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Experimental diabetes mellitus inhibits prostacyclin synthesis by the rat penis: pathological implications

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Summary. In view of the marked increase in blood flow into the penis during erection and the association of diabetes mellitus with impotence, we used the diabetic rat model to investigate the possibility that: (a) the penis may produce prostacyclin; and (b) prostacyclin secretion may be decreased in diabetes. Rats given a high dose of streptozotocin (120 mg/kg body weight) developed acute ketotic diabetes and were killed after 48 h. Animals given a low dose of streptozotocin (65 mg/kg body weight) developed non-ketonuric diabetes and were killed after 7 or 62 days. Aortic rings and penile tissue discs were incubated in buffer, which was assayed for 6-oxo-prostaglandin $F_{1\alpha}$, the stable and spontaneous breakdown product of prostacyclin. Penile tissue from control, ketotic and non-ketonuric (7 days) animals released similar quantities of prostacyclin, whereas that from long-term non-ketonuric animals (62 days) produced significantly less prostacyclin. Production of this prostanoid by the aortic rings paralleled these changes. We conclude that: (a) penile tissue releases prostacyclin in quantities comparable to those of the aorta; (b) long-term diabetes leads to diminished prostacyclin release by penile and aortic tissue: the former may contribute to the pathogenesis of diabetic impotence; and (c) since short-term ketotic diabetes does not inhibit aortic or penile prostacyclin release, duration of diabetes rather than its severity is responsible for diminished prostacyclin release.

Key words: Diabetes mellitus, impotence, prostacyclin, penis, rat.

The prevalence of sexual impotence in diabetic patients is appreciably greater than that in the normal population [1, 2]. Since penile erection involves considerable and acute vasodilation, with a concomitant increase in blood flow, it is likely that powerful vasodilators are released locally immediately before and during erection. One such vasodilator is vasoactive intestinal polypeptide (VIP). Its release into the penile circulation during erection has already been demonstrated and the relevance of diminished VIP release to the pathogenesis of impotence in diabetic patients has recently been highlighted [3, 4].

The present study examines the possibility that diabetes may affect any penile production of another vasodilator, prostacyclin (PGI_2). Previous data on rats rendered diabetic experimentally show that aortic PGI_2 secretion is significantly diminished [5], suggesting that synthesis of this prostanoid by other vascular organs may also be impaired. Furthermore vascular lesions (e.g. aneurysms, thickening of basement membranes) have been described in penile tissue of diabetic animal models and in patients suffering from impotence associated with diabetes [6]. The penis of the diabetic rat has also been shown to be a representative model for the diabetic human organ [7, 8]. Since the penis has hitherto not been shown to produce PGI_2, the present study investigates: (1) whether PGI_2 is produced in large amounts by penile tissue; and (2) the effect of diabetes on penile PGI_2 production.

Materials and methods

Acute "ketotic" study

Fourteen 200 g male Sprague Dawley rats were rendered ketotic by an intravenous (tail vein) injection of streptozotocin (120 mg/kg body weight) at approximately 11.00 h. Streptozotocin was obtained from Sigma Chemical Company, Poole, Dorset, UK. Urine testing with Multistix (Ames Division, Miles Laboratories, Stoke Poges, Bucks, UK) revealed glycosuria and ketonuria (large amount, > 15.7 mmol/l) within 24 h of administering streptozotocin.

The rats were randomly allocated to three groups:

Group 1 (n = 7). These rats were treated by subcutaneous administration of porcine neutral insulin (3–8 units Actrapid MC, Novo Industri, Copenhagen, Denmark) at 9.00 h and 18.00 h over 3 days, the first injection being given at 18.00 h on day 1, 7 h after inducing diabetes with streptozotocin. The dose of insulin was adjusted so as to reduce

Table 1. Plasma glucose concentrations in the rat groups studied. Blood was collected at the time of tissue sampling

Rat groups	Plasma glucose concentration (mmol/l)
Acute ketotic diabetic rats	
Untreated ($n=7$, group 1)	20.5[a,b] (16.8-25.8)
Insulin-treated ($n=7$, group 2)	6.7 (4.0-9.7)
Control rats ($n=7$, group 3)	8.3 (6.8-11.3)
Non-ketonuric diabetic rats of 7 days duration ($n=7$, group 4 A)	22.6[a,c] (17.1-32.1)
Control rats ($n=7$, group 5 A)	11.0 (8.4-11.7)
Non-ketonuric diabetic rats of 62 days duration ($n=7$, group 4 B)	39.0[a] (25.0-45.0)
Control rats ($n=7$, group 5 B)	7.8 (6.4-10.7)

