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Selective impairment of hindquarters vasodilator responses to bradykinin in conscious Wistar rats with streptozotocin-induced diabetes mellitus

R.J. Kiff, [1]S.M. Gardiner, A.M. Compton & T. Bennett

Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH

1 Male, Wistar rats were treated with streptozotocin (STZ, 70 mg kg^{-1}, i.p.) or saline and chronically instrumented with pulsed Doppler probes and intravascular catheters (implanted under sodium methohexitone anaesthesia) to allow assessment of haemodynamics in the conscious state 28 days later.

2 Control and STZ-treated rats received bolus doses of glyceryl trinitrate (10–80 nmol kg^{-1}), acetylcholine (0.1–5 nmol kg^{-1}) and bradykinin (0.3–30 nmol kg^{-1}).

3 Although, as reported previously, STZ-treated rats had normal mean arterial blood pressure together with renal and mesenteric vasodilatations and hindquarters vasoconstriction relative to control rats, both groups showed similar hypotensive and regional haemodynamic responses to glyceryl trinitrate and acetylcholine. However, while the depressor effects of bradykinin were similar in control and STZ-treated rats, the former showed a hindquarters vasodilator response to bradykinin that was absent in the STZ-treated rats.

4 A loss of bradykinin-mediated vasodilatation in the hindquarters vascular bed in STZ-treated rats in the presence of normal, hindquarters vasodilator responses to other agents and normal bradykinin-mediated vasodilator responses in other vascular beds is consistent with existing evidence that the vasodilatation elicited by bradykinin in the hindquarters vascular bed is particularly dependent on nitric oxide synthesis and that this is impaired selectively in STZ-treated rats.

Keywords: Bradykinin; glyceryl trinitrate; acetylcholine; haemodynamics; streptozotocin-induced diabetes

Introduction

There is evidence for endothelial cell dysfunction in clinical diabetes mellitus (Porta *et al.*, 1987; Stout, 1987) and it is, therefore, possible that this abnormality contributes to the several cardiovascular complications that can occur in people with this disease (Porta *et al.*, 1987; Stout, 1987). Growing awareness of the importance of endothelial cell integrity for the maintenance of normal cardiovascular homeostasis (Kaiser & Sparks, 1987; Gryglewski *et al.*, 1988; Moncada *et al.*, 1989) has led to a series of studies concerned with the possibility that endothelium-dependent vasorelaxation is abnormal in diabetes mellitus (e.g. Oyama *et al.*, 1986; Bhardwaj & Moore, 1988; Altan *et al.*, 1989). While the majority of these investigations has been concerned with *in vitro* vasorelaxation of tissues obtained from rats with streptozotocin (STZ)-induced diabetes mellitus, the results are not consistent. For example, aortic rings isolated from rats with STZ-induced diabetes mellitus, when precontracted with noradrenaline, have been found to show reduced relaxation to acetylcholine, ADP, histamine and the calcium ionophore, A23187, but not to sodium nitroprusside, glyceryl trinitrate, papaverine or atrial natriuretic peptide (Oyama *et al.*, 1986; Pieper & Gross, 1988; Kamata *et al.*, 1989). These findings indicate selective impairment of endothelium-dependent vasorelaxation. However, in other studies, also concerned with acetylcholine-induced relaxations on aortic rings precontracted with noradrenaline, responses of tissues from STZ-treated rats have been found to be normal (Head *et al.*, 1987; Wakabayashi *et al.*, 1987; Harris & MacLeod, 1988; Mulhern & Docherty, 1989). There are no systematic differences between these two groups of studies regarding dose of STZ or duration of diabetes mellitus.

It is feasible that the disparity between these results is due to the *in vitro* experimental conditions. Thus, none of the investigations cited allowed for the marked differences in composition of extracellular fluid in STZ-treated and control rats (Hebden *et al.*, 1986). In this context it is notable that *in vivo* studies have shown impaired renal vasodilator responses to acetylcholine and bradykinin (Ha & Dunham, 1987) and diminished cerebral arteriolar vasodilator responses to adenosine 5'-diphosphate (ADP) and 5-hydroxytryptamine (Mayhan, 1989) in STZ-treated rats. However, Ha & Dunham (1987) found that the renal vasodilator response to sodium nitroprusside was also impaired in STZ-treated rats, whereas Mayhan (1989) reported a normal cerebral vasodilator response to glyceryl trinitrate in rats with STZ-induced diabetes mellitus. It is noteworthy that both these investigations were performed in anaesthetized animals.

