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Diabetes and Renal Calcium Binding Protein in the Rat[1] (41929)

H. P. SCHEDL,* S. CHRISTAKOS,† H. D. WILSON,*
L. MALKOWITZ,† AND R. L. HORST**

*Veterans Administration Medical Center and Gastroenterology–Hepatology Research Laboratories, Department of Medicine, College of Medicine, University of Iowa, Iowa City, Iowa 52240; †University of Medicine and Dentistry of New Jersey, Newark, New Jersey 07103; and **National Animal Disease Laboratory, Ames, Iowa 50010

Abstract. Renal calcium binding protein (CaBP), a vitamin D-dependent protein of 28,000 M_r, may be involved in calcium transport by cells of the renal tubule. The streptozotocin-diabetic rat is hypercalciuric and shows markedly decreased concentration of 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$] in serum and of CaBP in small intestine. To examine the relationship of renal CaBP in diabetes to 1,25-$(OH)_2D_3$ and urinary calcium excretion, renal CaBP, serum 1,25-$(OH)_2D_3$, and urinary calcium were measured in control, diabetic, and insulin-treated diabetic rats. Treatment of the diabetic rat with insulin decreased urinary calcium excretion and elevated 1,25-$(OH)_2D_3$ toward normal. Renal CaBP was found to be the same in controls and diabetics despite a tenfold difference in concentration of 1,25-$(OH)_2D_3$ in serum, and to be unaffected by insulin treatment, which elevated 1,25-$(OH)_2D_3$ by a factor of 7 above untreated diabetics. It is concluded that in the diabetic rat either (1) the threshold concentration of 1,25-$(OH)_2D_3$ for inducing synthesis of renal CaBP is set at a much lower level than that for intestinal CaBP, or (2) since both 1,25-$(OH)_2D_3$ and renal CaBP are produced in the kidney, 1,25-$(OH)_2D_3$ exerts a paracrine effect on renal CaBP production because of its high local concentration. The increased urinary calcium excretion in the untreated streptozotocin-diabetic rat is not secondary to an alteration in renal CaBP.

Vitamin D-dependent calcium binding proteins (CaBP) are present in several organs, including gut and the kidney of birds and mammals. In the chick, the identical protein of molecular weight 28,000 is found in both gut and kidney (1). In the rat, the most studied mammalian species, the intestinal CaBP has a molecular weight of 9000 and the renal protein a molecular weight of 28,000. Renal CaBP of the rat measured by radioimmunoassay is markedly decreased by vitamin D depletion as compared with control values (2, 3). The small intestinal CaBP measured by Chelex assay is markedly decreased in the vitamin-D-depleted rat and in the streptozotocin-diabetic rat (4), in association with very low concentrations of 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$] in serum (5, 6). In the present study we measured renal CaBP by radioimmunoassay in control, diabetic, and insulin-treated diabetic rats and sought correlations with urinary calcium and serum concentrations of 1,25-$(OH)_2D_3$ and its precursor 25-hydroxycholecalciferol (25-OH-D_3).

Materials and Methods. Male Sprague–Dawley rats (Harlan Sprague–Dawley) weighing approximately 200 g were fed Purina rat chow *ad lib* (Purina Chow 5012: 1.01% calcium, 0.74% phosphorus, 0.21% magnesium, 3.3 IU vitamin D_3/g). The animals were divided randomly into control and experimental groups. Rats to be made diabetic were injected intraperitoneally with streptozotocin (kindly provided by the Upjohn Co., Kalamazoo, Mich.) freshly dissolved in citrate buffer, pH 4.5 (50 mg/ml; 80 mg/kg body wt), while the control group was injected with buffer only. Body weight was measured daily and urine was collected in 24-hr samples. The diabetic state was verified 2 days postinjection by weight loss and glucosuria, as measured with TesTape (Eli Lilly Co., Indianapolis, Ind.). Beginning on the third day after injection, diabetics were subdivided into an untreated group and an insulin-treated group. Insulin-treated diabetic rats received 1 U insulin/50 g body wt (NPH, Iletin, Lilly) at 1600 hr daily. All animals

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were housed individually in metabolic cages. Controls and diabetics were pair-fed and the insulin-treated diabetic group was fed *ad lib.* For the pair-fed animals, the amount of food provided to the control animals during the first 4 days after streptozotocin injection was restricted to the amount of food consumed by the matched group of diabetic animals on the previous day. Beginning with the fourth day after streptozotocin treatment, when the diabetic animals began to exhibit hyperphagia, the amount of food provided to the diabetic animals was restricted to the amount consumed by the control animals on the previous day.

