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Regulation of the Receptor-Mediated Cyclic AMP Response of Kidney to Parathyroid Hormone in the Vitamin D-Deficient Rat

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Rats fed a diet deficient in vitamin D were found to exhibit a refractory cyclic AMP response of kidney slices to parathyroid hormone and a marked decrease in membrane parathyroid hormone-dependent adenylate cyclase activity. Both the characteristic calcium deficiency (hypocalcemia) and secondary elevation of circulating parathyroid hormone appeared before the first noticeable decrease in hormone-dependent enzyme activity. After repletion of D-deficient rats with vitamin D_2, we found that serum calcium and parathyroid hormone were both restored to normal levels before the depressed enzyme response to the hormone was reversed. Moreover, infusion of parathyroid hormone into vitamin D-replete rats led to a marked reduction in parathyroid hormone-dependent adenylate cyclase activity, which was partly restored to control level 3 hours after discontinuing the hormone infusion. Taken as a whole, this study suggests that the elevated endogenous parathyroid hormone in the vitamin D-deficient rat is involved in the "down-regulation" of renal cyclic AMP responsiveness to the hormone. However, these experiments do not rule out the possibility that calcium deficiency and/or vitamin D per se participate in the regulation of the renal cyclic AMP response to parathyroid hormone.

Key words: kidney, vitamin D, parathroid hormone, cyclic AMP

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The vitamin D-deficient rat exhibits a profound reduction in the physiologic responses of kidney and bone to parathyroid hormone (PTH). Specifically, the in vivo manifestations of the vitamin D-deficient state are noted as a refractory phosphaturic response to PTH [1–3] and an impaired calcemic response to the hormone [3–5]. These observations have been interpreted to mean that vitamin D (metabolites) plays a "permissive" role in PTH regulation of target cell function. However, it has been demonstrated that the calcium-deficient, vitamin D-replete animal also shows a reduction in renal [6] and bone [7] responsiveness to PTH similar to that observed in the –D animal. This implies that factors other than vitamin D deficiency per se may contribute to the depression of target cell responsiveness to PTH. The vitamin D-deficient [8] and calcium-deficient [6] rat models exhibit marked hypocalcemia as a reflection of calcium deficiency, which in turn results in a substantial elevation of circulating PTH. Therefore, one or both of these factors may play a role in the "down-regulation" of kidney and bone responsiveness to PTH.

We reported that a locus of impairment of renal responsivity to the peptide hormone was at the level of the membrane PTH-dependent adenylate cyclase in both the vitamin D-deficient rat [9] and the calcium-deficient, vitamin D-replete rat [6]. Both experimental groups exhibited substantial reductions in the level of PTH-dependent adenylate cyclase activity in plasma membrane preparations from renal cortex. The refractory kidney cyclic AMP response to the hormone was a result of an impairment of the PTH receptor-mediated activation of the adenylate cyclase, since the vitamin D-deficient and calcium-deficient animal models did not show any change in the activity of the catalytic unit of the enzyme (fluoride activation). Moreover, we found that the reduction in hormone-dependent renal adenylate cyclase activity of plasma membranes prepared from both these animal models was specific for PTH activation, whereas calcitonin- and vasopressin-dependent adenylate cyclase activities were unchanged from control [6, 9].

The information to date suggests that the mechanism of the impaired cyclic AMP response of kidney to PTH may be a "down-regulation" of PTH receptor-mediated responsiveness of the membrane adenylate cyclase. This regulation of PTH receptors may be associated with, or perhaps is due to, the secondary hyperparathyroid state of the vitamin D-deficient and calcium-deficient (+D) animal models. In the present study, we have explored the interrelationship between the calcium deficiency (hypocalcemia), elevated endogenous PTH, and the refractory renal cyclic AMP response to PTH in the vitamin D-deficient rat.