Recently, we found that conscious, Wistar rats with STZ-induced diabetes mellitus had resting renal and mesenteric vasodilatations and hindquarters vasoconstriction, relative to control rats (Kiff *et al.*, 1991). Furthermore, there appeared to be a selective impairment of the hindquarters vasoconstrictor response to N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide production from L-arginine (Moore *et al.*, 1990; Rees *et al.*, 1990), consistent with the resting hindquarters vasoconstriction being due to reduced production of, and/or sensitivity to, endothelium-derived nitric oxide. In the present work our objective was to investigate the possibility that administration of a range of vasodilator agents might reveal additional abnormalities in STZ-treated rats. Therefore, we extended our previous observations by comparing the depressor and the regional vasodilator responses to glyceryl trinitrate, acetylcholine and bradykinin in control and STZ-treated Wistar rats. These vasodilators were chosen because they have differential regional haemodynamic actions and we have background information on the influence of L-NAME on their effects in normal rats (Gardiner *et al.*, 1990b).

Methods

Male Wistar rats (3–4 months old) were injected with STZ (70 mg kg^{-1}) dissolved in 0.9% NaCl ($n = 22$) or with saline

[1] Author for correspondence.

alone ($n = 9$). Rats that had blood glucose levels of greater than 12 mmol l^{-1}, measured on a tail vein sample with a Reflomat (Boehringer, Mannheim) 4 days after STZ injection, were considered to have diabetes mellitus and were entered into the experiment. Fourteen days later rats were anaesthetized (sodium methohexitone 40–60 mg kg^{-1}, i.p., supplemented as required) and pulsed Doppler flow probes (Haywood *et al.*, 1981) were sutured around the left renal and superior mesenteric arteries and the distal abdominal aorta (below the ileocaecal artery to measure hindquarters flow). Following surgery, the rats were kept in their own cages. After a further 14 days the animals were re-anaesthetized (sodium methohexitone, 40 mg kg^{-1} i.p., supplemented as required). Venous catheters were implanted into the right jugular vein and an intra-arterial catheter was placed in the distal abdominal aorta (via the ventral caudal artery). The animals were allowed to recover and were housed individually in their home cages. The following day the animals were connected to a pressure transducer and a pulsed Doppler flow-meter (Crystal Biotech, VF-1, Holliston, USA) for continuous recordings of heart rate, blood pressure and Doppler shift, which was taken as an index of flow (Haywood *et al.*, 1981). The Doppler flow-meter was modified to operate with a pulse repetition frequency of 125 kHz and was fitted with high velocity modules to avoid the problem of signal aliasing (Gardiner *et al.*, 1990a). Vascular conductances were calculated by dividing the mean Doppler shift values by the mean blood pressure (Gardiner *et al.*, 1990b) and were expressed as arbitrary units.

After a 30 min baseline recording period, incremental bolus doses of glyceryl trinitrate (10–80 nmol kg^{-1}), acetylcholine (0.1–5 nmol kg^{-1}) and bradykinin (0.3–30 nmol kg^{-1}) were given in random order, with at least 10 min between each dose.

Blood glucose levels were determined at the end of the experimental period. All the STZ-treated rats had blood glucose levels in excess of 20 mmol l^{-1} (i.e. the upper limit of the Reflomat).

Data analysis

All agents used caused dose-dependent hypotension, but they influenced regional blood flows differently. Thus, glyceryl trinitrate caused marked mesenteric hyperaemia with little change in renal and hindquarters blood flow; acetylcholine increased renal blood flow but not mesenteric or hindquarters blood flows whereas bradykinin increased all blood flows (in control rats), with the most marked effect on the mesenteric vascular bed. Hence, we elected to compare the results in control and STZ-treated rats by considering the nadirs in mean arterial blood pressure and the peaks in regional vascular conductances, irrespective of when these occurred. However, in both groups these events generally coincided, with the notable exception that the maximum rise in mesenteric blood flow and vascular conductance following administration of glyceryl trinitrate occurred when mean arterial blood pressure had returned to baseline.

