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NEPHROGENOUS CYCLIC AMP IN STREPTOZOTOCIN-INDUCED DIABETIC RAT

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SUMMARY

The effect of streptozotocin-induced diabetes, in the rat, on the excretion of nephrogenous cyclic AMP was investigated. This excretion was significantly less than that observed in control animals ($P < 0.001$). Administration of insulin partly restored the excretion of nephrogenous cyclic AMP to control values. In view of the reported elevated parathyroid hormone levels in the diabetic rat, the present findings suggest target organ resistance to parathyroid hormone in this diabetic animal model.

The streptozotocin diabetic rat shows both decreased duodenal calcium absorption and serum $1,25(OH)_2D_3$* (1, 2). Administration of $1,25(OH)_2D_3$ to this animal corrects the subnormal calcium transport (3) and treatment with insulin restores to normal serum $1,25(OH)_2D_3$ levels (2), as well as duodenal calcium absorption (4). On the basis of these observations, an insulin-dependent defect in the metabolism of vitamin D leading to a deficiency of $1,25(OH)_2D_3$ production has been proposed (2, 3). Previously, we reported that administration of (^3H) $25\text{-}OHD_3$ to streptozotocin-induced diabetic rats is followed by a decreased appearance of (^3H) $1,25(OH)_2D_3$ in plasma, liver, kidney and small intestine (5). Since the catabolism of $1,25(OH)_2D_3$ in the diabetic rat is not accelerated (6), the decreased appearance of (^3H) $1,25(OH)_2D_3$ (5, 6) suggested an impaired activity of renal 1α-hydroxylase. The mechanism for this inhibition is not understood. In view of the role of PTH in regulating renal 1α-hydroxylase activity (7), the reports of elevated serum PTH levels in the diabetic rat (8, 9) raises the possibility of target organ resistance to this hormone. The excretion of nephrogenous cyclic AMP

*Abbreviations: $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; $25\text{-}OHD_3$, 25-hydroxyvitamin D_3; PTH, parathyroid hormone; NcAMP, nephrogenous cyclic AMP; cAMP, cyclic AMP; GF, glomerular filtrate.

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(NcAMP) increases in response to PTH (10). This measurement is considered a reliable and sensitive index of parathyroid function (10, 11). The present study was, therefore, designed to investigate the effects of streptozotocin-induced diabetes in rats on NcAMP excretion, and the ability of insulin to reverse these changes.

MATERIALS AND METHODS

The sodium salt of cyclic AMP was obtained from Sigma (St. Louis, Mo). 8(^{3}H) adenosine 3',5'-cyclic phosphate ammonium salt (spec. activ. 20 Ci/mmol) was purchased from Amersham Corporation (Arlington Heights, Ill). Streptozotocin was kindly supplied by Dr. Dulin of Upjohn Co. (Kalamazoo, Mi).

Male albino rats 50-60g (Holzman Co., Madison, Wi) from vitamin D-deficient mothers were used in all experiments. After weaning, the animals were shielded from ultraviolet light and fed a vitamin D-deficient diet (Nutritional Biochemical, Cleveland, Ohio) with water supplemented ad libitum for 4-6 weeks. At that time the rats had no detectable circulating levels of 25-OHD$_3$ (12). For each experiment rats were randomly divided into two groups. One was made diabetic by intraperitoneal injection of 120 and 25 mg/Kg of streptozotocin freshly dissolved in 0.1 ml citrate buffer (pH 4.5) on two successive days. The second received buffer only. The criteria for diabetes were decreased growth rate, polyuria, persistent glucosuria (Tes-Tape, Lilly Co.) and increased levels of blood glucose (13). The streptozotocin-injected rats who did not develop diabetes were included as streptozotocin-treated controls. On the fourth day following streptozotocin, half of the diabetic rats were treated with insulin (Lente, Squibb), 1 u/animal administered subcutaneously once daily for seven days. At that time, rats were placed individually in metabolic cages for 24-hour urine collections and then exsanguinated from the bifurcation of the aorta under light ether anesthesia. Plasma and urinary cyclic AMP were measured by the protein binding method of Gilman (14), using charcoal treated with bovine serum albumin (2% w/v) to separate the bound from free nucleotide (15). The binding protein was prepared from rabbit muscle (16) and could be stored at -20^{o}C in 0.5 ml portions for periods of up to six months without loss of activity. Cyclic AMP was measured in urinary aliquots after dilution with assay buffer (1/100-1/150 (controls) or 1/20-1/30 (diabetics)). (^{3}H) cyclic AMP (0.001 µCi) was added to 750 µl of plasma to estimate recovery and was then mixed with 75 µl perchloric acid (27% solution) and centrifuged at 3000 rpm for 15 min. The supernatant was neutralized with 100 µl 2.2 N potassium hydroxyde (pH 6.5-8.0). Samples were recentrifuged and the protein-free supernatant collected and frozen at -20^{o}C prior to assay (17). The specificity of the assay was verified by measurement of cAMP content of samples after incubation for 12 hours with cyclic 3',5' nucleotide phosphodiesterase (Sigma) 0.25 U/ml plasma at 30^{o}C, pH 7.5 and 20 mM $MgSO_4$. These phosphodiesterase-treated samples were found to contain no detectable cAMP. The accuracy of this method calculated from recovery experiments varied from 82-95%. Individual samples were corrected for recovery. The sensitivity of the assay was 0.1-0.2 pmoles. Intra-assay coefficient of variations between three different dilutions was 7.5% for urinary and 10.7% for plasma values. NcAMP was calculated as a function of glomerular filtration rate and expressed as nmoles/100 ml (glomerular filtrate) as described by Broadus et al (11). Urinary creatinine was measured after the technique of Anker (18) and serum creatinine by an ASA Dupont, automatic analyzer. Serum calcium was determined by atomic absorption spectro-