MATERIALS AND METHODS

Animal Preparation

Male, Sprague-Dawley rats were obtained from Holtzman Co, Madison, Wisconsin, at 21 days of age (weanling) and immediately placed on the experimental diets [9, 10]. These were: control – 0.47% Ca, 0.36% P, and 70 units vitamin D_2 p.o. twice weekly; vitamin D-deficient – same as control but received no vitamin D_2. The experimental diets were fed for periods up to 4 weeks.

Experimental Procedures

At various times the animals were killed by decapitation, blood was collected for preparation of serum, and kidneys were rapidly excised and placed in ice-cold 0.9% NaCl. One cortical slice (0.5 mm) was then prepared from each kidney using a Stadie-Riggs microtome, and the remaining cortical tissue was dissected free of medulla. This cortical tissue was used in the preparation of a subcellular fraction enriched in plasma membranes according to the method of Fitzpatrick et al [11]. The cortical slice was divided in half, and each half-slice was incubated for 15 min at 37° in 2 ml Krebs-Ringer-HCO_3, pH 7.4, buffer containing 1 mM methylisobutylxanthine. Then 1 μg/ml synthetic bovine PTH (1–34) (Beckman Instruments, Bioproducts Department, Palo Alto, California) was added to one of the half-slice vessels and vehicle was added to the paired half-slice (basal). After 10 min further incubation (peak response time) each half-slice was transferred to 0.5 ml 50 mM acetate buffer, pH 4.0, at 100° for 3 min. Approximately 5,000 CPM cyclic (^{3}H)-AMP (New England Nuclear) was added to each tube, and the tissue was homogenized, centrifuged, and the supernatant extract employed for assay of cyclic AMP by radioimmunoassay [12, 13]. The adenylate cyclase activity of renal plasma membranes was assayed as previously described [6, 14]. Serum levels of Ca, Mg, P, and immunoreactive (i) PTH were measured as previously reported [6, 15]. The iPTH values were expressed as pg-equivalents to native bovine PTH (1-84).

RESULTS

The data in Table I demonstrate the effect of the vitamin D-deficient diet on the renal cyclic AMP response to PTH in vitro. Renal tissue from the control group responds to a maximal activation level of 1 μg/ml PTH (1-34), with an approximate 20-fold increase in cyclic AMP content in the tissue slice. However, cortical slices from the vitamin D-deficient group exhibit a pronounced reduction in the cyclic AMP response to PTH, which is highly significant relative to the control cyclic AMP response ($P < 0.01$). The basal cyclic AMP level of slices from vitamin D-deficient animals was significantly ($P < 0.01$) elevated over control.

The refractory cyclic AMP response to PTH in vitro was explored further by assaying the cortical plasma membranes of control and vitamin D-deficient rat kidney for PTH-dependent adenylate cyclase activity. Figure 1 demonstrates that the reduced tissue slice

TABLE I. Renal Cortical Cyclic AMP Response to Parathyroid Hormone

	Slice cyclic AMP pmole/mg tissue	
	Basal	PTH 1 μg/ml
Control	1.07 ± 0.08	21.9 ± 2.0
Vitamin D-deficient	1.69 ± 0.06[a]	10.5 ± 0.7[a]

Animals were fed the diets described in Materials and Methods for 4 weeks and then killed to obtain cortical slices for incubation in vitro. The data for tissue cyclic AMP levels are expressed as the mean ± SEM of 6 control and 6 vitamin D-deficient rats.

[a]Significantly different from control, with $P < 0.01$.

