Concentration of phosphoribosyl pyrophosphate in renal hypertrophy

Contrasting effects of early diabetes and unilateral nephrectomy

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Studies were made of the renal phosphoribosyl pyrophosphate (*PPRibP*) content and *PPRibP* synthetase (EC 2.7.6.1) activity in rats diabetic for 5, 14 or 20 days, or unilaterally nephrectomized (UN) for 5 days, and in doubly lesioned animals. Approximately equal degrees of renal enlargement were found after 5 days diabetes or 5 days UN. In the doubly lesioned animals the increment of growth was additive. Unilateral nephrectomy of 5 days duration, in contrast with diabetes, had no effect on the *PPRibP* content of the contralateral kidney, nor did it modify the renal *PPRibP* content when performed on animals diabetic for 5, 14 or 20 days. The activity of *PPRibP* synthetase was unaffected by diabetes, UN or diabetes + UN. The results pinpoint a stage of nucleotide synthesis which is differentially affected by the two stimuli, in line with evidence for differences in regulation of nucleic acid turnover in the two conditions.

INTRODUCTION

We have previously reported that the growth of the kidney in normal development and in the initial renal hypertrophy of diabetes is associated with a decreased tissue content of phosphoribosyl pyrophosphate (*PPRibP*) (Kunjara *et al.*, 1986; Sochor *et al.*, 1986), and that a negative correlation exists between the renal content of *PPRibP* and the rate of kidney growth. It was suggested (Kunjara *et al.*, 1986) that was the requirement for *PPRibP* for RNA and DNA syntheses, which increase in diabetes (Seyer-Hansen, 1978, 1983; Cortes *et al.*, 1980), that led to depletion of *PPRibP*.

A second type of kidney growth, which is also characterized by an increase in the tissue RNA and DNA (principally the former), is that found in the compensatory hypertrophy of the contralateral kidney after unilateral nephrectomy (UN) (Malt, 1969; Ross *et al.*, 1975; Cortes *et al.*, 1976, 1980; Seyer-Hansen, 1978, 1983). There is some evidence that the mechanism of accretion of nucleic acids in this latter type of growth differs from that found in diabetic renal hypertrophy in that the increased content may result from a diminished rate of breakdown (Hill *et al.*, 1974; Ross *et al.*, 1975) as well as from an increased synthesis (Cortes *et al.*, 1976, 1980). For this reason it was interesting to study the effect of UN as a single lesion and, in addition, its effect in the double lesion of diabetes followed by UN on the tissue content of *PPRibP* to see whether the association of increased growth rate and falling tissue content of *PPRibP*, as found in the diabetic rat kidney, is replicated in compensatory hypertrophy and whether the effect of the two growth stimuli is cumulative.

METHODS

Animals

Albino rats of the Wistar strain, with an initial body weight of 200–220 g, were used. Diabetes was induced by the injection, intraperitoneally, of alloxan (200 mg/kg body wt.) into the rats which had been starved overnight. Food and water were allowed *ad lib.* thereafter. Unilateral nephrectomy was performed through a dorsal incision, and the left kidney was removed under nembutal anaesthesia.

Two major groups were studied, the first in which UN or sham-operation was performed on day 5 of diabetes and the rats were killed 1, 3 or 5 days later, and a second group in which rats diabetic for 5, 14 or 20 days were subjected to either UN or sham-operation and then killed 5 days later. Each set of animals included a control group subjected to either UN or sham-operation. All rats were fed *ad lib.* and given free access to water.

Determination of *PPRibP* content and *PPRibP* synthetase activity

The rats were killed by cervical dislocation, the kidneys removed and tissue extracts prepared as previously described by Kunjara *et al.* (1986). These extracts were used directly for the measurement of *PPRibP* and *PPRibP* synthetase as described by Kunjara *et al.* (1986). The results are presented on the basis of *PPRibP* content or *PPRibP* synthetase activity/g wet wt. of tissue. Expressing these results on the basis of mg of protein or of RNA would not materially alter the comparative values, since, as shown

Abbreviations used: *PPRibP*, phosphoribosyl pyrophosphate; UN, unilateral nephrectomy.

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Table 1. Effect of unilateral nephrectomy of normal and diabetic rats on the growth and PPRibP content of the residual kidney

Values are given as means $\pm$ S.E.M. Fisher's *P* values are given as * < 0.05 , ** < 0.01 , *** < 0.001 ; NS, not significant. The initial body weights of the rats in all groups were 200–220 g.

