Diabetologia 18, 65–68 (1980)

Diabetologia

Effect of Insulin Treatment on Prostacyclin in Experimental Diabetes

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Summary. Diabetic patients have a high susceptibility to microvascular complications, atherosclerosis and thrombosis. Platelet hyperreactivity possibly related to an imbalance in arachidonic acid metabolism may be involved. Aortic rings or renal cortex produced a potent inhibitor of platelet aggregation, identified as prostacyclin (PGI_2). Release of PGI_2 by tissues from streptozotocin – diabetic rats (aorta: 0.07 ± 0.1 ng/mg wet weight; renal cortex 0.004 ± 0.001 ng/mg wet weight) was significantly depressed when compared with controls (aorta: 0.26 ± 0.07 ng/mg wet weight; renal cortex: 0.009 ± 0.001 ng/mg wet weight). Treatment of diabetic animals with insulin for 8 days restored PGI_2 production to normal. The finding that PGI_2 is depressed in the aorta and in the kidney, tissues which develop angiopathy, and that this is normalised by insulin, suggests that impaired PGI_2 production, perhaps associated with platelet hyperreactivity may play a role in the vascular complications of diabetes.

Key words: Streptozotocin diabetic rats, prostacyclin, aorta, renal cortex, insulin treatment.

Patients with diabetes mellitus develop microvascular complications and have an increased susceptibility to atherosclerosis [1] and arterial thrombosis [2]. Disorders of blood coagulation and platelet function are reported to be particularly marked in diabetics with vascular disease [3]. This hypercoagulable state may be related to an imbalance in platelet [4] and vessel wall prostaglandin metabolism. We have previously demonstrated that the release of prostacyclin (PGI_2), a potent inhibitor of platelet aggregation, is significantly depressed in experimental diabetes [5] whilst the platelet production of thromboxane is significantly elevated [6]. In this study two approaches have been used to investigate the possible relevance of abnormal PGI_2 to diabetic vascular disease. First, PGI_2 levels in the kidney, a tissue susceptible to microangiopathy, have been estimated and secondly, the effect of insulin treatment on prostacyclin release by aorta and kidney from diabetic rats has been examined.

Methods

Animals

Male rats (Alderley Park strain, 90–120 g body weight) were made diabetic by IV injection of streptozotocin (80 mg/kg) 1–3 months prior to use. Glucosuria was confirmed ('Clinistix') and blood samples (0.5 ml) were taken from the dorsal aorta under ether anaesthesia at sacrifice, for glucose estimation by 'Technicon autoanalyser' [7]. Sex and age-matched rats were used as controls. In experiments to examine the effects of chronic insulin treatment, groups of diabetic rats were given single daily injections of a combination of semilente and ultralente (Wellcome) insulin for a period of eight days.

The animals had been diabetic for 27–30 days when insulin treatment was started. They received 60 U semilente insulin per kg bodyweight and 60 U ultralente insulin per kg on the first day, and 20 U semilente/kg and 80 U ultralente/kg on subsequent days. Physiological (0.154 mol/l) saline was administered to groups of diabetic and non-diabetic controls. All injections were given subcutaneously in the morning (~ 10 a. m.). Blood samples (25–50 µl) were taken for glucose estimation from the retro-orbital sinus before treatment and 23 h after each injection, and from the dorsal aorta at sacrifice. In one experiment, blood glucose levels were also estimated 4 h after injection. One animal from each group was sacrificed (by cervical dislocation) at hourly intervals throughout the day.