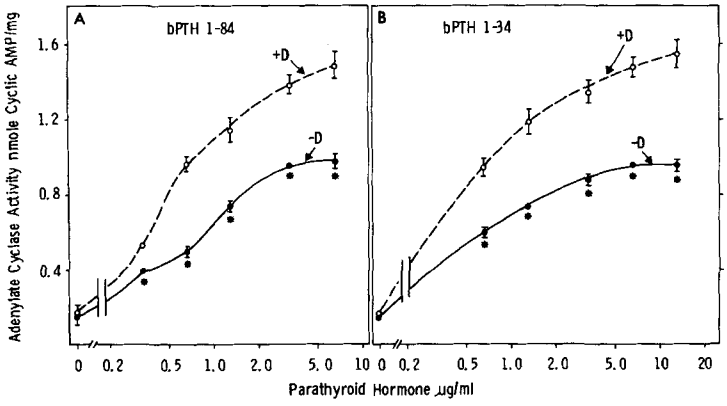

Fig 1. Comparison of the activation of renal cortical adenylate cyclase by native bovine PTH (1-84) and synthetic bovine PTH (1-34). Male weanling rats were fed the control (+D) or vitamin D-deficient (–D) diets described in Materials and Methods for 4 weeks and then killed to obtain renal cortex for preparation of plasma membranes. Adenylate cyclase activity is expressed as the mean ± SEM (vertical bar) of 3 +D and 3 –D preparations, each assayed in duplicate. The values are nmole per mg protein per 10 min reaction at 30°. *$P < 0.01$ relative to the control condition. Fluoride-dependent (NaF 10 mM) enzyme activities were: +D 1.7 ± 0.1, –D 1.5 ± 0.1 ($P > 0.05$).

response to PTH in vitamin D-deficient kidney was associated with a pronounced and highly significant ($P < 0.01$) decrease in PTH-dependent adenylate cyclase activity. Furthermore, this depression in the enzyme response to the hormone was observed whether the native 84-amino acid molecule was used as the agonist or the synthetic, amino-terminal portion (1-34) of the PTH molecule was employed to activate the membrane adenylate cyclase. Other relevant data depicted in Figure 1 show that vitamin D deficiency does not reduce the basal enzyme activity, whereas the stimulation at each concentration of PTH is significantly less than control for either the native 1-84 or synthetic 1-34 PTH peptides in regard to their activation of the adenylate cyclase in vitro. The effect of vitamin D deficiency, therefore, was observed at all levels of PTH, and a marked reduction in the maximal velocity of the PTH-dependent enzyme activity was a prominent feature at concentrations of PTH that saturate the available receptors.

We next explored the relationship between the effects of the vitamin D-deficient diet on the serum total calcium and iPTH with the earliest appearance of the reduction in renal PTH-dependent adenylate cyclase activity. Figure 2 shows the relative changes in circulating levels of Ca and iPTH in rats fed a vitamin D-deficient diet beginning at 21 days of age (time zero). There was a progressive reduction in serum Ca beginning at day 7 and continuing throughout the 28-day period of this experiment. This hypocalcemic response to the vitamin D-deficient diet was mirrored by a progressive increase in circulating iPTH, which was first observed at day 7 and then appeared to plateau at 21 days of D-deficient diet. Levels of serum Mg and P were not changed in vitamin D-deficient animals relative to control.

The PTH-dependent adenylate cyclase activity of cortical membranes from control and vitamin D-deficient animals after 0, 1, 2, and 3 weeks of diet are depicted in Figure 3. Concentration-response curves for activation of the enzyme by PTH reveals that the response of the adenylate cyclase to PTH is not changed by the D-deficient diet at one week, whereas there was a noticeable but insignificant ($P > 0.05$) decrease in PTH-dependent