Results expressed as mean with range in parentheses. [a] $p<0.001$: control versus diabetic rats; [b] $p<0.002$; non-ketonuric diabetes of 62 days duration versus acute ketotic diabetes (untreated); [c] $p<0.004$: non-ketonuric diabetes of 62 days duration versus non-ketonuric diabetes of 7 days duration

Table 2. Effect of acute ketotic diabetes (treated/untreated) on PGI_2 release by the rat penis and aorta

	Release of 6-oxo-$PGF_{1\alpha}$ ($ng \cdot 50\,mg\ tissue^{-1} \cdot h^{-1}$)		
	Rats with ketotic diabetes (untreated) (group 1) ($n=7$)	Rats with ketotic diabetes (treated) (group 2) ($n=7$)	Control rats (group 3) ($n=7$)
Penis	18.5 (10.6-30.0)	18.3 (8.4-28.0)	19.7 (8.0-27.2)
Aorta	185 (160-220)	190 (160-270)	190 (150-230)

Results expressed as median with ranges in parentheses

ketonuria to <0.5 mmol/l (trace) without abolishing glycosuria. On day 3, 48 h after streptozocin and 3 h after their morning insulin injection (09.00 hours), the rats were anaesthetised intraperitoneally with pentobarbital (90 mg/kg, Sagatal, May & Baker, Dagenham, Essex, UK), and tissue and blood samples obtained as described below.

Sampling at 48 h was selected, since others [9] as well as ourselves have found that several rats cannot survive for long periods after injection of high doses of streptozotocin.

Group 2 ($n=7$). These rats were not treated with insulin, but were otherwise treated in the same manner as the rats in group 1.

Group 3 ($n=7$). This was the control group and consisted of seven matched rats not given streptozotocin. These rats were injected with an appropriate volume of the citrate buffer (pH 4.5) which was used to prepare the streptozocin solutions.

Rats in all groups were allowed free access to food (standard rat chow) and water ad libitum.

Blood was collected by cardiac puncture. Plasma was stored for glucose estimations [10]. Plasma glucose concentrations for each group are shown in Table 1. Penises were excised and the sheath and fatty tissue removed. The penile tissue was then cut cross-sectionally into 3 mm diameter discs using a scalpel blade and a Teflon block. The rings were rinsed in ice-cold Krebs Ringer bicarbonate buffer (KRB) [11, 12], which had been pre-gassed to pH 7.4. The discs were randomised and kept in KRB. Discs (50 mg), in duplicate, were incubated in 1 ml KRB at 37 °C for 60 min in a shaking water bath. After incubation, the tubes were centrifuged (2000 *g* for 5 min) and the supernatant stored at −70 °C. The samples were defrosted simultaneously and the concentration of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$), the stable, spontaneous breakdown product of PGI_2, was measured using a specific radioimmunoassay [11, 12].

Aortic rings from the same animals were also prepared as previously described [11, 12]. The rings were incubated and assayed for 6-oxo-$PGF_{1\alpha}$ as described for the penile discs.

Chronic "non-ketotic" study

Rats ($n=14$, group 4) were rendered diabetic with a lower dose of streptozotocin (65 mg/kg body weight) than that described in groups 1 and 2. Urine was monitored as described above and revealed glycosuria, but no appreciable ketonuria (negative to trace only; 0-0.5 mmol/l). After 7 days, seven of these diabetic rats (group 4 A) were processed as described above and the results were compared with those observed in seven matched non-diabetic control rats (group 5 A). After 62 days, the remaining seven diabetic rats from group 4 (group 4 B) were processed as described above and the results compared with another seven matched non-diabetic control rats (group 5 B).

All rats were allowed free access to food and water; none received any form of treatment. Urine samples were monitored periodically to demonstrate the continued absence of appreciable ketonuria.

Statistical analysis

Results are expressed as median and range and are compared using non-parametric unpaired Mann-Whitney tests (two-tailed) [13].

Results

The plasma glucose concentrations in all groups are shown in Table 1.

Acute "ketotic" study

6-oxo-$PGF_{1\alpha}$ production by penile tissue and aortic rings was similar in the control rats (group 3) and in the insulin-treated and non-treated ketotic rats (groups 1 and 2) (Table 2).

