SHORT COMMUNICATION

Prostaglandin synthesis and inactivation in kidneys and lungs of rats with experimental diabetes

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Summary

1. Microsomal prostaglandin synthesis and tissue prostaglandin concentrations in the kidneys of rats made diabetic by pretreatment with alloxan or streptozotocin were significantly decreased but were unchanged in lungs.

2. Prostaglandin inactivation was increased in kidneys of alloxan-diabetic rats but not changed in streptozotocin-diabetic kidneys or in the lungs of either group.

3. These changes in prostaglandin metabolism may cause a renal prostaglandin deficit and could contribute to certain pathological sequelae observed in diabetes mellitus.

Key words: diabetes, kidney, lungs, prostaglandin-metabolizing enzymes, prostaglandin synthase.

Introduction

Abnormalities in prostaglandin metabolism may contribute to the haematological and cardiovascular complications of diabetes mellitus such as microangiopathy, atherosclerotic vascular changes and the increased tendency to myocardial infarction (Keen & Jarrett, 1975). Platelets from diabetic patients show increased aggregability (Heath, Brigden, Canever, Pollock, Hunter, Kelsey & Bloom, 1971; Sagel, Colwell, Crook & Laimans, 1975), produce more prostaglandin-like material when aggregating and are more sensitive to aggregation induced by arachidonic acid (Halushka, Lurie & Colwell, 1977).

On the other hand, synthesis of prostacyclin in biopsy specimens of human-forearm veins and arteries is decreased (Johnson, Harrison, Raftery & Elder, 1979; Silberbauer, Schernthaner, Sinzinger, Piza-Icatzer & Winter, 1979). Studies of prostaglandin metabolism in experimental models of diabetes may help to clarify the causes and consequences of these defects; thus we have measured the activities of enzymes which synthesize and inactivate prostaglandins in the kidneys and lungs of rats with two types of experimental diabetes.

Methods

Experimental diabetes was induced in male Wistar rats (160–200 g body weight) by intravenous injection of alloxan (50 mg/kg, 3 days before killing) or streptozotocin (60 mg/kg, 12 days before killing). In both groups blood sugar concentrations were greater than 19.5 mmol/l. Control animals were injected intravenously with sodium chloride solution (150 mmol/l: saline) (1·0 ml/kg, 3 days before killing); their blood sugar concentration was 4·6 ± 0·2 mmol/l (n = 6). Kidneys and lungs of the rats were removed and stored at −20°C until use. After thawing the organs were homogenized at 4°C in 4 vol. of phosphate buffer (50 mmol/l; pH 7·4) containing EDTA and cysteine (both 1 mmol/l) and centrifuged (4°C) at 3000 and 100 000 g for assay (a) of prostaglandin synthesis [the resuspended microsomal pellets were incubated for 60 min at 37°C with arachidonic acid (10 μg/ml) and reduced glutathione (3 mmol/l), and the resulting prostaglandin-like material was extracted and assayed on the isolated rat-stomach fundus-strip preparation in terms of prosta-
glandin \( F_{2\alpha} \) equivalents] and (b) of prostaglandin inactivation [the 100 000 g supernatant was incubated at 37°C with prostaglandin \( F_{2\alpha} \) (10 \( \mu g/ml \)) containing 0.1 \( \mu Ci \) of \([\beta^3H]\)-prostaglandin \( F_{2\alpha} \) (The Radiochemical Centre, Amersham, Bucks.; specific radioactivity 10-9 \( Ci/mmol \)) and NAD\(^{+} \) (5 mmol/l), extracted and the proportion of metabolites determined by thin-layer radiochromatographic separation]. These methods have been described in detail before (Hoult & Moore, 1977, 1980a). Both the formation of prostaglandin-like material from arachidonic acid and the breakdown of prostaglandin \( F_{2\alpha} \) were negligible when incubated similarly in buffer alone or with boiled microsomal fraction (for synthesis) or boiled 100 000 g supernatant (for breakdown).

A crude estimate of tissue prostaglandin content was obtained by extracting a portion of the homogenate within 15 s of homogenization in the cold (five strokes in an Ultra-Turrax homogenizer type 18/2N, about 5 s in all), and, after evaporation of the solvent and resuspension of the residue in Krebs bicarbonate solution, the spasmogenic prostaglandin-like material was assayed in terms of prostaglandin \( F_{2\alpha} \) equivalents by use of the rat fundus-strip preparation. Under these conditions the efficiency of the extraction was 93.8 \( \pm \) 1.8% \( (n = 10) \). Student's paired \( t \)-test was used to determine the significance of differences.