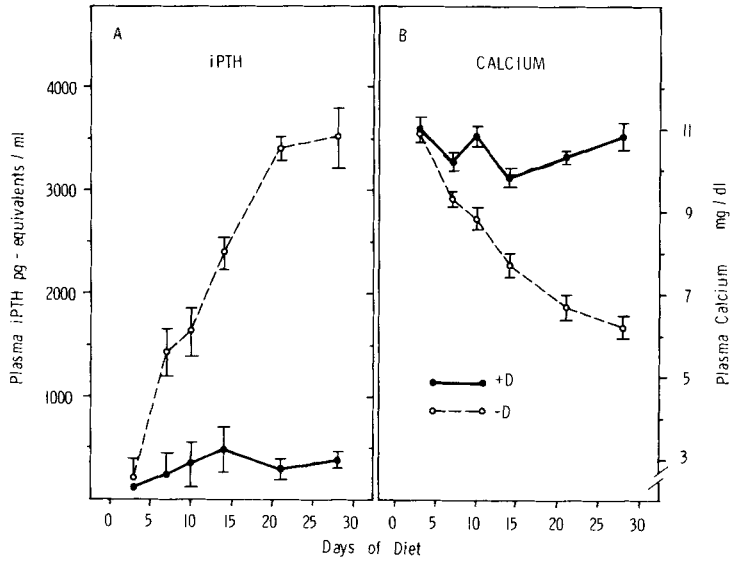

Fig 2. Effect of vitamin D deficiency on circulating iPTH and calcium levels. Male weanling rats were fed the experimental diets beginning at time zero, and then 4–6 animals per group were killed at the indicated times for measurement of iPTH and total calcium. The values are expressed as the mean ± SEM (vertical bars) with calcium assayed in duplicate and iPTH in triplicate. Both iPTH and calcium levels of serum from –D rats were significantly different from +D by 7 days of diet ($P < 0.01$) as were the values between groups thereafter.

activity by two weeks and a marked decrease in responsiveness of the enzyme to the hormone by 3 weeks of D-deficient diet relative to control ($P < 0.01$). Therefore, both the hypocalcemia and elevated endogenous PTH observed in D deficiency (Fig 2) precedes the earliest reduction in renal PTH-dependent adenylate cycalse activity by as much as 1 to 2 weeks. Furthermore, the data in Figure 3 also demonstrated that the PTH-dependent enzyme activity is not fully developed in the weanling rat (21 days of age). The PTH-dependent and fluoride-stimulated (data not shown) adenylate cyclase activities increased progressively until reaching an apparent maximum at 5 weeks of age (week 2 of diet). This type of enzyme development in rat kidney has been reported previously [16]. In this experiment both the control and D-deficient groups exhibited this development in the enzyme activity until 2 weeks of D deficiency when the PTH-dependent enzyme activity began to diverge from control.

To study the kinetics of the reversal of the effects of vitamin D deficiency on serum Ca and iPTH and on the renal cyclic AMP response to PTH, we administered 3,000 units of vitamin D_2 p.o. to a group of animals that had been fed the D-deficient diet for 3 weeks. Figure 4 depicts the time course after repletion with this dose of vitamin D_2. The markedly elevated iPTH at zero time progressively decreased at 3 and 6 hours after repletion but then was increased at 12 hours to a level higher than the initial iPTH value. However, the serum iPTH then progressively fell over the next 36 hours and approached the euparathyroid state 48 hours after vitamin D_2 administration. The increase in serum Ca began between 6 and 12 hours after vitamin D_2 and was restored to a normocalcemic level by 24 hours. In contrast, restoration of the depressed PTH-dependent adenylate cyclase to a level equivalent

