Increased Prostacyclin Release from Perfused Hearts of Acutely Diabetic Rats

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Summary. The release of prostacyclin and PGE_2 from the isolated perfused hearts of acutely diabetic (streptozotocin 100 mg/kg) rats was studied and compared with hearts from control animals. Prostacyclin and PGE_2 were measured by a differential bioassay technique. No basal release of either prostaglandin was detected. However, after addition of arachidonic acid, a dose dependent release of prostacyclin and PGE_2 was noted. Prostacyclin was identified as the major prostaglandin. Release of prostacyclin and PGE_2 from acutely diabetic rat hearts was increased 2–3 times compared to control hearts. No release of prostaglandin endoperoxides was observed in either group of hearts.

Key words: Prostaglandins, prostacyclin, PGE_2 , perfused rat heart, prostaglandin endoperoxides, coronary flow, platelet aggregation, streptozotocin diabetes, bioassay.

Diabetic patients develop microvascular lesions and have a high incidence of thromboembolic disorders [1, 2]. Insulin deficiency has been proposed as a factor in the pathogenesis of these events [3]. Both disturbed vascular function and increased platelet aggregability observed in diabetic patients [2] could be explained [4, 5, 6] by increased formation of thromboxane A_2 by the platelets, or decreased synthesis of prostacyclin, an inhibitor of platelet aggregation and deposition [7], by the vessel wall.

Prostacyclin is a potent vasodilator and changes in the pattern of arachidonic acid metabolites could, therefore, influence the homeostatic regulation of the microcirculation [6]. Stam and Hülsmann [8] have recently observed increased release of PGE_2 -like

activity from the acutely-diabetic rat heart, accompanied by enhanced coronary flow. Treatment with indomethacin abolished both these effects. Because prostacyclin is known to be the major prostaglandin-like substance released by the rat heart in-vitro [9], increased prostacyclin release would be a more probable explanation for the increased flow. To test whether diabetes has a direct effect on the release of prostacyclin and prostaglandin-like substances, we have investigated prostacyclin and PGE_2 release from acutely-diabetic rat hearts.

Materials

Arachidonic acid (Nu-Chek Inc., Elysian, Minnesota, > 99% pure) was converted to the sodium salt and finally dissolved in 50 mmol/l Tris buffer (pH 7.4). Prostacyclin and PGE_2 (Upjohn Comp., Kalamazoo, Michigan) were dissolved in 0.1 mmol/l NaOH or 50 mmol/l phosphate buffer (pH 7.2), respectively, at 1 mg/ml. Indomethacin (Merck, Sharp and Dohme, München) was dissolved in 1 mol/l Tris buffer (pH 8.4) and diluted with distilled water to a concentration of 0.2 mg/ml. The preparation of the blocking agents is described elsewhere in detail [12, 13]. Final concentrations were methysergide-bimaleate 20 mg/l, phenoxybenzamine-hydrochloride 10 mg/l, propranolol-hydrochloride 200 mg/l, hyoscyamine-hydrobromide 10 mg/ml, and mepyr-amine-maleate 10 mg/ml. Streptozotocin (Upjohn Comp.) was freshly dissolved in 0.1 mol/l citrate buffer immediately before use.

Methods

Diabetes was induced in male rats (200–260 g) with streptozotocin (100 mg/kg IP). Blood glucose was determined by a glucose oxidase method (glucose analyser, Beckman Instr.), and urinary glucose and ketones by Gluketur sticks (Boehringer, Mannheim). The rats were fasted for 20 h before sacrifice but had free access to water. Three days after administration of streptozotocin hearts were removed from the heparinised (250 IU) and anaesthetized (1500 mg/kg urethane) rats. They were perfused through the aorta