Results were analyzed by Wilcoxon's ranks sums test in the Mann-Whitney U test for paired and unpaired data, respectively. $P < 0.05$ was taken as significant.

Drugs and peptides

Acetylcholine chloride (Sigma), bradykinin (Bachem U.K.), glyceryl trinitrate (Tridil, DuPont, U.K.) and streptozotocin (Sigma) were all dissolved (or diluted in the case of glyceryl trinitrate) in isotonic saline. For bradykinin, the saline contained 1% bovine serum albumin (Sigma). Steptozotocin was made up immediately before use.

All i.v. injections were given in 0.1 ml aliquots and flushed in with 0.15 ml isotonic saline. The volume of saline alone had no consistent cardiovascular effects.

Results

Changes in mean arterial blood pressure

Glyceryl trinitrate, acetylcholine and bradykinin all caused dose-dependent reductions in mean arterial blood pressure (Figure 1). There were no significant differences between the depressor responses to each of these agents in control and STZ-treated rats (Figure 1).

Changes in renal vascular conductance

Although there was a resting renal vasodilatation in STZ-treated rats relative to the control animals (Figure 2), both control and experimental groups showed vasodilator

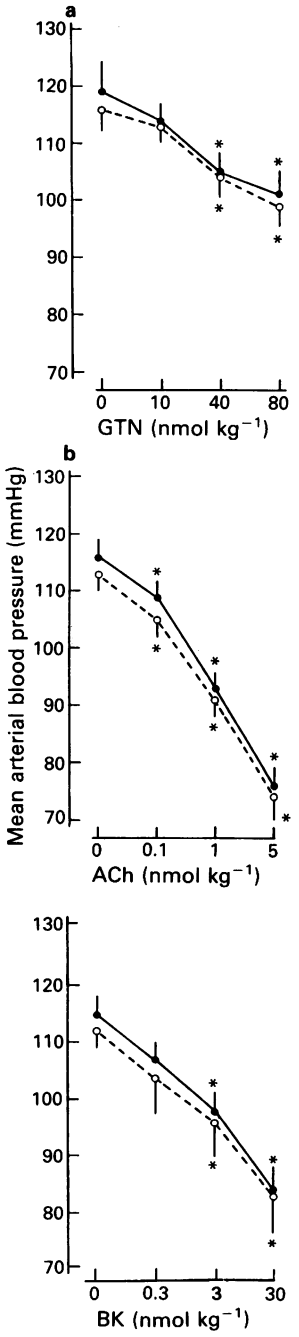

Figure 1 Resting values and maximum changes in mean arterial blood pressure in response to bolus doses of (a) glyceryl trinitrate (GTN), (b) acetylcholine (ACh) and (c) bradykinin (BK) in control (●, $n = 9$) and in streptozotocin-treated (○, $n = 11$), Wistar rats. * $P < 0.05$ versus baseline.

