Renal hypertrophy in experimental diabetes

The activity of the 'de novo' and salvage pathways of protein synthesis

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Measurements were made of the activity of phosphoribosyl pyrophosphate amidotransferase (*PPRibP*-At, EC 2.4.2.14) and of adenine (APRT, EC 2.4.2.7) and hypoxanthine (HPRT, EC 2.4.2.8) phosphoribosyltransferases, representing the 'de novo' and salvage pathways respectively. *PPRibP*-At activity increased within 3 days of diabetes, whereas APRT and HPRT increased later. Incorporation of [¹⁴C]formate and of [8-¹⁴C]adenine into the nucleic acids of kidney slices showed that formate was incorporated earlier, and to a greater extent, than was adenine. These results indicate that, although the 'de novo' pathway for nucleotide synthesis is the main route in early diabetes, the salvage pathway assumes greater importance at later stages.

INTRODUCTION

The renal hypertrophy that occurs in the early stages of experimental diabetes is associated with an increase in both RNA and DNA [1,2], with an increase in the flux of glucose through the pentose phosphate pathway and the activity of the enzymes of the pathway [3], as well as with a fall in the tissue content of phosphoribosyl pyrophosphate (*PPRibP*) [4]. That the increase in RNA is due to a new synthesis, as opposed to a depressed breakdown, has been established by the work of Cortes and his group, who have infused precursors of purine and pyrimidine nucleotides and measured their incorporation into nucleotides and nucleic acids [5–8].

It has been suggested [4] that the fall in tissue content of *PPRibP*, despite an increase in the rate of provision of its immediate precursor, ribose 5-phosphate, reflects a cross-over point in the pathway of *PPRibP* production and utilization consequent on an increased rate of nucleic acid synthesis.

Since the synthesis of nucleic acids involves the phosphoribosylation of purine bases, which can arise from either, or both, of the 'de novo' or salvage pathways, the potential activity of these pathways has been studied by the measurement of the activity of the rate-limiting enzyme of the 'de novo' pathway, phosphoribosyl pyrophosphate amidotransferase (*PPRibP*-At, EC 2.4.2.14) and of the two phosphoribosylating enzymes of the salvage pathway, adenine phosphoribosyltransferase (APRT, EC 2.4.2.7) and hypoxanthine phosphoribosyltransferase (HPRT, EC 2.4.2.8), which also converts guanine into GMP. Parallel studies of the rates of incorporation of [¹⁴C]formate and of [8-¹⁴C]adenine into nucleic acids have been included to establish whether the relative balance of incorporation via the 'de novo' and salvage pathways is modified in diabetes.

METHODS

Animals

Male Wistar rats, with initial body weights in the

range 170–190 g, were used. Diabetes was induced by the intravenous injection of streptozotocin (60 mg/kg body wt.) to animals as previously described [4], and the animals were killed 3, 5 or 14 days later.

Incorporation studies

Approx. 300 mg of kidney slices was incubated in 5 ml of Eagle's Minimal Essential Medium, containing Earle's Balanced Salt Solution (Gibco; 041-1010) as described by Brosh *et al.* [9], but supplemented with phosphate to give 10 mM final concentration. The additions were either 20 μ Ci of sodium [¹⁴C]formate (58 mCi/mmol) or 1 μ Ci of [8-¹⁴C]adenine (53 mCi/mmol) (Amersham International). The glucose concentration was 5 mM, the gas phase was O₂/CO₂ (19:1) and the incubation time was 60 min at 37 °C. The use of 10 mM-P_i in the incubation medium requires some justification, in the light of the activating effect of this ion on *PPRibP* synthetase [10] and the fact that it is considerably higher than the physiological concentration. This value was chosen because of the high gradient of P_i across the cell membrane and because Planet & Fox [11] have shown, in erythrocytes, that an external concentration of 10 mM-P_i gives an intracellular concentration of 1 mM, which more closely approximates to the physiological value.

At the end of the incubation period, the contents of the flask were transferred to a homogenizer tube, and 0.5 ml of 10 M-HClO₄ was added. After blending in an Ultra-Turrax blender (Janke und Kunkel, Stauffen-in-Breisgau, Germany), the whole was centrifuged at 2000 *g* for 10 min. The extent of incorporation into nucleic acids was measured essentially as described by Brosh *et al.* [9].

