Kidney International, Vol. 36 (1989), pp. 802–809

Altered response of adenylate cyclase to parathyroid hormone during compensatory renal growth

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Altered response of adenylate cyclase to parathyroid hormone during compensatory renal growth. The loss of renal mass is associated with functional adaptations in the remaining nephrons to maintain homeostasis. Although parathyroid hormone (PTH) is important in the adaptations to phosphate, the mechanisms are not completely defined. In the present studies we examined the response of the adenylate cyclase system to PTH in renal cortical membranes of rat kidneys ten days after unilateral nephrectomy. The kidneys obtained at the time of the initial nephrectomy were used as controls. Unilateral nephrectomy resulted in contralateral compensatory renal growth, as demonstrated by a 24 ± 4.7% ($P < 0.01$) increase in weight in the remaining kidney. Glomerular filtration rate (GFR) after unilateral nephrectomy was 62% of the control, while basal fractional phosphate excretion was higher in rats with unilateral nephrectomy (7.7 ± 2.1% vs. 2.9 ± 0.8%, $P < 0.05$). PTH infusion resulted in a similar increase of fractional phosphate excretion and urinary cAMP in both groups. In the absence of added guanine nucleotides, PTH-dependent adenylate cyclase activity in cortical membranes from kidneys with compensatory growth was decreased as compared to controls (V_{max} 807.5 ± 62.7 pmol cAMP/mg protein/30 min vs. 1,384.8 ± 116.1, respectively, $P < 0.01$). The apparent affinity for PTH stimulation of adenylate cyclase (Kact) was unchanged. Magnesium-dependent adenylate cyclase activity was also decreased in the membranes from kidneys with compensatory growth. However, the kinetics of adenylate cyclase for the substrates ATP-Mg or ATP-Mn were similar. The addition of Gpp(NH)p resulted in a similar maximal response to PTH in the two groups, indicating an increased response of the enzyme to PTH in the presence of the guanine nucleotide. Cholera toxin-dependent ADP-ribosylation of the stimulatory guanine nucleotide binding protein (Gs) showed a marked decrease in the apparent content of the alpha subunit in membranes from kidneys with compensatory growth compared to controls. On the contrary, pertussis toxin-dependent ADP-ribosylation of the inhibitory guanine nucleotide binding protein (Gi) did not show differences in the content of the alpha subunit in both groups of membranes. Since the transduction of the hormone signal from the receptor is mediated by G proteins, the present studies suggest that during compensatory renal growth a decrease in the alpha subunit of Gs could account for the impaired response of adenylate cyclase to PTH in vitro, which could be overcome by high concentrations of guanine nucleotides.

The loss of renal mass is followed by compensatory growth of the remaining kidney [1, 2]. This growth response occurs by virtue of both hypertrophy and hyperplasia and is associated with functional adaptations in tubular transport processes to preserve homeostasis [3, 4]. The adaptations to phosphate are characterized by a progressive reduction of fractional phosphate reabsorption as renal mass decreases [5, 6].

The response to loss of renal mass has been studied after unilateral nephrectomy. It has been established that similar to the compensatory response observed in progressive renal failure, unilateral nephrectomy results in growth and functional adaptations in the remaining kidney, which include alterations in the actions of PTH on phosphate excretion [7, 8]. Since renal cortical adenylate cyclase plays an important role in the action of PTH in the kidney, it is possible that the mechanism of renal adaptation to phosphate during the loss of renal mass may be related with changes in the interaction of the hormone with the enzymatic system. Therefore, to get insight into this possibility, the present studies were undertaken to characterize the actions of PTH on kidneys with compensatory growth after unilateral nephrectomy. This model permits the study of hormone interactions with the adenylate cyclase system without the interference of the possible effects of uremia [7, 8].

Methods

Materials

Synthetic bPTH (1-34), ATP, GTP, Gpp(NH)p, cholera toxin, pertussis toxin and other reagents were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA) and [32-P]-NAD from New England Nuclear (Boston, Massachusetts, USA). ^{125}I was obtained from Amersham/Searle (Arlington Heights, Illinois, USA). ^{125}I-cAMP was prepared in our laboratory as previously described [9].