Chronic "non-ketotic" study

6-oxo-$PGF_{1\alpha}$ production by penile tissue and aortic rings in non-ketonuric rats was significantly reduced after 62 days ($p<0.002$) (group 4 B), but not after 7 days of diabetes (group 4 A) (Table 3).

Comparison of all three diabetic groups revealed that the 6-oxo-$PGF_{1\alpha}$ production by penile tissue and aortic rings of the non-ketonuric diabetic rats of 62 days duration (group 4 B) was significantly ($p<0.002$) reduced when compared with the acute ketotic diabetic

Table 3. Effect of non-ketonuric diabetes (7 and 62 days duration) on PGI_2 release by the rat penis and aorta

	Release of 6-oxo-$PGF_{1\alpha}$ ($ng \cdot 50\,mg\ tissue^{-1} \cdot h^{-1}$)	
	Non-ketotic diabetic rats ($n=7$)	Control rats ($n=7$)
Non-ketonuric diabetes of 7 days duration (groups 4a and 5a)		
Penis	16.5 (9.2-28.0)	17.8 (8.7-26.0)
Aorta	185 (165-220)	200 (150-220)
Non-ketonuric diabetes of 62 days duration (groups 4b and 5b)		
Penis	9.2[a] (6.6-12.0)	19.8 (14.1-27.5)
Aorta	110[a] (90-185)	200 (150-240)

Results expressed as mean with ranges in parentheses. [a] $p<0.002$: control rats versus non-ketonuric diabetic rats of 62 days duration

model (groups 1 and 2, insulin-treated and untreated), or with the non-ketonuric diabetic rats of 7 days duration (group 4A) (Table 3) ($p<0.002$).

Discussion

Our results show that: (1) rat penile tissue releases substantial amounts of PGI_2; (2) penile and aortic PGI_2 secretion is not affected by the metabolic disturbance caused by acute ketotic diabetes; and (3) long-standing non-ketonuric diabetes leads to a marked diminution in PGI_2 secretion by the rat penis and aorta. Rat aorta appears to produce substantially more PGI_2, weight for weight, than the penis. However, direct comparisons must by made with some caution, since we have shown that various forms of trauma, which may occur during preparation of the relevant tissues, influence spontaneous PGI_2 release [14].

That a vascular organ like the penis should produce PGI_2 is not surprising. However the secretion of this prostanoid by the penis has, to our knowledge, not been demonstrated previously. The exact anatomical location of PGI_2 synthesis in the rat penis remains unclear; however, it is of interest that the structure of the corpora cavernosa is very similar to that of blood vessels (smooth muscle; endothelial cells [15]), which are an established source of PGI_2. It is therefore possible that the corpora cavernosa are the principal source of PGI_2 in the penis. This view is supported by our preliminary findings that human corpora cavernosa, obtained during penile surgery, release PGI_2 (J. Y. Jeremy, unpublished observations). Animal studies involving larger organs are also in progress.

Prostacyclin may well play a functional role in the penis by mediating the acute vasodilation and increase in blood flow during erection. Recent work has shown that the release of another vasodilator, vasoactive intestinal polypeptide (VIP), occurs during penile erection [3, 4]. Furthermore release of VIP, as well as the population of Vipergic nerve endings in the penis, is markedly diminished in impotent males, especially those who have diabetes mellitus [4]. It would clearly be of interest to establish whether PGI_2 behaves in a similar manner, and whether VIP (or other substances) stimulates PGI_2 release from penile tissue.

Prostacyclin may also play a role in sustaining erection, since it is possible that penile vascular tissue behaves in a manner similar to that of the urinary bladder [12], and portal [16] and peripheral veins [17]. These respond with an increase in PGI_2 production following distension. PGI_2, by virtue of its capacity to inhibit platelet aggregation and adhesion [18], may also play a vital role in maintaining the vascular integrity of the engorged cavernous body.

"Long-term" non-ketonuric diabetes led to a marked diminution in PGI_2 release, a result which is in accord with those previously reported for aortic PGI_2 production [5] and with the epidemiology of impotence in diabetics [1, 2]. However, the acute and severe metabolic disturbance created by ketotic diabetes had no effect on penile PGI_2 production. Any direct analogy with ketoacidosis occurring in vivo has to be guarded, however, since in our experimental model plasma insulin levels are, in fact, raised or within the normal fasting range during the first 24 h [9]. Long-term diabetes may mediate its action by a depletion of PGI_2 precursors or by progressive changes in the activities of the enzymes involved in its biosynthesis (e.g., by glycosylation of the enzymes). Alternatively, the "metabolic environment" in the non-ketonuric diabetes of 62 days duration model may be such as to inhibit PGI_2 synthesis [19]. This latter view is supported by the observation that the plasma glucose concentrations were significantly higher in the latter group when compared with the other groups of diabetic rats (Table 1).