Enzyme measurements

(1) **Preparation of the tissue extracts.** The rats were killed by cervical dislocation and the kidneys rapidly removed. The right kidney was used for incorporation studies as described above, and the left kidney for the preparation of tissue extracts. The tissue suspensions were 1:10 (w/v) homogenates of the whole kidney in

Abbreviations used: *PPRibP*, phosphoribosyl pyrophosphate; *PPRibP*-AT, phosphoribosyl pyrophosphate amidotransferase; APRT, adenine phosphoribosyltransferase; HPRT, hypoxanthine phosphoribosyltransferase.

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either 0.29 M-sucrose/0.01 M-Tris/HCl, pH 7.0 (*PPRibP*-At), or 0.25 M-sucrose/0.1 mM-EDTA, pH 7.4 (APRT and HPRT). These homogenates were centrifuged at 12000 *g* for 30 min and the supernatants dialysed against 100 vol. of the appropriate homogenizing medium for 1 h before use in the enzyme assays.

(2) Assay procedures.

(a) *PPRibP*-At. The activity of this enzyme was measured as described by Wood & Seegmiller [12]. The separation of the product, [¹⁴C]glutamate, from the substrate, [U-¹⁴C]glutamine, by adsorption on to DEAE-cellulose discs, was as described by Martin [13].

(b) APRT. This was measured by the method of Kizaki & Sakurada [14], except for the addition of 3 mM-TTP, as described by Allsop & Watts [15], to inhibit the action of 5'-nucleotidase (EC 3.1.3.5), as suggested by Gutensohn & Guroff [16].

(c) HPRT. This enzyme was also measured by the method of Kizaki & Sakurada [14], but with the addition of 3 mM-TTP.

Chemical methods

(a) **Nucleic acids.** RNA and DNA were measured on a HClO₄ pellet from tissue suspensions prepared as above, after the pellet had been extracted with 80% (v/v) ethanol and then hydrolysed with 5% trichloroacetic acid for 30 min at 90 °C. RNA was measured by the method of Schneider [17] and DNA by the method of Burton [18].

(b) **Protein.** Protein was measured in the total tissue homogenate by the method of Lowry *et al.* [19], with bovine serum albumin (fraction V) as standard.

RESULTS

The changes in the tissue contents of RNA, DNA and *PPRibP* resulting from diabetes are shown in Table 1. The changes in nucleic acid content are in agreement with previous reports [1,8], which show that short-term diabetes (2–5 days) has little effect on the RNA content, but decreases DNA by some 10%. They also agree with the previous report [4] in showing that the kidney growth after the induction of diabetes is accompanied by a fall in *PPRibP* content.

Enzyme activities

In view of the difficulty of finding an equitable basis on which to compare values in a rapidly growing tissue, such as kidney in early diabetes, with one which is essentially in a steady-state relationship to the whole animal, as in untreated adult animals, the results given in Table 2 are presented in three ways: as $\mu\text{mol/h}$ per g, as $\mu\text{mol/h}$ per total kidney mass and as $\mu\text{mol/h}$ per mg of DNA.

On the first of these bases, there are only small changes in the activity of APRT or HPRT at any time studied, but *PPRibP*-At activity is raised at all times examined, significantly so at 5 and 14 days. When the results are expressed as activity/mg of DNA, APRT is significantly raised at 5 days, but not at either 3 or 14 days; HPRT is significantly raised at all three stages of diabetes studied, as is *PPRibP*-At, the latter most markedly at the longer time intervals. Finally, when the results are expressed as activity in the total kidney mass, HPRT and *PPRibP*-At are increased at all three time intervals, and APRT only

Table 1. Vital data for control rats and for rats diabetic (streptozotocin) for 3, 5 or 14 days

Results are given as means $\pm$ S.E.M. *P* values (Fisher's test) are shown by: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Condition	No. of animals	Initial body wt. (g)	Final body wt. (g)	Wt. of two kidneys (g)	Kidney wt. (g/100 g body wt.)	Protein (mg/g wet wt.)	RNA (mg/g wet wt.)	DNA (mg/g wet wt.)	<i>PPRibP</i> (nmol/g)	Blood glucose (mm)
Control (C)	16	170–190	232 $\pm$ 8	2.069 $\pm$ 0.057	0.898 $\pm$ 0.016	196 $\pm$ 10	8.19 $\pm$ 0.38	4.25 $\pm$ 0.20	9.93 $\pm$ 0.15	8.3 $\pm$ 3
3 days diabetic (D)	12	170–190	189 $\pm$ 3	2.165 $\pm$ 0.071	1.157 $\pm$ 0.053	194 $\pm$ 3.5	7.45 $\pm$ 0.44	3.74 $\pm$ 0.14	6.73 $\pm$ 0.35	25.7 $\pm$ 7
D/C (%)			81***	105	129***	99	91	88*	68***	310***
5 days diabetic (D)	12	170–190	201 $\pm$ 5	2.338 $\pm$ 0.087	1.166 $\pm$ 0.035	181 $\pm$ 25	7.86 $\pm$ 0.39	3.95 $\pm$ 0.24	7.18 $\pm$ 0.50	24.2 $\pm$ 1.6
D/C (%)			87**	113*	130***	92	96	93	72**	292***
14 days diabetic (D)	10	170–190	220 $\pm$ 11	2.724 $\pm$ 0.139	1.248 $\pm$ 0.050	192 $\pm$ 20	7.53 $\pm$ 0.34	4.04 $\pm$ 0.22	7.87 $\pm$ 0.36	23.2 $\pm$ 1.8
D/C (%)			95	132***	139***	98	92	95	79***	280***