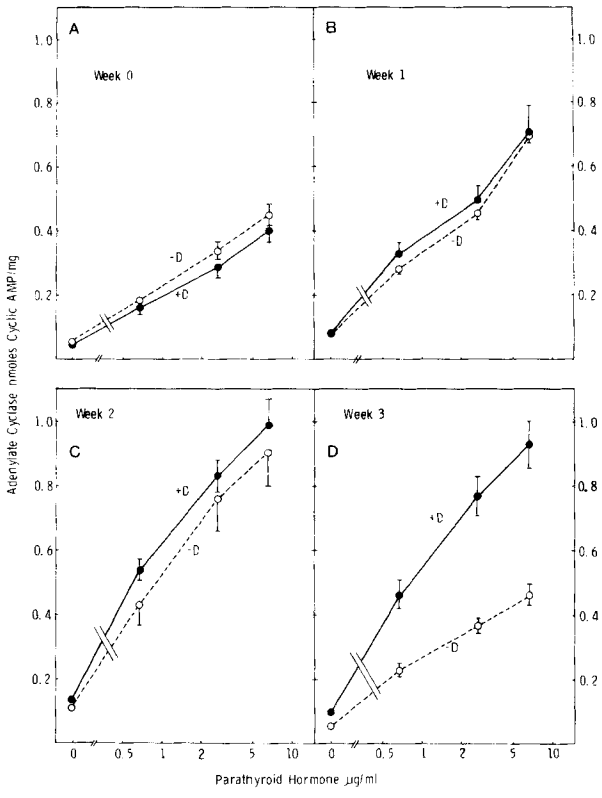

Fig 3. Effect of vitamin D deficiency on the renal cortical PTH-dependent adenylate cyclase activity. Male weanling rats were fed as previously described (Figs 1 and 2) and then killed at 0, 1, 2, and 3 weeks of experimental diet. Cortical plasma membranes were prepared from each pair of kidneys and assayed for basal and PTH-dependent adenylate cyclase activity. Each value is the mean ± SEM of 3 animals (ie, 3 membrane preparations), each assayed in duplicate.

to +D membranes was not observed until 48 hours after repletion with vitamin D_2. The increases in PTH-stimulated enzyme activity noted at 3 and 6 hours after vitamin D administration were not significantly different from the enzyme activity at time zero ($P > 0.05$). Therefore, a 12-hour lag period at minimum was observed prior to the first significant ($P < 0.05$) increase in PTH-dependent adenylate cyclase, which was found 24 hours after repletion with vitamin D_2.

Since the exposure of target cells to elevated endogenous hormone or exogenous hormone has been implicated in subsequent alterations in receptor-mediated regulation of cyclic AMP formation [17], we reasoned that the effect of vitamin D deficiency to elevate endogenous PTH in the rat may contribute to the depressed renal PTH-dependent adenylate cyclase activity. To test the effect of an acute elevation of circulating PTH on subsequent renal response to the hormone, we infused bovine PTH into conscious, restrained rats at a rate of 35 units of PTH per hour for a total of 8 hours and then examined the PTH-dependent adenylate cyclase activity of renal membranes prepared from animals killed immediately at the end of the infusion or 1 and 3 hours after the hormone infusion was discontinued. Figure 5 depicts the data from such an experiment. The 8-hour PTH infusion