Methods

For the studies with isolated membranes, twenty-four adult Sprague-Dawley rats with a mean weight of 235.9 ± 24.6 g were studied. Animals underwent right nephrectomy under general anesthesia with ether. The kidneys were weighed and processed for isolation of renal cortical membranes as described below. Each membrane preparation was obtained from a pool of four kidneys removed simultaneously from four animals. These preparations constituted the control group. After surgery, the rats were fed standard rat chow and allowed water ad libitum. After 10 days the left kidneys were removed in all animals. Kidneys were pooled and processed for isolation of renal

Received for publication August 1, 1988
and in revised form April 26, 1989
Accepted for publication June 16, 1989

cortical membranes as in the control group. These preparations constituted the group with compensatory renal growth. Blood samples were obtained from all animals at the time of right and left nephrectomy for determinations of serum creatinine and phosphorus.

A second group of 12 normal rats were sham operated, and ten days later the left kidneys were surgically removed and weighed. The values obtained were used as time controls for normal kidney growth.

Clearance studies

Clearance studies were performed in an additional group of 16 rats. Eight of these animals were studied prior to right nephrectomy and the other eight animals were studied ten days after the nephrectomy. Rats were anesthetized with pentobarbital (60 mg/k body wt) and a tracheostomy performed. A jugular catheter was used for infusion of 1.2 ml/hr of 5% DW during surgery and experimentation. A femoral artery catheter was used for blood sampling. Urine was collected from a catheter inserted into the bladder. After an equilibration period of 30 minutes, two consecutive urine collections (30 min each) were performed, and arterial blood samples were drawn at the midpoint of each period. Then, a priming dose of 2.0 μg of synthetic bPTH (1-34) dissolved in 0.5 ml of isotonic saline was administered in five minutes, followed by 7 μg of the hormone in 4 ml of isotonic saline given at a rate of 10 μl per minute. After an equilibration period of 30 minutes, two additional clearance periods were obtained. Plasma and urine samples were analyzed for creatinine, phosphate and cAMP for determinations of creatinine clearance, fractional phosphate excretion and urinary cAMP.

Preparation of renal cortical membranes

Kidneys were decapsulated, the cortex was dissected from the medulla and minced with fine scissors. All steps were carried out at 4°C. Cortical membranes were prepared by the method of Ebel, Aulbert and Merker [10] to the point of partial purification. The minced kidney tissue was homogenized in 250 mM sucrose, 20 mM Tris-HCl, pH 7.6, 1 mM EDTA (ratio 1:5 wt/vol) using a teflon-glass homogenizer. The homogenate was centrifuged at 700 × g for 10 minutes in a refrigerated centrifuge. The pellet was discarded and the supernatant centrifuged again in a similar manner. The resulting supernatant was centrifuged at 1,350 × g for 10 minutes and the pellet was discarded. The supernatant was centrifuged at 2,500 × g for 20 minutes. The resulting supernatant was removed. The pellet was resuspended in the homogenization buffer and centrifuged at 2,500 × g for 30 minutes. The final pellet was resuspended in the homogenization buffer and stored at −70°C in small aliquots for later use. Protein concentration in preparations was measured by the method of Lowry et al [11].

Na-K ATPase activity in the membranes was determined as previously described [12]. The enrichment in Na-K ATPase was 3.47 ± 0.72 times in compensatory renal growth versus 3.59 ± 0.69 in controls, P = NS. However, the enzyme activity in the membrane preparations of kidneys with compensatory growth was fivefold higher than in controls (0.190 ± 0.057 μM Pi/μg protein/min vs. 0.035 ± 0.006, respectively, $P < 0.01$).

RNA and DNA content in kidney homogenates were determined as described by Blobel and Potter [13].

Adenylate cyclase assay

Adenylate cyclase activity in the membrane preparations was measured by the determination of cAMP formed from ATP according to the method of Steiner et al [14] with some modifications as previously described [15]. Incubations were carried out at 30°C for 30 minutes in a mixture containing 50 mM Tris-HCl, pH 7.6, 25 mM KCl, 0.2 mM EDTA, 1 mM IBMX, 5% serum, 0.2 mM ATP, 20 mM $MgCl_2$, 1 mg/ml creatinine kinase, 25 mM phosphocreatinine and 20 to 30 μg of membrane protein in a total volume of 100 μl. When required synthetic bPTH (1-34), Gpp(NH)p, NaF, $MnCl_2$, or ATP at variable concentrations were added to the incubation mixture. The reaction was terminated by boiling the samples for three minutes. The cAMP was measured by radioimmunoassay as previously described [9].