Fig. 1 a and b. Original traces of the effects of arachidonic acid injections on the coronary perfusion pressure of a diabetic heart **a**, and on the tone of isolated bovine coronary artery strip **b** superfused with the cardiac effluent. After an equilibration period of 30 min arachidonic acid was injected into the aortic inflow at doses and times indicated ($\uparrow$). Calibrating doses of prostacyclin applied directly to the assay tissue are marked ($\blacktriangle$)

using the Langendorff technique at constant flow (8 ml/min) with Krebs-bicarbonate buffer equilibrated with 5% CO_2 in O_2 at 37°C (pH 7.4). The hearts beat spontaneously and their viability was assessed by measuring creatine phosphate (6.2 ± 0.3 $\mu\text{moles/g}$ wet weight) and ATP (4.0 ± 0.2 $\mu\text{moles/g}$ wet weight, $n = 10$) content after 60 min perfusion. No difference in creatine phosphate or ATP content of diabetic and control hearts was observed. Coronary perfusion pressure was monitored in a branch of the aortic inflow tract.

Experiments were started (time 0) after a 20–30 min equilibration period, and did not exceed 120 min. Prostaglandin-like activities were determined in the cardiac effluent by differential bioassay using the cascade technique of Vane [10, 11]. Bovine coronary artery strips [10] relaxed in a dose-dependent way to prostacyclin (10–200 ng), and contracted slightly in response to PGE_2 at doses above 50 ng. Chick rectum [11] was dose-dependently contracted by PGE_2 (5–100 ng) and did not respond to prostacyclin. As controls, rat-stomach strips, and in some experiments a rabbit-aorta strip, were also included. The tissues were treated with a mixture of blocking agents according to Block et al. [12], with the addition of indomethacin (2 $\mu\text{g/ml}$) to inhibit the endogenous formation of prostaglandin-like activities. Arachidonic acid was injected in a constant volume into a branch of the aortic inflow.

Statistical evaluation was performed by the U-test. Results are given as mean $\pm$ SEM. Statistical significance was assumed if $p \leq 0.05$.

Results

Streptozotocin caused a markedly elevated blood glucose (349 ± 31 mg/100 ml; $n = 12$) as compared to controls (76 ± 3 mg/100 ml; $n = 12$).

Basal release of prostaglandin-like substances could not be detected either from acutely-diabetic or control hearts using the differential bioassay technique. However, arachidonic acid administration to control and diabetic hearts was followed by an immediate decrease in coronary perfusion pressure

Fig. 2. Release of prostacyclin (PGI_2) from control ($\bullet$, $n = 4-6$) and acutely-diabetic ($\circ$, $n = 3-7$) rat hearts as measured by the relaxation of bovine coronary artery strip. After an equilibration period of 20–30 min the hearts were perfused as described and arachidonic acid was injected into the aortic inflow at the time intervals given in Figure 1. Mean $\pm$ SEM of n observations are indicated. *, $p < 0.05$ when compared to the appropriate control

and the release of a substance which contracted chick rectum and rat stomach, but relaxed bovine coronary arteries. There was no contraction of rabbit aorta ($n = 3$). A typical example is shown in Figure 1. Coronary perfusion pressure was already maximally reduced by the smallest dose (3 μg) of arachidonic acid. The relaxation of bovine coronary artery was

Fig. 3. Release of PGE₂ from control (●, *n* = 4–6) and acutely-diabetic (○, *n* = 3–7) rat hearts as measured by the contraction of chick rectum. Further details are given in the legend to Figure 2. Mean ± SEM of *n* observations are indicated. *, *p* < 0.05 when compared to the appropriate control

Table 1. Release of prostacyclin and PGE₂ from control and acutely-diabetic rat hearts after the application of 45 μg arachidonic acid. Prostacyclin and PGE₂ expressed as ng, mean ± SEM