Table 2. Activities of phosphoribosyl-transfer enzymes in the kidneys of control rats from diabetic (streptozotocin) for 3, 5 or 14 days

The numbers in each group are as given in Table 1. Results are given as means $\pm$ s.e.m. Fisher's *P* values are shown by: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Condition	PPRibP-At activity			APRT activity			HPRT activity		
	(μ mol/h per g)	(μ mol/h per total kidney†)	(μ mol/h per mg of DNA)	(μ mol/h per g)	(μ mol/h per total kidney†)	(μ mol/h per mg of DNA)	(μ mol/h per g)	(μ mol/h per total kidney†)	(μ mol/h per mg of DNA)
Control	2.35 $\pm$ 0.11	4.86 $\pm$ 0.24	0.552 $\pm$ 0.026	23.6 $\pm$ 0.58	48.8 $\pm$ 1.27	5.55 $\pm$ 0.14	7.91 $\pm$ 0.23	16.4 $\pm$ 0.46	1.86 $\pm$ 0.05
3 days diabetic (D)	2.61 $\pm$ 0.18	5.95 $\pm$ 0.30	0.697 $\pm$ 0.050	21.2 $\pm$ 0.68	45.9 $\pm$ 1.54	5.67 $\pm$ 0.20	8.62 $\pm$ 0.27	18.7 $\pm$ 0.54	2.30 $\pm$ 0.07
D/C (%)	111	122*	126*	90*	94	102	109	114**	124***
5 days diabetic (D)	3.22 $\pm$ 0.29	7.53 $\pm$ 0.65	0.815 $\pm$ 0.07	24.9 $\pm$ 0.42	58.2 $\pm$ 0.97	6.30 $\pm$ 0.09	9.14 $\pm$ 0.26	21.4 $\pm$ 0.63	2.31 $\pm$ 0.07
D/C (%)	137*	155***	148***	105	119***	114**	116**	130***	124***
14 days diabetic (D)	3.13 $\pm$ 0.12	8.53 $\pm$ 0.33	0.775 $\pm$ 0.031	24.0 $\pm$ 1.04	65.4 $\pm$ 2.83	5.94 $\pm$ 0.27	8.39 $\pm$ 0.32	22.9 $\pm$ 0.88	2.08 $\pm$ 0.08
D/C (%)	133***	176***	140***	102	134***	107	106	140***	112*

† Activities in the total of both kidneys.

at the two longer times. It is noteworthy that, no matter which mode of presentation is used, the effect of diabetes on PPRibP-At is greater than on the other two enzymes of the salvage pathway, and tends to occur earlier.

Incorporation of [14 C]-formate or -adenine into nucleic acids

The rate of incorporation of labelled substrates into nucleic acids of normal and diabetic-rat kidneys is shown in Table 3. The incorporation of formate is sharply increased, even at the shortest time studied, and only increases slightly with the length of duration of the diabetes. The increase is apparent on all three modes of expression, and is greater than the rate of increase of either RNA or DNA at each time.

Adenine was incorporated at approximately half the rate of formate, and its rate of incorporation was essentially unchanged by diabetes when the results are expressed on a unit weight basis. Significant increases were observed when the incorporation into the total kidney mass is considered, although the increment of incorporation was appreciably less than that observed when formate was the substrate.

No measurements were made of either hypoxanthine or guanine incorporation, since the changes in the activity of APRT and of HPRT follow very similar courses, and it seemed reasonable to believe that hypoxanthine incorporation would therefore probably parallel the pattern of that shown by adenine.