Cholera toxin-dependent ADP-ribosylation of stimulatory guanine nucleotide binding proteins (Gs)

Cholera toxin-dependent ADP-ribosylation of Gs was examined as described by Downs et al [16]. Cholera toxin was activated by incubation with 20 mM dithiotreitol for 10 minutes at 30°C. Membrane preparations (200 μg of protein) were centrifuged at 11,000 × g for five minutes at 4°C, and the pellet resuspended and incubated for 20 minutes at 30°C in 100 μl of 100 mM potassium phosphate pH 7.5, with 100 U/ml Trasylol, 20 mM thymidine, 0.1 mM GTP, 25 μM [32-P]-NAD (specific activity 10 Ci/mmol) and 2 mM dithiotreitol in the presence or the absence of 10 μg cholera toxin. The reaction was terminated by dilution with 10 volumes of cold 100 mM potassium phosphate and centrifugation at 11,000 × g for two minutes. The pellet was washed and resuspended in Laemmli's sample buffer for SDS-PAGE [17].

Pertussis toxin-dependent-ADP ribosylation of inhibitory guanine nucleotide binding proteins (Gi)

Pertussis toxin dependent-ADP ribosylation of renal cortical membranes was performed as described by Codina et al [18] with some modifications [19]. Briefly, 50 μg of membrane proteins were incubated in 50 μl of 50 mM Tris-HCl pH 7.6, 10 μM 32-P-NAD (specific activity 5 Ci/mmol), 1 mM ATP, 20 mM dithiothreitol, 10 mM thymidine, 1 mM EDTA in the presence or absence of 5 μg of activated pertussis toxin, for 30 minutes at 37°C. The reaction was terminated by dilution with 10 volumes of ice-cold 50 mM Tris-HCl and centrifugation at 11,000 × g for 10 minutes at 4°C. The supernatant was discarded and the pellet washed and resuspended in Laemmli's sample buffer for SDS-PAGE [17].

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Samples (100 μg of protein) of radiolabeled membranes in the presence of cholera toxin or pertussis toxin were analyzed by SDS-PAGE on slab gels 1.5 mm thick with a 10% separating gel and 5% stacking gel. Electrophoresis was performed at 25 mA per gel [17]. Gels were stained with Coomasie brilliant blue (R-250), destained, dried and exposed to Kodak X-AR5 films (Eastman Kodak, Rochester, New York, USA) for four days at −80°C. The relative incorporation of 32-P in the protein bands was estimated by densitometry of the autoradiograms using a

Table 1. Rat and kidney weight prior (control) and 10 days after unilateral nephrectomy compared to sham operated animals

Group	Rat weight	Kidney weight	% Renal growth	Kidney wt/ Rat wt × 100
Control $N = 24$	235 ± 24.6	1.071 ± 0.13	—	0.452 ± 0.13
Unilateral nephrectomy $N = 24$	272.0 ± 24.4	1.530 ± 0.13	24.0 ± 4.7	0.558 ± 0.17
Sham operated $N = 12$	263.3 ± 19.8	0.995 ± 0.06	—	0.420 ± 0.01
P[a]	< 0.02	< 0.01		< 0.01
P[b]	NS	< 0.02		< 0.01

Rat and kidney weight are expressed in grams.
[a] Control vs. unilateral nephrectomy
[b] Sham operated vs. unilateral nephrectomy. Control vs. sham operated were non-significantly different.

Table 2. Glomerular filtration rate (GFR), fractional excretion of phosphate (FE_{PO_4}) and urinary cyclic AMP (U cAMP) in control and unilaterally nephrectomized rats

Group	GFR *μl/min*	FE_{PO_4} %	U cAMP *μmol cAMP/g Cr*
Control $N = 8$			
Pre-PTH	692 ± 59[a]	2.9 ± 0.8[b]	13.3 ± 2.5
Post-PTH	679 ± 197	25.7 ± 7.2	26.5 ± 4.1
P	NS	< 0.01	< 0.01
Unilateral nephrectomy $N = 8$			
Pre-PTH	429 ± 52	7.7 ± 2.1	15.8 ± 3.8
Post-PTH	409 ± 50	31.5 ± 6.3	43.2 ± 7.2
P	NS	< 0.01	< 0.05

[a] $P < 0.01$ control vs. unilaterally nephrectomized animals
[b] $P < 0.05$ control vs. unilaterally nephrectomized animals

Biomed Soft Laser densitometer SL-TRFF (Biomed Instruments Inc. Fullerton, California, USA).

Statistical methods

The results are expressed as mean ± SEM. Students' *t*-test was used to determine the significance of differences between groups.