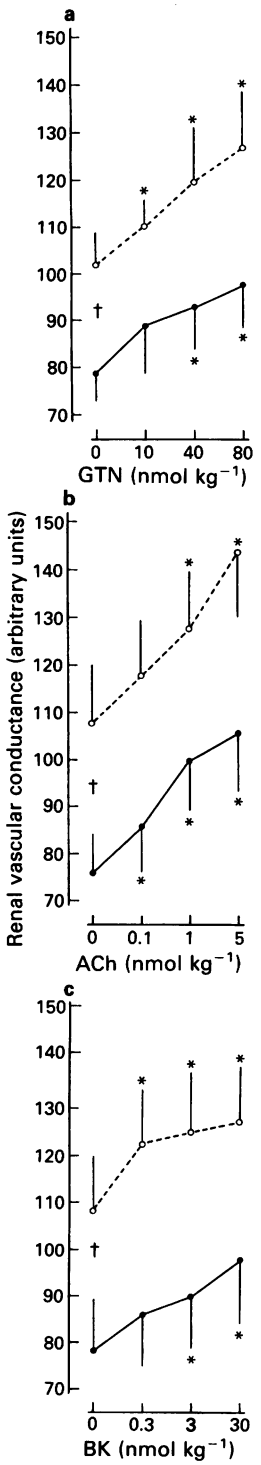

Figure 2 Resting values and maximum changes in renal vascular conductance in response to bolus doses of (a) glyceryl trinitrate (GTN), (b) acetylcholine (ACh) and (c) bradykinin (BK) in control (●, $n = 9$) and in streptozotocin (STZ)-treated (○, $n = 11$), Wistar rats. * $P < 0.05$ versus baseline; † $P < 0.05$ control versus STZ-treated (resting values).

responses to glyceryl trinitrate, acetylcholine and bradykinin. The responses in the two groups were not significantly different, except for the response to bradykinin which reached a maximum in the STZ-treated group but not in the control group (Figure 2).

Changes in mesenteric vascular conductance

There was a difference in mesenteric vascular conductance between control and STZ-treated rats that persisted in the presence of the vasodilators (Figure 3). Glyceryl trinitrate, acetylcholine and bradykinin elicited similar mesenteric vasodilatations in control and STZ-treated rats (Figure 3), although the mesenteric vasodilator response to bradykinin came to a plateau at the higher doses in STZ-treated rats (Figure 3).

Changes in hindquarters vascular conductance

There was a resting hindquarters vasoconstriction in the STZ-treated rats relative to the control animals (Figure 4). In

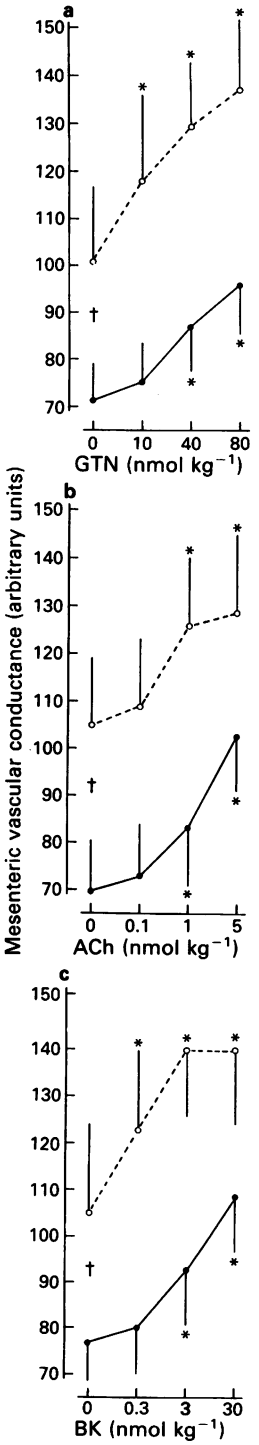

Figure 3 Resting values and maximum changes in mesenteric vascular conductance in response to bolus doses of (a) glyceryl trinitrate (GTN), (b) acetylcholine (ACh) and (c) bradykinin (BK) in control (●, $n = 9$) and in streptozotocin (STZ)-treated (○, $n = 11$), Wistar rats. * $P < 0.05$ versus baseline; † $P < 0.05$ control versus STZ-treated (resting values).