Condition	Group	No. of animals	Final body wt. (g)	Right kidney wt. (g)	P for group comparisons				PPRibP content (nmol/g)	P for group comparisons				Blood glucose (mm)	
					P for group comparisons					P for group comparisons					
					(a)–(b)	(a)–(c)	(b)–(d)	(c)–(d)		(a)–(b)	(a)–(c)	(b)–(d)	(c)–(d)		
1. Initial duration of diabetes 5 days; variable UN (1 – 5 days)															
Control, 1 day sham op.	(a)	6	228 ± 5	1.128 ± 0.053	NS				9.52 ± 0.53	NS				7.8 ± 0.7	
Control, 1 day UN	(b)	6	227 ± 5	1.120 ± 0.048							10.37 ± 0.72				7.1 ± 0.4
5 days diabetic, 1 day sham op.	(c)	4	200 ± 8	1.494 ± 0.100		*	**	NS			7.64 ± 0.81	NS	NS	NS	25.2 ± 4.5
5 days diabetic, 1 day UN	(d)	8	200 ± 8	1.423 ± 0.054							7.43 ± 0.51				25.2 ± 2.5
Control, 3 days sham op.	(a)	6	245 ± 2	1.072 ± 0.033	***				9.21 ± 0.36	NS				6.3 ± 0.9	
Control, 3 days UN	(b)	7	238 ± 5	1.260 ± 0.021							8.88 ± 0.39				6.3 ± 0.7
5 days diabetic, 3 days sham op.	(c)	5	216 ± 10	1.358 ± 0.067		***	***	*			7.49 ± 0.36	**	NS	NS	25.9 ± 2.4
5 days diabetic, 3 days UN	(d)	5	208 ± 7	1.542 ± 0.022							6.81 ± 0.48				25.0 ± 2.0
Control, 5 days sham op.	(a)	10	236 ± 7	1.048 ± 0.038	***				9.61 ± 0.31	NS				6.9 ± 0.2	
Control 5 days UN	(b)	7	237 ± 6	1.344 ± 0.041							9.38 ± 0.39				7.4 ± 0.5
5 days diabetic, 5 days sham op.	(c)	7	201 ± 10	1.307 ± 0.047		***	NS	*			7.75 ± 0.49	**	NS	NS	23.1 ± 1.8
5 days diabetic, 5 days UN	(d)	5	200 ± 10	1.538 ± 0.084							8.09 ± 0.65				19.8 ± 3.0
2. Initial duration of diabetes 5–20 days; constant UN (5 days)															
Control, 5 days sham op.	(a)	10	236 ± 7	1.048 ± 0.038	—				9.61 ± 0.31	—				6.9 ± 0.2	
5 days diabetic, 5 days sham op.	(c)	7	201 ± 10	1.307 ± 0.041		***	—	*			7.75 ± 0.49	**	—	NS	23.1 ± 1.8
5 days diabetic, 5 days UN	(d)	5	200 ± 10	1.538 ± 0.084							8.09 ± 0.65				19.8 ± 3.0
Control, 5 days sham op.	(a)	9	267 ± 3	1.191 ± 0.026							9.50 ± 0.54				6.3 ± 0.4
14 days diabetic, 5 days sham op.	(c)	13	198 ± 7	1.400 ± 0.046	—	***	—	***	9.32 ± 0.87	—	NS	—	NS	16.4 ± 2.4	
14 days diabetic, 5 days UN	(d)	5	209 ± 6	1.909 ± 0.120							7.49 ± 0.75				16.0 ± 2.3
Control, 5 days sham op.	(a)	5	271 ± 20	1.215 ± 0.075							9.48 ± 1.24				5.9 ± 0.1
20 days diabetic, 5 days sham op.	(c)	5	261 ± 7	1.516 ± 0.101		*	—	**			10.20 ± 0.79	NS	—	NS	17.9 ± 1.8
20 days diabetic, 5 days UN	(d)	8	248 ± 7	1.946 ± 0.064					9.09 ± 0.59				24.3 ± 1.9		

by Seyer-Hansen (1978), the renal contents of RNA and protein remain essentially the same per g of tissue in kidneys of diabetic, UN or diabetic + UN rats as in those from control rats. The same is true for the DNA content in the kidneys of diabetic and UN rats, but the DNA in kidneys of diabetic + UN rats is lower by some 20% (Seyer-Hansen, 1978).

RESULTS

Contrasting effect of short-term unilateral nephrectomy and diabetes on the *PPRibP* concentration of the rat kidney

As shown in Table 1, in the normal rat there is a significant increase in the weight of the contralateral kidney at 3 and 5 days after UN. At none of the times studied, either before (1 day) or during (3–5 days) the renal growth phase after the operation, was there any change in the *PPRibP* concentration in the remaining kidney (Table 1). This lack of response contrasts sharply with the highly significant fall in *PPRibP* concentration in the kidneys of short-term-diabetic rats [5 days after alloxan treatment (Table 1), and see Kunjara *et al.* (1986)], which exhibited a similar increment in growth per kidney. Thus, for an equivalent growth response to two different stimuli, there is a different pattern of change in the concentration of renal *PPRibP*.

These observations led to the study of the effects of the double lesion of UN and diabetes on renal growth and *PPRibP* concentration, in which measurements were made (1) when the diabetic-rat kidney was in a phase of rapid growth, i.e. 5 days after alloxan administration, and (2) when the early rapid hypertrophy had slowed down, at 14 and 20 days of diabetes.