Results

Effects of unilateral nephrectomy on contralateral kidney growth and function

As demonstrated in Table 1, kidneys removed 10 days after unilateral nephrectomy showed an absolute weight increase of 43% above the contralateral kidneys (control group) obtained previously (1.53 ± 0.13 g vs. 1.07 ± 0.13, respectively, $P < 0.01$). The whole animal weight increment during the same period of time was only 15.3%. Furthermore, the relative increase in kidney mass calculated as the ratio of kidney weight/rat weight was 24 ± 4.7% ($P < 0.01$). In contrast, while the whole animal weight increment in sham operated animals used as time controls was similar to that in the unilaterally nephrectomized rats, kidney weight was significantly lower (0.995 ± 0.06 g vs. 1.530 ± 0.13, $P < 0.02$, in sham and unilaterally nephrectomized animals, respectively), suggesting that the elevation in the remaining kidney weight was mainly a consequence of organ growth.

In both groups the RNA content in the kidney homogenates was similar (0.031 ± 0.002 μg RNA/μg protein vs. 0.030 ± 0.002 in compensatory renal growth and controls, respectively). In contrast, the DNA content was significantly higher in compensatory renal growth (0.034 ± 0.003 μg DNA/μg protein vs. 0.017 ± 0.002 in control, $P < 0.01$). The RNA/DNA was 0.935 ± 0.073 μg in compensatory renal growth versus 1.902 ± 0.162 μg in control, $P < 0.01$, suggesting that kidney growth results, to an appreciable extent, from hyperplasia rather than hypertrophy.

As illustrated in Table 2, GFR in the unilaterally nephrectomized animals was 62% of the control. In this group baseline fractional phosphate excretion was higher compared to controls (7.7 ± 2.1% vs. 2.9 ± 0.7, $P < 0.05$). However, the increment after PTH infusion was similar in both groups (31.5 ± 6.3 vs. 25.7 ± 7.2, NS, in unilaterally nephrectomized animals and controls, respectively). Infusion of PTH resulted in a similar increase in urinary cAMP in both groups.

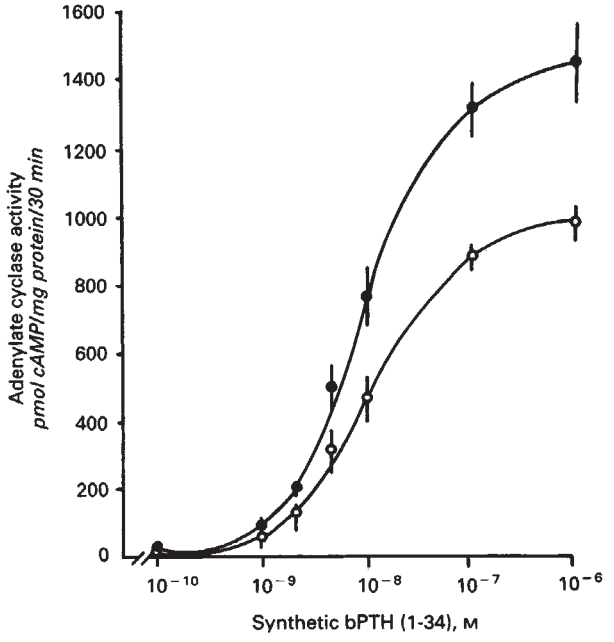

Fig. 1. *Adenylate cyclase activity in response to increasing concentrations of synthetic bPTH (1-34) in renal cortical membranes from kidneys with compensatory growth* (○) *and controls* (●). Points are means ± SE of duplicate determinations in 6 membrane preparations from each group. Basal enzyme activity (261.1 ± 12 pmol cAMP/mg protein/30 min in compensatory renal growth and 326.2 ± 11.2 in controls) was subtracted from each point.