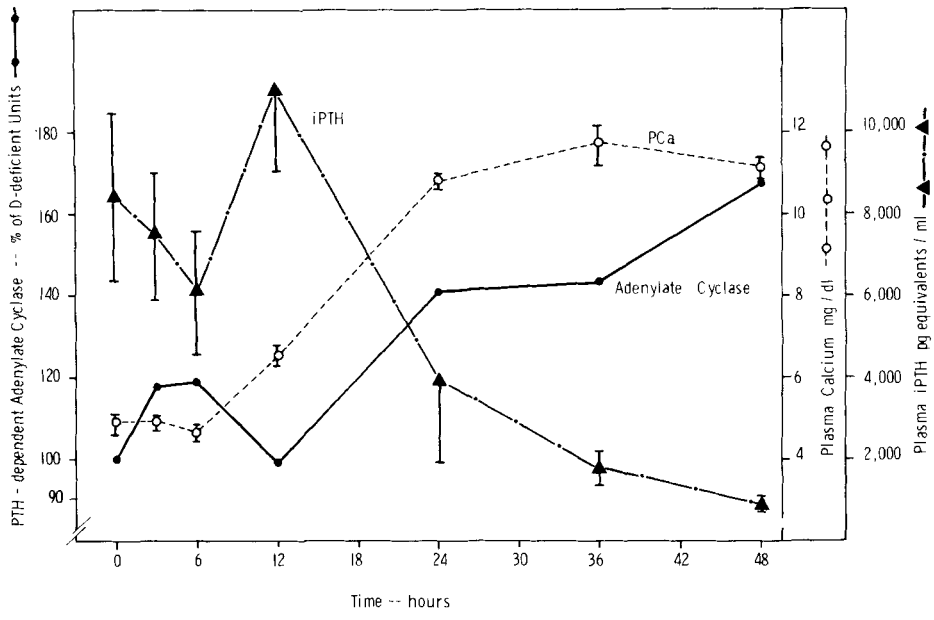

Fig 4. Effects of repletion with vitamin D_2 on the iPTH, calcium and renal PTH-dependent adenylate cyclase levels in vitamin D-deficient rats. Male weanling rats were fed the vitaminD-deficient diet for 3 weeks and then treated with 3,000 units of vitamin D_2 p.o. at time zero (above). At the indicated intervals, 4 rats were killed and the iPTH, calcium and renal membrane adenylate cyclase assayed as previously described. The values are the mean ± SEM (vertical bars) of those measurements at each time. PTH-dependent adenylate cyclase activity of membranes from D-deficient rats at time zero is expressed as 100%, with each subsequent enzyme activity after repletion expressed relative to time zero activity.

resulted in a marked and highly significant ($P < 0.01$) reduction in the response of the membrane adenylate cyclase to PTH. Moreover, the reduction in PTH-dependent enzyme activity was rapidly restored toward control in a time-dependent manner 1 and 3 hours after the PTH infusion was discontinued. This demonstrated that the acute effect of PTH treatment in vivo induced a refractoriness of the kidney to subsequent stimulation of the adenylate cyclase by the hormone in vitro, which appeared to be a dynamic regulation of the cyclic AMP system since it was rapidly reversed after discontinuation of the hormone infusion. However, the acute elevation of PTH in this experiment resulted in a significant reduction in the fluoride-stimulated adenylate cyclase activity of the cortical membrane preparation. This too was reversed after discontinuation of the PTH infusion. These data suggest a different mechanism for the acute action of PTH, since the fluoride-stimulated adenylate cyclase activity was not depressed in either the vitamin D-deficient [9] or the calcium-deficient, vitamin D-replete rat [6].

DISCUSSION

The present study confirms our previous report [9] that vitamin D-deficient rats exhibit a profound reduction in renal PTH-dependent adenylate cyclase activity and extends that observation to demonstrate a refractory cyclic AMP response to PTH in cortical slices from D-deficient animals. In addition, the experiments reported here showed that the

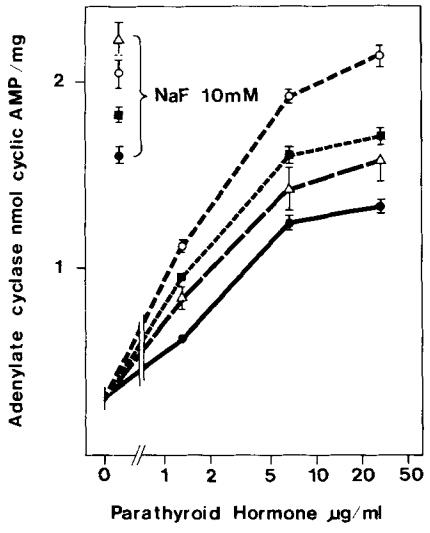

Fig 5. Depression of the renal PTH-dependent adenylate cyclase induced by infusion of PTH in vivo. Intact male rats (150 gm) were infused with a solution containing 5 mM $CaCl_2$, 20 mM NaCl, 2.5 mM KCl and 0.22 M glucose [9] at 3 ml/hour for 5 hours and then some of the animals were infused with 35 units of PTH per hour for 8 hours and the remaining (control) animals received the infusate containing no hormone. Rats were killed at the end of the hormone infusion period or at 1 and 3 hours after discontinuation of PTH treatment (recovery periods). Membranes were prepared from each animal (n = 4 per group) and assayed for adenylate cyclase activity. These data are expressed as the mean ± SEM for each condition. Control ○ - - - ○, PTH-infused and killed at the completion of hormone treatment ●—●, PTH-infused and 1-hour recovery △- - -△, PTH-infused and 3-hour recovery ■ - - - ■.