TABLE 1. BODY WEIGHT, SERUM GLUCOSE, CALCIUM AND PHOSPHORUS CONCENTRATIONS IN GROUPS STUDIED. (MEAN ± SEM)

Groups*	Body Wt (g) Initial	Body Wt (g) Final	Glucose mg/dl	Ca^{++} mg/dl	P mg/dl
C	64 ± 2	69 ± 2	120 ± 10	9.7 ± 0.2	3.1 ± 0.05
SC	65 ± 1	68 ± 2	130 ± 10	9.5 ± 0.2	3.4 ± 0.05
D	63 ± 2	47 ± 1**	548 ± 25***	9.4 ± 0.2	3.2 ± 0.04
DI	65 ± 2	65 ± 2	260 ± 15***	9.5 ± 0.2	3.3 ± 0.04

* 6-10 rats per group. C: control; SC: streptozotocin control; D: diabetic; DI: diabetic insulin treated.
** significantly different from control (C), streptozotocin control (SC) and insulin treated (DI). $p < 0.05$.
*** significantly different from control (C) and streptozotocin control (SC). $p < 0.001$.

photometry (Perking Elmer, 370) and serum phosphorus by a colormetric technique (19). All data are presented as the mean ± SEM. Statistical significance between means was assessed using student's two-tailed test.

RESULTS

The data on body weight, serum glucose, calcium and phosphorus are given in Table 1. Following streptozotocin, body weights in the diabetic group were significantly less than in control. Insulin was effective in restoring body weights to normal. Serum glucose, significantly elevated in the diabetic group, was partially restored with insulin to control values. Serum calcium and phosphorus were similar in control, diabetic and insulin-treated diabetic rats. NcAMP excretion was significantly decreased in diabetic as compared to control animals (Table 2). Administration of insulin restored this excretion to almost control values. In order to determine if the decline in NcAMP in the diabetic rats was related to the observed reduced weight of these animals, control rats were restricted in food intake to match the reduced weights of the diabetic animals. The results in Table 2 (Exp. IV) demonstrate that weight loss alone in the absence of diabetes was not responsible for the decreased excretion of NcAMP in the diabetic animals. The metabolic data of individual animals of experiment I (Table 1) is summarized in Table 3.

TABLE 2. NEPHROGENOUS cAMP IN CONTROL (C), STREPTOZOTOCIN CONTROL (SC), DIABETIC (D) AND DIABETIC INSULIN-TREATED (DI) RATS

	NcAMP nmoles/100 ml GF
Experiment I	
C (6)	5.6 ± 0.6
SC (6)	5.7 ± 0.6
D (5)	2.4 ± 0.5*
DI (5)	4.4 ± 0.6
Experiment II	
C (6)	5.9 ± 0.6
SC (6)	6.1 ± 0.7
D (5)	3.0 ± 0.7*
DI (5)	4.2 ± 0.4
Experiment III	
C (5)	4.1 ± 0.5
D (5)	1.4 ± 0.3*
DI (5)	3.1 ± 0.3
Experiment IV**	
C (6)	4.9 ± 0.5
D (6)	2.5 ± 0.4*

* Significantly different from control and streptozotocin control. $P < 0.001$

** Food restricted from C to match weight of D group. Experiments were carried out on the 12th day after first streptozotocin injection. The body weight of C was 50 ± 2g and of D 48 ± 2g.