Adenylate cyclase activity

Figure 1 illustrates adenylate cyclase activity in renal cortical membrane preparations in response to increasing concentrations of synthetic bPTH (1-34) in the incubation media. Membrane fractions from kidneys with compensatory growth after unilateral nephrectomy showed a marked reduction in PTH-dependent adenylate cyclase activity compared with that in membrane preparations from the normal contralateral kidneys used as controls. The decrease in enzyme activity was a

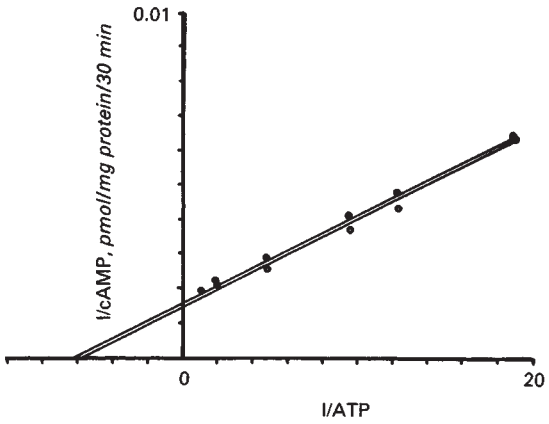

Fig. 2. *Double reciprocal plot of adenylate cyclase activity in membrane preparations from kidneys with compensatory growth* (○) *and controls* (●) *at increasing concentrations of ATP.* Km for ATP were respectively 0.20 mM ± 0.07 vs. 0.18 ± 0.04 mM (P = NS), and V_{max} 473 ± 42 pmol cAMP/mg protein/30 min vs. 580 ± 63 (P = NS). N = 6 in each group.

consequence of reduction of V_{max} (807.5 ± 62.7 pmol cAMP/mg protein/30 min vs. 1,384.8 ± 116.1 in controls, $P < 0.01$). The Kact for PTH-stimulated enzyme activity (PTH-concentration giving half maximal stimulation of enzyme activity) was not altered (9.45 ± 2.10 nM synthetic bPTH vs. 6.52 ± 0.92, P = NS, respectively).

Effect of substrate on adenylate cyclase activity

Basal enzyme activity in the absence of PTH was also lower in membranes from kidneys with compensatory growth. Therefore, to examine whether the altered response of adenylate cyclase to PTH in these membrane preparations was a consequence of abnormal regulation of the catalytic unit of the enzyme system, experiments were performed to study the kinetics of adenylate cyclase activity at increasing concentrations of the substrate ATP. As shown in Figure 2, the affinity of the enzyme for ATP in the presence of magnesium was almost identical (km 0.20 ± 0.07 mM ATP vs. 0.18 ± 0.04 in compensatory renal growth and controls, respectively, NS). V_{max} was also similar (473 ± 42 pmol cAMP/mg protein/30 min vs. 580 ± 63.2, respectively).

It has been shown that in the presence of manganese as the sole divalent cation in the medium, an uncoupling of the adenylate cyclase system occurs, so that enzyme activity reflects the activity of the catalytic unit without the influence of other components of the system [20, 21]. Therefore, experiments were performed substituting manganese (20 mM) for magnesium. The results showed that under these conditions basal enzyme activity in membrane preparations from kidneys with compensatory growth and control kidneys were similar (301 ± 40.5 pmol cAMP/mg protein/30 min vs. 393 ± 19.4, P = NS).

Effect of magnesium on basal adenylate cyclase activity

Since the regulation of adenylate cyclase activity by hormones and guanine nucleotides is closely related to the action of magnesium [20, 22, 23], in the next series of experiments we studied the magnesium dependence of adenylate cyclase activity in membrane preparations from kidneys with compensatory growth as compared to controls. As shown in Figure 3A, basal enzyme activity increased as magnesium concentration was raised from 0 to 40 mM in both groups. However, enzyme activity was significantly lower in compensatory renal growth. In the presence of 0.1 mM Gpp(NH)p, a synthetic analogue of GTP, enzyme activity increased to the same extent in both groups, and the curves of magnesium dependent activation were shifted to the left (Fig. 3B). Thus, affinity for magnesium was increased similarly by Gpp(NH)p in both groups.

Effect of guanine nucleotides on adenylate cyclase activity

Since guanine nucleotides are necessary for the regulation of adenylate cyclase in the intact cell, the next series of studies were performed to examine the effects of Gpp(NH)p, on the regulation of basal and PTH-dependent adenylate cyclase activity. In preliminary experiments it was determined that in both groups of membranes the maximal response of adenylate cyclase to this nucleotide was obtained at a concentration of 0.1 mM. The addition of Gpp(NH)p at this concentration to the incubation medium resulted in an increase in basal enzyme activity in both groups of membranes (806 ± 108 pmol cAMP/mg protein/30 min in compensatory renal growth vs. 977 ± 78 in control, P = NS). Figure 4 shows the effects of Gpp(NH)p on PTH-dependent adenylate cyclase activity in the membrane preparations. The addition of 0.1 mM Gpp(NH)p resulted in a higher absolute increase in PTH-dependent enzyme activity in compensatory renal growth (1,291.6 ± 179 pmol cAMP/mg protein/30 min vs. 762.7 ± 121, $P < 0.05$). As a consequence the defective response of adenylate cyclase to PTH in membranes from kidneys with compensatory growth was corrected compared to controls (V_{max}: 2,167 ± 165 pmol cAMP/mg protein/30 min vs. 2,191 ± 70, respectively, P = NS).