In conclusion, our findings suggest that diabetes is associated with impaired PGI_2 production in penile tissue. This deficiency may in turn contribute to the pathogenesis of impotence in diabetics.

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References

1. McCulloch DK, Campbell IW, Wu FC, Prescott RJ, Clarke BF (1980) The prevalence of diabetic impotence. Diabetologia 18: 279-283
2. McCulloch DK, Young RJ, Prescott RJ, Campbell IW, Clarke BF (1984) The natural history of impotence in diabetic men. Diabetologia 26: 437-444
3. Ottesen B, Wagner G, Virag R, Fahrenkrug J (1984) Penile erection: possible role for vasoactive intestinal polypeptide as a neurotransmitter. Br Med J 288: 9-11

4. Gu J, Polak JM, Lazarides M, Morgan R, Pryor JP, Marangos PJ, Blank MA, Bloom SR (1984) Decrease of vasoactive intestinal polypeptide (VIP) in the penises from impotent men. Lancet 2: 315–317
5. Colwell JA, Winocour PD, Lopes-Virella M, Halushka PV (1983) New concepts about the pathogenesis of atherosclerosis in diabetes mellitus. Am J Med 75 (Suppl 5B): 67–80
6. Fani K, Lundin AP- Beyer MM, Jimenez FA, Friedman EA (1983) Pathology of the penis in long-term diabetic rats. Diabetologia 25: 424–428
7. Crowe R, Lincoln J, Blackley PF, Pryor JP, Lumley JSP, Burnstock G (1983) Vasoactive intestinal polypeptide-like immunoreactive nerves in diabetic penis. Diabetes 32: 1075–1077
8. Benson GS, McConnell JA, Lipshultz LI (1980) Neuromorphology and neuropharmacology of the human penis. An in vitro study. J Clin Invest 65: 506–513
9. Junod A, Lambert AE, Stauffacher W, Renold AE (1969) Diabetogenic action of streptozotocin: relationship of dose of metabolic reponse. J Clin Invest 48: 2129–2139
10. Mikhailidis DP, Foo Y, Ramdial L, Kirk RM, Rosalki SB, Dandona P (1981) Pancreatic exocrine function after truncal and highly selective vagotomy. J Clin Pathol 34: 963–964
11. Mikhailidis DP, Jeremy JY, Barradas MA, Green N, Dandona P (1983) Effect of ethanol on vascular prostacyclin (prostaglandin I_2) synthesis, platelet aggregation and platelet thromboxane release. Br Med J 287: 1495–1498
12. Jeremy JY, Mikhailidis DP, Dandona P (1984) The rat urinary bladder produces prostacyclin as well as other prostaglandins. Prost Leukotr Med 16: 235–248
13. Altman DG, Gore SM, Gardner MJ, Pocock SJ (1983) Statistical guidelines for contributors to medical journals. Br Med J 286: 1489–1493
14. Jeremy JY, Mikhailidis DP, Dandona P (1984) Vascular trauma and prostacyclin release. Microcircul Endothel Lymph 1: 629–644
15. Williams PL, Warwick R (eds) (1980) Gray's anatomy, 36th edn. Churchill Livingstone, Edinburgh, p 1420
16. Hamilton G, Chow FPR, Hutton RA, Dandona P, Hobbs KEF (1982) The relationship between prostacyclin activity and pressure in the portal vein. Hepatology 2: 236–242
17. Ritter JM, Barrow SE, Blair IA, Dollery CT (1983) Release of prostacyclin in vivo and its role in man. Lancet 1: 317–319
18. Moncada S, Vane JR (1979) Prostacyclin formation and effects. In: Roberts SM, Schneiman F (eds) Chemistry, biochemistry and pharmacological activity of prostanoids. Pergamon Press, Oxford, pp 258–273
19. Jeremy JY, Mikhailidis DP, Dandona P (1983) Simulating the diabetic environment modifies in vitro prostacylin synthesis. Diabetes 32: 217–221

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