	Control rats <i>n</i> = 3	Diabetic rats <i>n</i> = 4	Ratio diabetic/ control	P (U-test)
Prostacyclin	130 ± 19	297 ± 50	2.3	< 0.05
PGE ₂	15 ± 5	36 ± 8	2.4	< 0.05
Ratio prostacyclin/PGE ₂	8.7	8.2	0.9	< 0.05

dose-dependently increased by 3 to 27 μg of arachidonic acid. Treating the hearts with indomethacin (2 μg/ml) completely prevented these effects. The dose response curves for the release of prostacyclin and PGE₂-like activities by hearts from acutely diabetic and control rats are shown in Figures 2 and 3. At all doses of arachidonic acid, prostacyclin was the major prostaglandin-like substance released from normal and diabetic heart. For diabetic hearts the prostacyclin release increased from below 10 ng up to 300 ng depending on the amount of arachidonic acid, whereas the maximum PGE₂-like activity detected was 36–40 ng. The ratio of prostacyclin to PGE₂ was the same in diabetic and control hearts. At all arachidonic acid doses the responses of the diabetic hearts were greater than that of the controls. Arachidonic acid (45 μg) released 297 ± 50 ng of

prostacyclin-like activity from diabetic hearts, significantly more than the 130 ± 19 ng released by control hearts (*p* < 0.05). At the same dose there was a release of 36 ± 8 ng of PGE₂-like activity from the diabetic hearts, compared to only 15 ± 5 ng in controls (*p* < 0.05). Table 1 summarises the results for the 45 μg dose of arachidonic acid.

Discussion

Infusion of arachidonic acid produces a dose-dependent release of prostacyclin and PGE₂ from both acutely diabetic and control hearts. Prostacyclin was the major prostaglandin released from hearts of both groups, confirming results from other studies of prostaglandin release from isolated hearts of several animal species [9, 13, 14]. The ratio prostacyclin/PGE₂ ranged between 8 and 9 being apparently independent of the dose of arachidonic acid applied. These results, and the lack of prostaglandin endoperoxide release (as evidenced by the absence of rabbit aorta contracting substance), suggest that in the dose range studied, saturation of the metabolic conversion of prostaglandin endoperoxides into prostacyclin and PGE₂ was not achieved. These findings differ from earlier studies with rabbit and guinea-pig hearts, in which maximum release of prostacyclin and PGE₂ was obtained at lower doses of arachidonic acid (10 μg), and release of prostaglandin endoperoxides occurred [13, 14]. The higher capacity of rat hearts to produce prostaglandins cannot be explained. Measurements of the appropriate enzymes (prostaglandin-cyclooxygenase, prostacyclin synthetase, PGE₂-isomerase) should clarify this.

The increased release of prostacyclin and PGE₂ in the acutely-diabetic rat heart might be explained by better availability of the exogenous arachidonic acid because of increased vascular permeability [1]. Basal release of prostaglandin-like substances was not measured because of the low concentrations released, but there are some indications that the basal release is increased in acutely-diabetic hearts. Stam and Hülsmann [8] have shown inhibition of the higher coronary flow of diabetic hearts with indomethacin. The higher coronary flow of diabetic hearts may be explained by the observed increase in the formation of prostacyclin rather than the synthesis of PGE₂. Furthermore, these findings could help to explain the diminished aggregability of platelets of streptozotocin-diabetic rats [15].

There are several reports dealing with decreased prostacyclin formation in diabetes [16, 19, 20]. It should be noted, however, that these experiments have been performed on preparations of large ves-

sels, whereas our experiments were done on a complete organ circulation including small arteries and veins. It has been shown that the endothelial surface is enhanced in arterioles of diabetic animals but diminished in the larger vessels [18]. This morphological data could explain these apparently divergent observations. Moreover, because local perfusion is regulated by small vessel tone, changes in prostacyclin release by these vessels could be more relevant to the vascular disturbances of diabetes.

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Received: June 11, 1979,
and in revised form: November 19, 1979

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Note added in proof. Recently, Buchanan et al. (1979) reported marked variations in the prostacyclin synthesis depending on the time lapse between vessel wall preparation and measuring prostacyclin, presumably because of thrombin formation induced by mechanical trauma during the preparation procedure. Therefore, these authors recommend to heparinize the animals prior to their death. In contrast to the reported results, studies describing a decreased prostacyclin formation [16, 19, 20] were performed with vessel wall preparations from unheparinized animals. This methodological difference may be of relevance for the observed divergent results in our and the cited studies.

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