusside in the kidney was not increased in diabetic rats. Since EDRF has recently been identified as nitric oxide (Palmer et al 1987) and the response to nitroprusside, which depends upon degradation to nitric oxide for its vasodilator effect (Katsuki et al 1977), was unchanged in diabetic kidney, it seems unlikely that the present results are due to vascular hyperresponsiveness to EDRF.

Consequently, diabetic resistance blood vessels, at least in the rat kidney, may respond to ACh with an increased biosynthesis of EDRF. This conclusion is at odds with previous published reports in which the relaxant effect of ACh on aortic rings prepared from diabetic rats was either reduced (Oyama et al 1986) or unchanged (Fortes et al 1983; Head et al 1987) suggesting, in turn, either reduced or unaltered aortic EDRF biosynthesis. Clearly, several factors determine the response to ACh (and by definition the biosynthesis of EDRF) in blood vessels of diabetic rats. However, our demonstration of an increased sensitivity of resistance blood vessels to ACh strongly suggests that there are major differences in the response to diabetes in different parts of the vascular tree.

The perfused kidney from diabetic rats also exhibited increased responsiveness to the vasoconstrictor effect of noradrenaline as evidenced both by a reduction in the EC50 and by an increase in the maximum effect which could be obtained. According to the literature, responses to noradrenaline of vascular tissue taken from diabetic rats fails to conform to a consistent pattern. For example, some studies demonstrate reduced sensitivity to exogenous noradrenaline (Fortes et al 1983; Ramanadham et al 1984) whilst other workers report increased sensitivity (Owen & Carrier 1979) of the diabetic rat aorta to this amine. Clearly, the present results using the perfused diabetic rat kidney fall into the second category. As far as we are aware no entirely satisfactory explanation for the variable response to noradrenaline by blood vessels of diabetic rats has been obtained. No doubt, several factors determine the sensitivity of diabetic blood vessels to both vasoconstrictor and vasodilator drugs. Such factors may well include the strain and age of animals, the choice of diabetogenic drug (i.e. alloxan or streptozocin) and the period of time that animals were diabetic before sacrifice.

Since we have presented evidence for augmented formation of EDRF by diabetic renal blood vessels challenged with ACh we should consider the possibility that EDRF release may contribute to the effect of noradrenaline in these experiments. Eglème et al (1984) have reported that activation of rat aortic endothelial cell α_2-adrenoceptors by clonidine liberates EDRF which subsequently reduces its contractile effect in this preparation. A similar effect of noradrenaline in the diabetic rat kidney would tend to oppose the increased renal vasoconstriction by noradrenaline observed in this study. However, we consider it unlikely that EDRF release is substantially involved in the response of the diabetic rat kidney to noradrenaline in these experiments. Thus, the contractile response to this amine (unlike clonidine) is reportedly only marginally increased by removing the endothelium from the aorta of non-diabetic rats (Eglème et al 1984). Furthermore, de-endothelialization of aortae from streptozocin-diabetic rats did not increase the response to noradrenaline (Wakabayashi et al 1987).

The present results do not support the possibility that deficient EDRF formation by vascular endothelial cells contributes to the microvascular and macrovascular abnormalities of diabetes. In contrast, our data suggest an augmented release of EDRF by the diabetic rat kidney challenged with ACh. Whether this reflects a biochemical adaptation to protect the kidney against excessive vasoconstriction in diabetes remains to be seen. Finally, these experiments highlight the difficulty of extrapolat-

ing the effects of EDRF on large arteries to the control of blood flow within individual organs in the physiological or disease situation.

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