Animals were killed by exsanguination from the aorta 10 days after streptozotocin or vehicle and 16 hr after the final daily injection of insulin. The left kidney was removed and frozen immediately in dry ice for renal CaBP radioimmunoassay (2). 1,25-$(OH)_2D_3$ and 25-OH-D_3 in serum were measured by the method of Horst (8), glucose in serum was determined by the method of Somogyi (9), and urinary calcium was measured by atomic absorption spectrometry (Perkin–Elmer 303). Data analysis was by unpaired Student's *t* test.

Results. Body weight and serum glucose data are shown in Table I. Mean weights of all groups were similar at the start of the experiment. Controls gained weight as did insulin-treated diabetics after the postinjection period of weight loss. Diabetics lost weight. Serum glucose at the time of sacrifice was markedly elevated in untreated diabetics and was restored to control levels by insulin treatment.

TABLE I. BODY WEIGHT AND SERUM GLUCOSE OF GROUPS STUDIED (MEANS ± SE)

Group[a]	Body weight (g)		Serum glucose (mg/100 ml)
	Initial	Final	
Control	203 ± 4	252 ± 5[c]	158 ± 9[c]
Diabetic	206 ± 4	175 ± 4	744 ± 47
Diabetic, insulin treated[b]	205 ± 5	234 ± 7[c]	147 ± 39[c]

[a] Nine animals in each group.
[b] Weight 190 ± 4 g on morning of Day 3, prior to initiation of insulin treatment.
[c] Control and diabetic, insulin-treated differ from diabetic, $P < 0.001$.

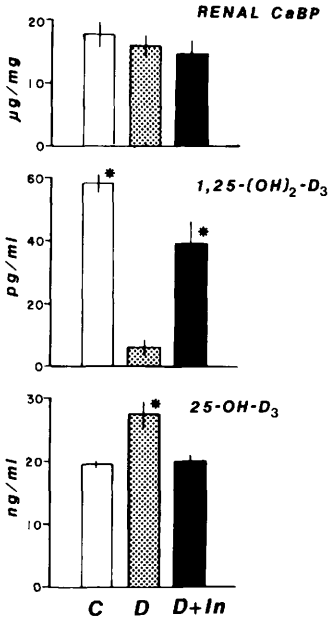

FIG. 1. Responses of concentration of calcium binding protein (CaBP) in kidney and of 1,25-$(OH)_2D_3$ and 25-OH-D_3 in serum to streptozotocin diabetes and insulin treatment. Renal CaBP is the same in control (C), diabetic (D), and insulin-treated diabetic (D + In) groups. 1,25-$(OH)_2D_3$ concentration in serum is depressed to one-tenth the control value by diabetes and increased sevenfold by insulin treatment. 25-OH-D_3 concentration in serum is greater in D than in C and D + In. *Significantly different from all other groups in data set, $P < 0.05$.

Renal CaBP concentration is shown in Fig. 1, with the corresponding serum concentrations of 1,25-$(OH)_2D_3$ and 25-OH-D_3, for the control, diabetic, and insulin-treated diabetic groups. Total renal CaBP content (not shown) demonstrated the same relationship as the CaBP concentrations for the various groups. Renal CaBP concentration and content did not differ among groups, although the serum 1,25-$(OH)_2D_3$ concentration differed significantly among all groups. The markedly reduced concentration of 1,25-$(OH)_2D_3$ in the diabetic group increased in response to treatment with insulin. Concentration of 25-OH-D_3, the precursor to 1,25-$(OH)_2D_3$, was greater in the diabetic group than the control and insulin-treated diabetic groups.

The effect of diabetes and insulin treatment on urinary calcium excretion is shown in

Fig. 2. Diabetes increased 24-hr urinary calcium excretion to several times the control value. Insulin treatment of diabetics decreased calcium excretion significantly but excretion rate remained above that of controls.