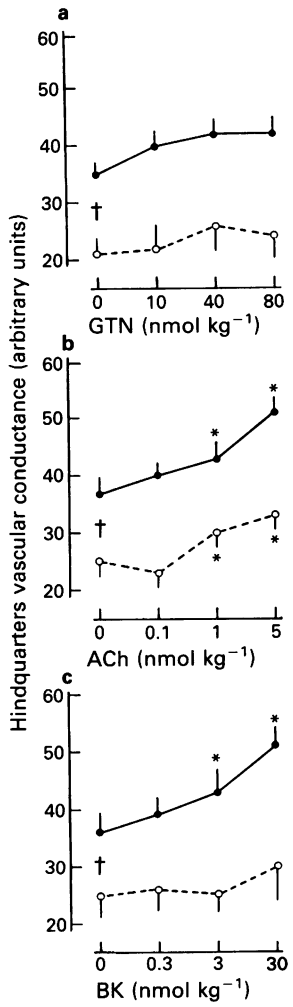

Figure 4 Resting values and maximum changes in hindquarters vascular conductance in response to bolus doses of (a) glyceryl trinitrate (GTN), (b) acetylcholine (ACh) and (c) bradykinin (BK) in control (●, $n = 9$) and in streptozotocin (STZ)-treated (○, $n = 11$), Wistar rats. * $P < 0.05$ versus baseline; † $P < 0.05$ control versus STZ-treated (resting values).

neither group did glyceryl trinitrate cause a significant hindquarters vasodilatation (Figure 4), whereas there was a small vasodilator response to acetylcholine that was similar in control and STZ-treated rats (Figure 4). However, while control rats showed a significant hindquarters vasodilator response to bradykinin, this was absent in the STZ-treated rats (Figure 4).

Discussion

The present work has shown that, although STZ-treated rats may have apparently normal hypotensive responses to glyceryl trinitrate, acetylcholine and bradykinin, there can be very specific abnormalities in the underlying haemodynamic changes. There were increases in renal vascular conductance in control and in STZ-treated rats in response to glyceryl trinitrate, acetylcholine and bradykinin, and in each case the responses in the two groups were not different (although the response to bradykinin in the STZ-treated group reached a plateau whereas it did not in the control rats). In contrast, Bhardwaj & Moore (1988) found that perfused kidneys from STZ-treated rats showed enhanced vasodilator responses to acetylcholine, whereas the vasodilator responses to sodium nitroprusside were the same as in control animals. However, these experiments were carried out in the presence of indomethacin and noradrenaline and so are difficult to compare to the present observations. Moreover, it is notable that Bhardwaj & Moore (1988) found no evidence of the resting renal vasodilatation observed here in conscious STZ-treated rats. However, their studies were performed only 12 days after STZ treatment, whereas our animals had been treated with STZ 28 days before measurements were made. Hence, it is possible that renal hyperaemia is a relatively late development following STZ-treatment. If renal hyperperfusion is associated with renal hypertrophy (Christiansen *et al.*, 1981) this would be consistent with the observation of Bhardwaj & Moore (1988) that kidney weight was not different in the control and STZ-treated animals they studied (i.e. there was no evidence of renal hypertrophy), although there is evidence for renal hypertrophy occurring as early as 2 days after induction of experimental diabetes mellitus (Steer *et al.*, 1985).

Ha & Dunham (1987) studied animals 4 weeks after STZ treatment and made measurements of renal blood flow *in vivo*. They found a significant reduction in resting renal blood flow and vascular conductance in STZ-treated rats but they made these measurements under pentobarbitone anaesthesia. Thus, their results cannot be extrapolated to the conscious state, particularly since it is evident that barbiturate anaesthesia abolishes the polyuria and high electrolyte excretion seen in conscious, STZ-treated rats (Musabayane, 1990). In addition, we have observed that renal blood flow in control and, particularly, in STZ-treated rats under barbiturate (sodium methohexitone) anaesthesia is substantially less than in the same animals when they are conscious (unpublished observations). The use of anaesthesia in the experiments of Ha & Dunham (1987) might account also for their observations of reduced renal vasodilator responses to acetylcholine, bradykinin and sodium nitroprusside in STZ-treated rats, since in the present experiments in conscious, STZ-treated animals, all renal vasodilator responses were normal. While there is discussion about the extent to which 'endothelium-dependent' vasodilatation in response to acetylcholine and bradykinin *in vivo* involves nitric oxide (Gardiner *et al.*, 1990b), the present findings of normal renal vasodilator responses to acetylcholine and to bradykinin in conscious, STZ-treated rats is consistent with the normal renal vasoconstrictor responses to L-NAME seen in these animals (Kiff *et al.*, 1991).