Prostacyclin Estimation

The abdominal aorta and right kidney were rapidly excised (approximately 2 mins) and immersed in Krebs Henseleit buffer (g/litre: – NaCl 6.92, KCl 0.35, $MgSO_4$ $7H_2O$ 0.29, $CaCl_2$ 0.28, KH_2PO_4 0.16, $NaHCO_3$ 2.1, Glucose 2.0; pH 7.4) at 4 °C containing 200 mg/100 ml glucose. The adventitial layer was removed from the aorta, and the vessel weighed and cut into fine rings (1–2 mg). The rings were then suspended in the buffer at 60 mg/200 µl. Following removal of the capsule, the kidney was bissected longitudinally, and the cortex was excised, weighed, and homogen-

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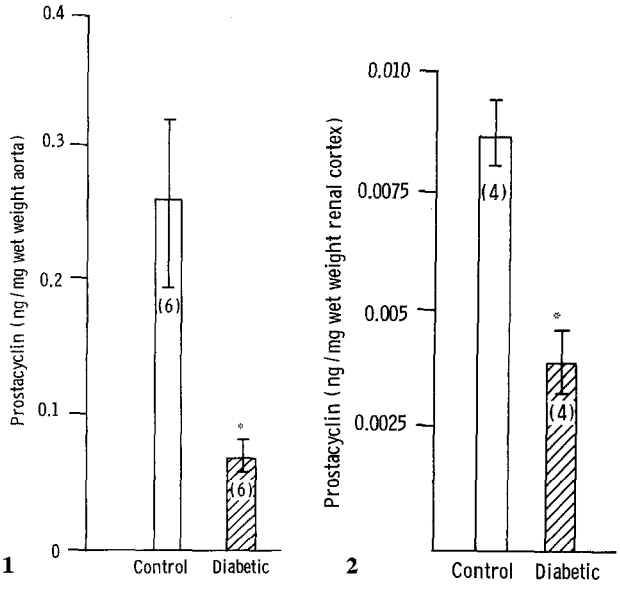

Fig. 1. Prostacyclin (ng/mg wet weight) released by the aorta of diabetic (blood glucose 412 ± 15 mg/100 ml) and control (blood glucose 77 ± 5 mg/100 ml) rats. Animals had been diabetic for 52 days at the time of the experiment. Numbers of animals in each group are shown in parentheses. Values represent mean ± SEM. * $p < 0.02$

Fig. 2. Prostacyclin (ng/mg wet weight) production by homogenates of renal cortex of diabetic (blood glucose 343 ± 31 mg/100 ml) and control (blood glucose 92 ± 7 mg/100 ml) animals. Animals had been diabetic for 49 days at the time of the experiment. Numbers of rats in each group are shown in parentheses. Values represent mean ± SEM.* $p < 0.01$

ised (60 mg/200 µl) in Krebs Henseleit buffer on ice. Incubations were carried out at 22 °C for 3 min. Aliquots of the supernatant (10–25 µl) were added to rat platelet rich plasma (containing 0.38% w/v trisodium citrate and ~ 10^9 platelets/ml), 30 s before the addition of a submaximal concentration of adenosine-5'-diphosphate (ADP, 2 µmol/l).

The activity of the supernatant in inhibiting platelet aggregation was measured in a double channel aggregometer ('Payton'). The quantity of PGI_2 was estimated from a standard curve obtained in each experiment using chemically defined PGI_2 (sodium salt), and expressed as ng/mg wet weight of tissue. Results are given as mean ± SEM. Levels of significance were assessed using the Student t test.

An antiserum was raised in rabbits to 5,6-dihydro PGI_2 as the hapten conjugated to bovine serum albumin [8]. The antiserum (1 µl) was incubated with platelet rich plasma for 30 s before the addition of either chemically defined PGI_2 or supernatant. ADP was then added after a further 30 s and aggregation recorded. The platelet inhibiting activity of both PGI_2 and of the supernatant was abolished by antiserum, whereas that of PGE_1 and PGD_2 was only slightly affected (< 15%).

Results

The incubated aortic rings and renal cortex released a potent inhibitor of platelet aggregation. This inhibitory activity was shown to be due to PGI_2 by (a) loss of effect after boiling for 15 s [9] (b) relaxation of bovine coronary artery [10] and (c) inhibition by a specific antiserum.

The abdominal aorta from normal rats produced greater amounts of PGI_2 (0.26 ± 0.07 ng/mg wet weight) than inferior vena cava (0.055 ± 0.003 ng/mg wet weight) under the same conditions.