Effect of unilateral nephrectomy on renal growth and *PPRibP* concentration in 5-day-diabetic rats

It is clear from the data in Table 1 that UN stimulated the growth of the 5-day-diabetic-rat kidney, the increment of growth being significant on days 3 and 5 after the removal of one kidney. However, there was no accompanying diminution in the *PPRibP* concentration at either time interval. Thus the additional growth due to the effect of UN is not reflected by any further change of the *PPRibP* over that due to diabetes alone [cf. Table 1, Group 1, (c) and (d)].

Effect of unilateral nephrectomy on renal growth and *PPRibP* concentration in rats diabetic for 14–20 days

At these longer times of diabetes the renal hypertrophy is considerably slower. When diabetic rats are subjected to UN in this slower phase of growth there is a further stimulation of the growth rate and, indeed, this UN-related growth appears more rapid when induced in the later stages of diabetes than is the case when the operation is performed in the early, fast-growing, stages of diabetic kidney hypertrophy. The percentage increase in kidney weight induced by 5 days UN in 5-, 14- and 20-day-diabetic rats were respectively 16%, 36% and 39% (Table 1). Nevertheless, UN at these longer times of diabetes produced no significant changes in the *PPRibP* concentration of the kidney (Table 1).

The activity of *PPRibP* synthetase was measured at the time when maximum effect of UN on renal growth in diabetes is manifest, i.e. after 14 days of diabetes + 5 days UN. As was the case when diabetes was the sole

stimulant (Kunjara *et al.*, 1986), there was no significant change in renal *PPRibP* synthetase activity [normal control 13.4 ± 1.2 , 14-day-diabetic 13.3 ± 0.8 , 14-day-diabetic + 5-day UN 12.1 ± 1.0 ($\mu\text{mol/h per g at } 37^\circ\text{C}$)].

DISCUSSION

Studies on the rates of RNA synthesis from labelled precursors have indicated that an increase in RNA synthesis determines the increase in RNA per cell in the kidney in early diabetes (Cortes *et al.*, 1980), whereas a concomitant decreased degradation of both pre-existing and newly formed rRNA could also be involved in the RNA accumulation in UN (Hill, 1975; Melvin *et al.*, 1976).

The present data, on the contrasting response of the *PPRibP* concentration in the kidney in renal hypertrophy after induction of diabetes and after UN, are consistent with two different mechanisms operating as a result of these two stimuli to renal growth at the time intervals studied here. The suggestion by Kunjara *et al.* (1986) that the decrease in renal *PPRibP* in the rapid phase of growth in experimental diabetes could be related to the increased rate of utilization for nucleotide and nucleic acid synthesis is consistent with the existence of a metabolic cross-over in this region, as indicated by the increased supply of the substrate ribose 5-phosphate (Steer *et al.*, 1985), an increased amount of product, nucleic acid (Seyer-Hansen, 1978), and a fall of the tissue content of an intermediate, *PPRibP*. The corollary of this would be that the lack of effect of UN on renal *PPRibP* concentration was due to the predominance of conservation of RNA over new synthesis.

Cortes *et al.* (1979, 1980, 1982) have stressed that diabetes-induced and compensatory renal growth are, almost certainly, triggered by different factors, although they produce similar alterations in uridine nucleotide metabolism and in RNA content. The present differential effects on *PPRibP* concentration are consistent with this view. Among the factors which might prove critical to the different responses of *PPRibP* are: (i) the higher rate of muscle wasting in diabetes relative to UN, a process that would provide a greater supply of precursors for nucleotide synthesis in the kidney in diabetes; (ii) differential effects of the two stimuli could be expected to influence a number of factors known to have a mitogenic effect on kidney cells in culture, e.g. concentrations of Na^+ , K^+ , vasopressin and purine bases (Kantha & Toback, 1985), and (iii) the concentration of plasma immunoreactive growth hormone rises sharply after UN of both normal and diabetic rats (Cortes *et al.*, 1980), again a factor that could provide the second signal for renal growth in the doubly lesioned group. Increased growth hormone could also provide the common signal for growth in the normal and diabetic rats.

Indirect evidence for a role for growth hormone in cell growth, and a possible regulatory mechanism increasing *PPRibP* formation, may be adduced from studies on rats bearing a growth-hormone-secreting pituitary tumour (MtTW5). The tumour-bearing rats showed a marked increase in hepatic oxidative and non-oxidative pentose phosphate pathways to ribose 5-phosphate formation (Gumaa *et al.*, 1969), which might thus promote *PPRibP* formation (Becker *et al.*, 1979; Kunjara *et al.*, 1986). Further studies on the effects of growth hormone and related factors on ribose 5-phosphate formation in

kidney might throw light on the differential response of renal *PPRibP* to diabetes and unilateral nephrectomy.

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