DISCUSSION

Increased renal growth in early diabetes is accompanied by an increase renal content of RNA [1,2,6,7]. An increased rate of synthesis of RNA has been proposed as contributing to the accretion of RNA, since the rate of incorporation of labelled orotate and adenine into RNA, measured by continuous infusion *in vivo*, was elevated in early diabetes [5-7]. Cortes and co-workers [5-8,20] have made detailed studies of the route of pyrimidine synthesis in the rat renal cortex and glomeruli in early diabetes and, in particular, have shown the expansion of the cellular pools of UTP and CTP, which appears to be the result of enhanced synthesis of pyrimidines through the orotate ('*de novo*') pathway. However, there appears to be less information on the effects of early diabetes on the pathway of purine synthesis or on the changes of the enzymes linked to the '*de novo*' and salvage pathways.

The present experiments support the concept that the '*de novo*' route of purine synthesis contributes to renal RNA synthesis in streptozotocin-diabetic rats. There is an elevated rate of [14 C]formate incorporation into the nucleic acids which precedes the marked increase in activity of PPRibP-At, which occurs at 5 days after treatment with streptozotocin. In contrast, parameters linked to the salvage pathway, [14 C]adenine incorporation and the activities of APRT and HPRT, essentially keep pace with the rate of renal growth and remain constant when expressed per g of kidney cortex. The different patterns of incorporation of [14 C]formate and of [14 C]adenine cannot be ascribed to different pool sizes of adenine, since direct measurement of the renal concentration of adenine showed no significant change in early diabetes. [Adenine concentrations (nmol/g) were: control, 80.7 $\pm$ 5.7; 3 days diabetic, 90.9 $\pm$ 5.7; 5 days diabetic,

Table 3. Incorporation of [1-¹⁴C]formate or of [8-¹⁴C]adenine into the nucleic acids of kidney slices taken from control rats and from rats diabetic (streptozotocin) for 3, 5 or 14 days

Results are given as means $\pm$ S.E.M., at 37 °C. Fisher's *P* values are shown by: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Condition	10 ⁻³ × [1- ¹⁴ C]Formate incorporation (c.p.m./h)			[8- ¹⁴ C]Adenine incorporation (nmol/h)		
	(per g wet wt.)	(per total kidney†)	(per mg of DNA)	(per g wet wt.)	(per total kidney†)	(per mg of DNA)
Control (C)	424 $\pm$ 39	878 $\pm$ 74	99.7 $\pm$ 9	2.29 $\pm$ 0.08	4.74 $\pm$ 0.17	0.539 $\pm$ 0.018
3 days diabetic	701 $\pm$ 32	1517 $\pm$ 58	188 $\pm$ 4	2.55 $\pm$ 0.08	5.52 $\pm$ 0.17	0.682 $\pm$ 0.021
D/C (%)	165***	173***	188***	111*	116**	126***
5 days diabetic (D)	685 $\pm$ 35	1601 $\pm$ 84	173 $\pm$ 9	2.35 $\pm$ 0.07	5.49 $\pm$ 0.16	0.595 $\pm$ 0.018
D/C (%)	162***	182***	174***	103	116**	110*
14 days diabetic (D)	876 $\pm$ 72	2385 $\pm$ 198	215 $\pm$ 18	2.22 $\pm$ 0.08	6.05 $\pm$ 0.22	0.550 $\pm$ 0.020
D/C (%)	206***	272***	215***	97	128***	102

† Incorporation in total of both kidneys.

84.9 $\pm$ 7.5; six values in each group. We are indebted to Dr. D. Perrett of the Department of Biochemistry, Medical School of St. Bartholomew's Hospital, for these determinations.]

Although the present experiments lend support to a role for both 'de novo' and salvage pathways in the increased RNA of the kidney in experimental diabetes, they do not provide data on the relative contributions of the two routes. Factors in favour of an important role for the salvage route are: the higher absolute activities of APRT and HPRT relative to PPRibP-At, and the increased adenine incorporation observed in the experiments *in vivo* by Cortes *et al.* [5]. Insofar as the data in Table 2 can be taken as indicative of the rates of the two pathways, it may be noted that the rise in activity of the salvage pathway follows that of the 'de novo' pathway, and it is tempting to suggest that, as diabetes develops, the salvage pathway assumes greater prominence as the substrates for the pathway, i.e. adenine and guanine, become available as a result of the increased rate of degradation of muscle RNA which occurs some 2–4 days after the induction of diabetes [21]. It is probably significant that the kidney has a notably high PPRibP-At/HPRT activity ratio [16], also indicative of the importance of the 'de novo' pathway in this tissue.

The present results, showing aspects relating to increased biosynthesis of purine nucleotides, taken in conjunction with the extensive evidence for elevated pyrimidine synthesis *de novo*, support the hypothesis previously advanced that the fall in renal PPRibP in early diabetes is a resultant effect of increased utilization [4].

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