In the mesenteric vascular bed there was no difference between the vasodilator responses to glyceryl trinitrate, to acetylcholine, or to bradykinin in the control and STZ-treated rats. However, the vasodilator responses to bradykinin reached a maximum with the higher doses of the peptide in the STZ-treated rats whereas no plateau was achieved in control animals, but this could be accounted for entirely by the elevation in resting mesenteric vascular conductance in the former group (Kiff *et al.*, 1991).

The hindquarters was the only vascular bed that showed evidence of impaired vasodilator responses in STZ-treated rats. Moreover, this defect was apparent only in response to bradykinin, with no difference in the hindquarters vasodilator responses to acetylcholine, or to endothelin-1 (Kiff *et al.*, 1991), and with neither control nor STZ-treated rats showing hindquarters vasodilatation to glyceryl trinitrate. These observations raise several questions. Why should there be a selective reduction only of the bradykinin-induced hindquarters vasodilator response in STZ-treated rats? Our previous findings of a resting hindquarters vasoconstriction and diminished vasoconstrictor response to L-NAME in STZ-treated rats led us to propose that there might be a localized impairment in the production of, and/or sensitivity to, nitric oxide in the hindquarters vascular bed (Kiff *et al.*, 1991), possibly due to local interference by L-glutamine of the synthesis of nitric oxide from L-arginine (Swierkosz *et al.*, 1990). The present findings of a selective diminution of the bradykinin-induced hindquarters vasodilatation in STZ-treated rats is consistent with the observation that, under normal conditions, this response is particularly sensitive to L-NAME (Gardiner *et al.*, 1990b), and hence may be more dependent on synthesis of nitric oxide than the responses to other vasodilators or the responses to bradykinin in other vascular beds (Gardiner *et al.*, 1990b). An observation that may be relevant to the latter

proposal is that vasodilator responses to bradykinin may involve more than one mechanism (Cowan & Cohen, 1990), and, hence, the extent of their involvements may vary in different vascular beds. The finding that hindquarters vasodilator responses to acetylcholine (this paper) and to endothelin-1 (Kiff *et al.*, 1991) were intact when bradykinin-mediated responses were impaired in STZ-treated rats is consistent with the relative resistance of the former responses to inhibition by L-NAME (Gardiner *et al.*, 1990b). Elsewhere (Gardiner *et al.*, 1990b) we have discussed the possibility that this is due to release of nitric oxide from a pre-formed pool and/or the involvement of nitric oxide-independent mechanisms (Aisaka *et al.*, 1989; Long & Berkowitz, 1989).

Another important question that arises from the present results is: what is the explanation for the lack of hindquarters vasodilator response to glyceryl trinitrate in control and in STZ-treated rats? Although this phenomenon might relate to dose and experimental protocol, it was clear from the experiments reported here that glyceryl trinitrate caused marked mesenteric hyperaemia and vasodilatation when hindquarters blood flow and vascular conductance were unchanged. There is evidence that the regional haemodynamic effects of glyceryl trinitrate are influenced by the reflex responses to the reductions in preload and afterload it causes (Vatner *et al.*, 1978), but it is notable that administration of a dose of endothelin-1 (Kiff *et al.*, 1991) that had similar hypotensive effects to glyceryl trinitrate in the present experiments did elicit marked hindquarters vasodilatation. Thus, it is possible that the differential regional vasodilator effects of glyceryl trinitrate relate more to differences in local biotransformation of the drug (Kawamoto *et al.*, 1990).

The regionally selective and agonist specific, vasodilator abnormalities noted in the present work highlight the problems associated with *in vitro* studies of aortic ring preparations when these are used as models of generalized endothelial dysfunction in diabetes mellitus (see Introduction). However, considering *in vitro* findings showing aortic endothelial cell abnormalities in STZ-treated rats, and the present results indicating normal vasodilator responses in renal and superior mesenteric vascular beds together with selective impairment of bradykinin-mediated vasodilatation only in the hindquarters vascular bed, it would be of interest to compare aortic compliance (Cruickshank *et al.*, 1991) in control and STZ-treated rats to determine if this is a more sensitive *in vivo* correlate with the *in vitro* findings.

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