DISCUSSION

The present data provide evidence that streptozotocin-induced diabetes in the rat decreases NcAMP. This fraction of the cyclic nucleotide has not been measured in the rat; however, administration of PTH to this animal has been shown to increase total urinary cAMP (20) and to stimulate renal adenylate cyclase (21). In man, urinary cAMP is derived from two sources, the glomerular filtrate and a quantity formed de novo in the kidney (10). This latter named, NcAMP, is controlled by PTH (10, 11). A decrease in PTH-dependent renal adenylate cyclase has been demonstrated in rats with secondary

TABLE 3. METABOLIC DATA IN CONTROL (C), DIABETIC (D), AND INSULIN DIABETIC (DI) RATS.

		Creatinine Clearance (ml/min)	Plasma cAMP (pmoles/ml)	Urinary cAMP* nmoles/ 100 ml GF	Urinary cAMP* nmoles/ mg creatinine	NcAMP** nmoles/ 100 ml GF	NcAMP** nmoles/ mg creatinine
C	Rat 1	0.23	16.6	7.8	11.2	6.2	8.8
	2	0.20	10.4	5.6	9.4	4.5	7.7
	3	0.09	23.0	9.1	12.9	6.9	9.7
	4	0.27	8.8	8.0	13.3	7.1	11.8
	5	0.20	11.7	6.6	11.0	5.4	9.1
	6	0.30	17.6	5.4	6.7	3.7	6.1
	Mean ± SEM	0.22 ± 0.03	14.68 ± 2.18	7.08 ± 0.60	10.75 ± 1	5.63 ± 0.55	8.87 ± 0.78
D	Rat 1	0.47	10.0	4.5	6.5	3.7	5.2
	2	0.94	18.6	4.1	5.9	2.1	3.2
	3	0.82	18.9	3.8	6.3	1.9	3.1
	4	0.58	16.6	5.0	7.1	3.3	4.8
	5	0.98	20.5	3.1	5.2	1.1	-
	Mean ± SEM	0.76 ± 0.10	16.92 ± 1.83	3.70 ± 0.29	6.20 ± 0.32	2.42 ± 0.47	4.10 ± 0.54
DI	Rat 1	0.47	10.0	4.5	6.5	3.7	5.2
	2	0.40	17.8	4.2	7.1	2.4	4.1
	3	0.35	11.0	6.7	11.2	5.6	9.3
	4	0.41	7.4	6.1	12.2	5.3	10.8
	5	0.29	13.2	6.4	9.2	5.0	7.5
	Mean ± SEM	0.38 ± 0.03	11.88 ± 1.75	5.58 ± 0.51	9.24 ± 1.10	4.40 ± 0.59	7.38 ± 1.24

* Urinary cAMP expressed as a function of glomerular filtration rate and as a function of urinary creatinine.
** NcAMP expressed as a function of glomerular filtration rate (nmoles/100 ml GF) and in relative terms provided by the clearance ratio (nmoles/mg creatinine).

hyperparathyroidism produced by either vitamin D or calcium deficiency (22). However, the decrease in the adenylate cyclase activity was directly related to the degree of hypocalcemia and not to the elevated PTH levels. In the present experiments, control, diabetic and insulin-treated animals were all normocalcemic (Table 1). Thus, in the absence of hypocalcemia, the finding of low NcAMP excretion in the diabetic rat in whom elevated levels of PTH have been reported (8-9) suggests target organ resistance to PTH in this animal. The effect of insulin in partially restoring NcAMP excretion to control values (Table 2), and the report of similar effect on PTH levels (8) provides additional support for this conclusion.

The possibility that streptozotocin nephrotoxicity was the cause for the observed decrease in NcAMP excretion is not likely due to the findings of no effect on the excretion of this fraction in those experiments in which the diabetegenic agent was administered and did not induce diabetes (Table 2). Furthermore, correction of NcAMP excretion by insulin would also rule out this possibility. In control rats, 70-85% of total urinary cAMP was excreted as NcAMP (Table 2). This is higher than that found in humans where 50% is of nephrogenous origin (10). This increased excretion in the vitamin D-deficient rat may be attributed to the higher secretion rate of PTH reported in this animal (23). The present findings of decreased NcAMP excretion is consistent with the inhibition of 1α-hydroxylation of vitamin D_3 observed in the diabetic rat. These results suggest renal resistance to PTH in this animal model. This phenomena is reminiscent to that observed in humans with type I pseudohypoparathyroidism (24). The significance of an accelerated catabolism of cAMP in the renal cortex of diabetic rat remains to be established (25).

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