"down-regulation" of renal responsiveness to PTH in this experimental animal is preceded by significant changes in both circulating calcium and iPTH levels. Although serum concentrations of 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol were not measured in these experiments, it is reasonable to assume from the studies of Rojanasathit and Haddad [18] that the hypocalcemic response of the D-deficient rat in our study reflects a progressive depletion of the active metabolites of vitamin D. Therefore, the decrease in vitamin D levels per se also preceded the reduction in PTH-dependent adenylate cyclase activity that was first noted 2 weeks after beginning the vitamin D-deficient diet and then fell markedly between 2 and 3 weeks of experimental diet. Therefore, any one of the three factors (vitamin D, calcium, and PTH) alone or in combination may be involved in the regulation of renal cyclic AMP responsiveness to PTH.

The phenomenon of target-tissue resistance to specific hormones, apparently resulting from increased endogenous hormone levels, has been well documented for insulin and growth hormone [17, 19]. However, there is an inherent danger in extrapolation, for example, of insulin's mode of action to regulate its own receptors to the situation observed in the vitamin D-deficient rat. This is due to the recent reports that elevated endogenous glucagon [20] and vasopressin [21] levels were associated with increased rather than decreased cyclic AMP responsiveness of liver and kidney to the relevant hormone. Therefore, elevated endogenous hormone does not only result in down-regulation of receptor-mediated responsiveness of the target cell but also may result in increased responsiveness. On the other hand, we have previously reported that the rat fed a low-calcium (0.02%), vitamin D-replete diet exhibits hypocalcemia, elevated PTH, and depressed renal cyclic

AMP responses to PTH [6]. This experimental animal received adequate vitamin D, so that down-regulation of renal PTH responsiveness can be demonstrated in the vitamin D-replete animal. Such an observation demonstrates that vitamin D deficiency is not a prerequisite for impaired responsiveness to PTH, but does not rule out the participation of vitamin D metabolites in the regulation of renal membrane PTH-dependent adenylate cyclase activity.

The most direct evidence that elevated PTH is involved in the regulation of renal cyclic AMP responsiveness was obtained in this study when PTH was infused acutely into the rat, resulting in a marked diminution of the PTH-dependent adenylate cyclase activity in the plasma membranes relative to control. Obviously, this type of experiment does not reproduce the physiologic state encountered in the vitamin D-deficient rat. Acute exposure to elevated PTH was associated with a significant reduction in both the PTH-dependent and fluoride-stimulated adenylate cyclase activities, whereas both the vitamin D-deficient [9] and calcium-deficient [6] animals exhibited a depression only in the PTH-dependent enzyme activity. Therefore, it may be reasoned that the progressive, slow changes in vivo in the chronic animal model leads to a qualitatively different modulation of the adenylate cyclase system.

The down-regulation of renal cyclic AMP responsiveness to PTH in both the acute and the chronic experimental models was reversible. However, the kinetics were dissimilar since the hormonal responsiveness was substantially restored toward control only a few hours after discontinuation of the hormone infusion, whereas the restoration of PTH responsivity in the vitamin D-deficient rat was relatively slow (ie, 48 hours) after repletion with vitamin D. The slower rate of restoration of the renal response to PTH after vitamin D repletion probably reflects the difference in complexity between the two experimental situations. Presumably, the sequence of events after citamin D repletion would be: (1) metabolism of vitamin D_2 to active metabolites; (2) enhanced intestinal calcium absorption and calcium resorption from bone, leading to increased extracellular calcium levels; (3) inhibition of PTH secretion due to the effect of vitamin D to restore calcium to normal and perhaps also because of a direct action of vitamin D metabolites to inhibit PTH secretion [22] with a reduction in circulating PTH levels; and (4) recovery of the depressed PTH-dependent adenylate cyclase system. Obviously, such a circuitous mechanism would be slow to complete in comparison to the events occurring after the acute PTH infusion is stopped, which may be related primarily to the rate of hormone degradation.

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