Discussion. Data of Table I define typical control, diabetic, and insulin-treated diabetic groups as characterized in prior studies (4–6). Serum 1,25-$(OH)_2D_3$ and 25-(OH)-D_3 concentrations are similar to findings of our previous studies (Fig. 1) (5, 6). Intestinal CaBP (4) and calcium transport (10, 11) are markedly depressed at the low serum 1,25-$(OH)_2D_3$ in diabetics (5, 6). Renal CaBP is the same in control, diabetic, and insulin-treated diabetic groups, despite tenfold differences in 1,25-$(OH)_2D_3$ concentrations in serum.

The role of vitamin D in renal calcium homeostasis has yet to be defined. Although not all studies are in agreement (12, 13), it is now established that renal CaBP is vitamin D dependent (2, 3) and responds appropriately to stimuli that increase 1,25-$(OH)_2D_3$. Calcium depletion causes a twofold increase, and phosphorus depletion a fourfold increase in renal CaBP (14). Calcium depletion is associated with hypocalciuria and phosphorus depletion with hypercalciuria (15). Streptozotocin diabetes in the rat (Fig. 2) (10, 11, 16) and diabetes mellitus in man (17, 18), are associated with hypercalciuria. This lack of correlation between renal CaBP, concentration of vitamin D metabolites in serum,

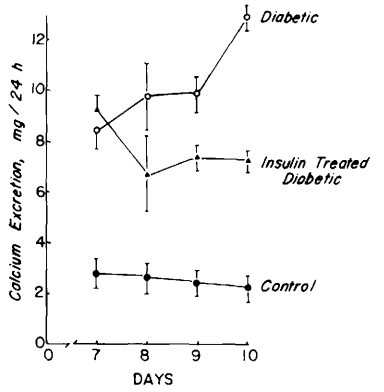

FIG. 2. Urinary calcium excretion in diabetes: Effects of insulin treatment. Diabetes increases urinary calcium excretion. The elevated urine calcium is decreased by insulin treatment but remains above control values.

and urinary calcium excretion demonstrates the need for further studies to define the role of renal CaBP and vitamin D metabolites in renal calcium transport.

The most interesting finding of our study is that renal CaBP is independent of concentration of 1,25-$(OH)_2D_3$ in serum. This suggests that the threshold concentration of 1,25-$(OH)_2D_3$ for inducing synthesis of renal CaBP is much lower than for intestinal CaBP. However, the kidney is the site of production of both 1,25-$(OH)_2D_3$ and the CaBP. Production of 1,25-$(OH)_2D_3$ by renal slices from the diabetic rat is less than one-third that produced by controls (16). Nevertheless, it is likely that the concentration of 1,25-$(OH)_2D_3$ in the kidney is greater than that in serum and in other organs such as the gut. Thus, the maintenance of renal CaBP in diabetics could be a paracrine type of response to high local 1,25-$(OH)_2D_3$ concentration near its site of production. It should be noted that streptozotocin diabetes is unique in that 25-OH-D_3 is normal in the setting of low 1,25-$(OH)_2D_3$, and this could be a factor in the normal renal CaBP.

Like the streptozotocin diabetic rat, patients with juvenile onset diabetes mellitus show disturbances of calcium homeostasis. Thus, diabetic patients show hypercalciuria responsive to insulin treatment (17, 18) and decreased concentrations of 1,25-$(OH)_2D_3$ in serum in conjunction with elevated 25-OH-D_3 (19, 20). Despite these parallels between the juvenile diabetic patient and the streptozotocin diabetic rat, findings with renal CaBP differ.

It has recently been reported that the renal vitamin D-dependent CaBP, measured by immunofluorescence in kidneys from juvenile diabetics and nondiabetic patients at necropsy, is greatly reduced in kidneys from diabetics (21). It is indeed possible that the decreased renal CaBP in kidneys from diabetic patients at necropsy might not be the consequence of metabolic effects of diabetes on the vitamin D–endocrine system and calcium homeostasis. Chronic renal disease is a major long-term complication of diabetes. In the human postmortem study, average duration of diabetes was 20 years. In our study, short-term diabetic rats had normal kidneys and renal CaBP in association with the defects

in calcium homeostasis and vitamin D metabolism (Figs. 1 and 2) of diabetes. The reason for the difference between our findings and those in human diabetics is unknown. The decrease in CaBP in patients with diabetes mellitus might be a species difference in metabolic effects of diabetes. Alternatively, long-term diabetes in man is complicated by loss of renal parenchyma, which in itself leads to a decrease in renal CaBP.

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