**Results**

Microsomal synthesis of prostaglandin-like material from arachidonic acid was substantially reduced in kidneys taken from both alloxan-diabetic rats (by 49.1 \( \pm \) 6.7% \( n = 10, P < 0.001 \)) and streptozotocin-diabetic rats (by 50.0 \( \pm \) 6.7% \( n = 11, P < 0.001 \)), but was the same in the lungs from both groups of diabetic rats (Fig. 1). The activity of prostaglandin-metabolizing enzymes (i.e. 'prostaglandinases', Hoult & Moore, 1980a) was increased in the kidneys of alloxan-diabetic rats by 134.5 \( \pm \) 20.7% \( (n = 10, P < 0.001) \), but was not changed in kidneys of animals given streptozotocin or in the lungs of either group (Fig. 1). The prostaglandin content of the freshly extracted homogenates (Fig. 1) was the same in the three groups of lungs but was much lower in kidneys of diabetic animals (reduced by 60.6 \( \pm \) 4.2% \( n = 10, P < 0.001 \) in alloxan-diabetic and by 59.0 \( \pm \) 4.4% \( n = 10, P < 0.001 \) in streptozotocin-diabetic rats). However, it is probable that much of this prostaglandin-like material is formed during homogenization and may be an indicator of prostaglandin

**Fig. 1.** Prostaglandin synthesis (a), breakdown (b) and concentrations (c) in kidneys and lungs from control (\( \square \)), alloxan-diabetic (\( \square \)) and streptozotocin-diabetic rats (\( \circ \)). In (a) synthesis is expressed as ng of prostaglandin \( F_{2\alpha} \)-like material formed per ml of microsomal suspension per hour, microsomes pooled from five rats in each group, \( n = 10-11 \) determinations; in (b) breakdown is expressed as % of prostaglandin \( F_{2\alpha} \) (10 \( \mu g/ml \)) broken down in 45 min (kidney) or 30 min (lung), supernatants prepared and tested separately from five rats in each group, \( n = 10 \); in (c) concentrations are expressed as \( \mu g \) of prostaglandin \( F_{2\alpha} \)-like material per gram wet weight of tissue, values obtained from homogenates prepared and extracted separately from five rats in each group, \( n = 10-13 \). Vertical lines show SEM values.
synthase activity rather than of the balance between synthesis and inactivation.

Discussion

These results show that experimental diabetes in the rat is associated with a striking decrease in the activity of prostaglandin synthase in the kidney but not in the lung. The concentrations of prostaglandins were also much lower in kidney but not in lung. It is therefore possible that there may be a substantial deficit in renal prostaglandins in vivo, and this may lead to, or contribute to, some of the renal manifestations of diabetes, such as altered blood flow, filtration and urine production, and the structural changes in blood vessels which lead to nephropa thy. For example, Foy & Salih (1979), by using rats prepared identically with those used in these experiments, have shown that renal blood flow per unit weight of tissue is decreased in both alloxan- and streptozotocin-diabetic rats and that creatinine and inulin clearances are increased. Such changes in renal function may be due to a reduction in vasodilator prostaglandins.

Prostaglandin synthase activity was determined in these experiments by measuring the transformation of arachidonic acid into bioassayable, stable prostaglandin-like material (i.e. prostaglandins E₂ and F₂α) under assay conditions which favour conversion of the intermediate prostaglandin endoperoxides into prostaglandin E₂ (Flower, Cheung & Cushman, 1973; Chan, Nagasawa, Takeguchi & Sih, 1975; Cottee, Flower, Moncada, Salmon & Vane, 1977). In the whole kidney it is known that the major metabolite of arachidonic acid is prostaglandin E₂ (Nasjletti & Colina-Chourio, 1976; Pace-Asciak & Rangaraj, 1977). It is probable that the formation of other prostaglandin-like substances derived from prostaglandin endoperoxides (e.g. thromboxane A₂ and prostacyclin, which have powerful and opposing effects on the cardiovascular system and platelets, Moncada & Vane, 1978) would also be decreased in the diabetic rat kidneys but this was not investigated in the present experiments. Furthermore, it was shown by Harrison, Reece & Johnson (1978) that the amount of prostacyclin in the renal cortex of streptozotocin-diabetic rats was reduced.

It is noteworthy that an increase in the activity of prostaglandinase may also contribute to a renal prostaglandin deficit, at least in the case of the alloxan-diabetic rats. We have shown previously that changes in the activity of prostaglandin synthase in certain pathological states are accompanied by inverse changes in the activities of prostaglandin-metabolizing enzymes (Houl t & Moore, 1980b). It is important to take into consideration the activity of prostaglandinases when considering the overall activity of the prostaglandin system, but this has not been attempted in previous studies on diabetes. It was surprising not to observe any change in the activity of prostaglandinase in the streptozotocin-diabetic rats and this merits further investigation; however, this form of diabetes is less severe than that induced by alloxan, in terms of survival and nephrotoxicity (J. M. Foy, personal communication), and this may be reflected by less-intense disruption of prostaglandin metabolism.

The mechanisms whereby these adaptational changes in prostaglandin synthesis and inactivation are brought about are not clear. For these experiments the simplest interpretation is that the absolute activities of synthetic and degradative enzymes are altered as a result of a metabolic signal, for example glucose excess, insulin deficiency or after a secondary hormonal or metabolic change.

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References


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