The production of PGI_2 by the aorta of diabetic rats (0.07 ± 0.01 ng/mg) was significantly depressed ($p < 0.02$) when compared with that of controls (0.26 ± 0.07 ng/mg) (Fig. 1).

Homogenates of rat renal cortex in vitro generated less PGI_2 (0.009 ± 0.001 ng/mg wet weight) than rings of abdominal aorta. As with aortic tissue, renal cortex from diabetic animals also released significantly less PGI_2 ($p < 0.01$) than normal (Fig. 2).

Effect of Insulin Treatment

a) Aorta: Blood glucose levels in diabetic rats fell during the first two days of insulin treatment and were maintained at low level from days 3 to 8 (Fig. 3). The mean blood glucose in these animals 24 hours after injection was 111 ± 14 mg/100 ml. Blood glucose levels remained high in diabetics treated with saline (298 ± 4 mg/100 ml) and low in control rats (85 ± 2 mg/100 ml).

PGI_2 release from the aorta was depressed ($p < 0.001$) in diabetic rats treated with saline (Fig. 4). Insulin treatment significantly increased PGI_2 production ($p < 0.05$) and there was no difference between values for these animals and controls.

b) Renal Cortex: In this experiment, mean blood glucose levels 24 hours after injection were 163 ± 15 and 395 ± 11 mg/100 ml in diabetic animals treated with insulin and saline respectively, and 99 ± 3 mg/100 ml in controls. Renal cortex from diabetic animals treated with saline produced significantly reduced amounts of PGI_2 ($p < 0.01$) (Fig. 5). Insulin treatment resulted in increased cortical PGI_2 production. There was no significant difference between PGI_2 production in insulin treated animals and non-diabetic controls.

Discussion

Platelet abnormalities including increased reactivity [4, 11], spontaneous aggregation [12], decreased survival [13], elevated prostaglandin (PG) [4] and malonyldialdehyde [14] production and β-thromboglobulin release [15], are found in diabetes and are particularly marked in patients with vascular complications.

The activation of platelets initiates the sequential conversion, by cyclo-oxygenase, of arachidonic acid to the intermediary PG endoperoxides. Endoperoxides can be converted to TxA_2 [16] or PGE_2, PGD_2 and $PGF_{2\alpha}$ [17] by platelet thromboxane synthetase and PG isomerases respectively, or to PGI_2 by

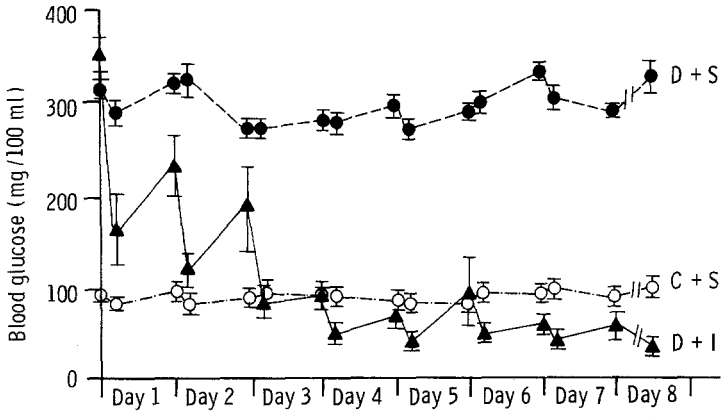

Fig. 3. Mean blood glucose levels (± SEM) in mg/100 ml, 4 and 24 hours after injection, in diabetic rats treated with saline (D + S), or insulin (D + I) and control rats treated with saline (C + S). Final values represent blood glucose levels at sacrifice. There were six animals in each group

endothelial cell prostacyclin synthetase [18]. TxA_2 is a potent constrictor of vascular smooth muscle [19] and induces platelet aggregation [20], whereas PGI_2 elicits vasodilation [21] and prevents platelet aggregation or induces disaggregation [22]. A biological balance is therefore maintained between activated platelets and the functional endothelial cell.

We have demonstrated that vascular PGI_2 is decreased and that platelet TxA_2 is elevated in experimental diabetes [5, 6] and in diabetic patients [23]. A deficiency in endothelial cell PGI_2 production, resulting in an inability to cope with the platelet release of TxA_2 and mitogenic factors [24] could be involved in the vascular complications of diabetes. This is supported by our finding that PGI_2 is depressed in the kidney, a tissue which develops microangiopathy.

High rates of PGI_2 production do not occur in the presence of high blood glucose [5]. Prostacyclin is restored to normal by chronic, but not by acute, insulin administration. The effectiveness of chronic insulin may be explained by the longer duration of treatment or by the greater decrease in blood glucose levels at sacrifice (chronic: 30 ± 3 mg/100 ml, acute: 179 ± 23 mg/100 ml). At present, we are unable to distinguish between these possibilities. However, if prolonged insulin treatment is necessary, then this suggests that a repair process is occurring and that the depressed PGI_2 in diabetes may be due to endothelial cell loss or damage. Alternatively, if the level of blood glucose is critical then hyperglycaemia or an associated metabolic abnormality of diabetes may be responsible. It has been previously shown that increased glucose concentrations [25] and osmolality [26] decrease PG synthesis.

Studies in diabetic animals have shown that insulin treatment or pancreatic islet transplantation decreases the severity of renal [27] and retinal [28] lesions and diminishes ocular fluorescein leakage [29]. There is also evidence from clinical studies that good metabolic control slows the progression of retinopathy [30] and nephropathy [31]. Furthermore, the development of microangiopathy in normal kidneys following transplantation into diabetic subjects [32] suggests that metabolic factors play a role in small vessel disease. Evidence for a favourable effect of good control on large vessel disease in the diabetic is lacking, although glucose intolerance is known to be a risk factor for cardiovascular disease [1].

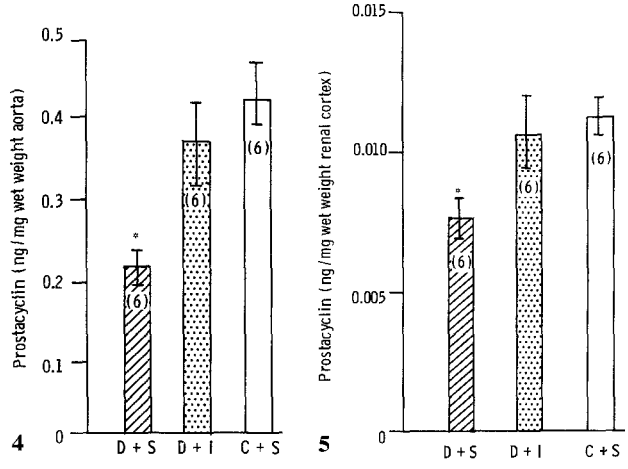

Fig. 4. Prostacyclin (ng/mg wet weight) released by the aorta of diabetic rats treated with saline (D + S) or insulin (D + I) and control rats treated with saline (C + S). The numbers of animals per group are shown in parentheses. Values represent mean ± SEM. $p < 0.001$ versus C + S and $p < 0.05$ versus D + I where indicated (*)

Fig. 5. Production of prostacyclin (ng/mg wet weight) by homogenates of renal cortex from saline-treated (D + S) and insulin-treated diabetic rats (D + I) and saline-treated controls (C + S). Values represent mean ± SEM. $p < 0.01$ versus C + S where indicated (*)

If, as these studies suggest, improvement of the metabolic abnormalities of diabetes prevents, or slows, the progression of vascular disease, then the finding that insulin treatment restores prostacyclin to normal is compatible with the suggestion that impaired vessel wall prostaglandin metabolism could play a role in the evolution of diabetic angiopathy.

Acknowledgement. We are indebted to Dr. K. Gibson, Chemistry Department for synthetic prostacyclin, Dr. B. Furr, Biology

Department for antiserum to prostacyclin and to Mrs. T. E. Robinson, Mr. M. J. A. Arnstein, Mrs. S. Jones and Miss S. Bowden for technical assistance.

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Received: January 30, 1979,
and in revised form: August 6, 1979

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