Effect of NaF on adenylate cyclase activity

NaF is a direct stimulator of adenylate cyclase by interacting with the stimulatory regulatory proteins (Gs) at a site different from guanine nucleotide and magnesium binding sites. Therefore, the effect of NaF was used as a probe to study the interaction of Gs with the catalytic unit of the enzyme system in membrane preparations from kidneys with compensatory growth and controls. Preliminary experiments at increasing concentrations of NaF from 2 to 20 mM showed that the optimal response of both groups of membranes was obtained at 10 mM NaF. As illustrated in Figure 5, at this concentration of the compound enzyme activity was not statistically different in compensatory growth and controls (1,903.2 ± 152.2 pmol cAMP/mg protein/30 min vs. 2,252.5 ± 240.1, respectively).

Cholera toxin-dependent ADP-ribosylation of membrane proteins

In the next series of experiments we examined cholera toxin-dependent ADP-ribosylation of proteins in membrane preparations from both groups of membranes. As illustrated in Figure 6, in the presence of the toxin and [32-P]-NAD there was AD-[32-P]-ADP-ribosylation of the 42,000 Mr alpha subunit of the stimulatory guanine nucleotide binding protein (Gs), and a protein band of 52,000 Mr corresponding to another molecular form of alpha subunits as reported by others for rat kidneys

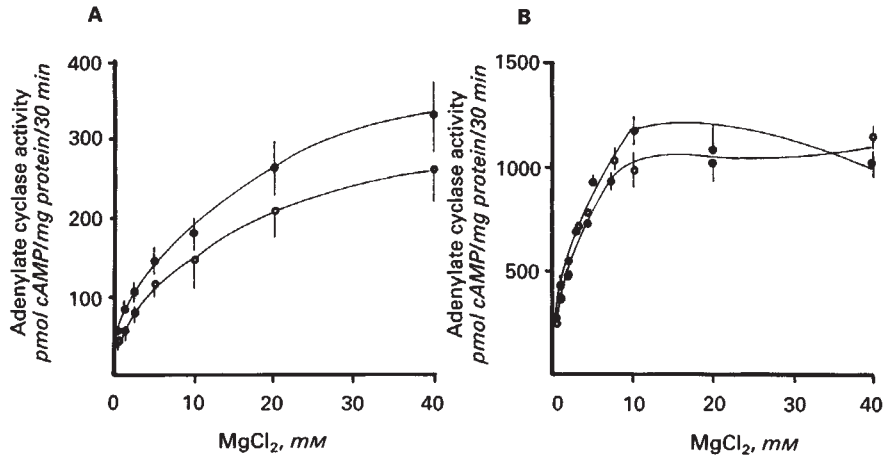

Fig. 3. *Magnesium-dependent adenylate cyclase activity in membrane preparations of compensatory renal growth* (○) *and controls* (●) *in the absence* (**A**) *or the presence* (**B**) *of 0.1 mM Gpp(NH)p.* Points are means ± SE of duplicate determinations in each group of membranes ($N = 6$). $^*P < 0.05$.

[24]. It was evident that membranes from kidney with compensatory growth showed a decrease in the apparent content of the two protein bands as compared to controls. The relative content of the alpha subunit of Gs as estimated by densitometry of the autoradiograms is shown in Table 3. In compensatory renal growth there was more than a 50% decrease in the relative content of both bands of the alpha subunit of Gs.

Pertussis toxin-dependent ADP-ribosylation of membrane proteins

In the last series of experiments we examined pertussis toxin-dependent ADP-ribosylation of proteins in the membrane preparations. In the presence of the toxin and [32-P]-NAD there was ADP-[32-P]-ADP-ribosylation of the 41,000 Mr alpha subunit of Gi and other, less prominent protein bands in both groups of membranes. However, as shown in Table 4, there was no difference in the relative content of the 41,000 Mr band in membranes from kidneys with compensatory growth and controls as stimated by densitometry of the autoradiograms.

Discussion

The loss of renal mass is characterized by adaptive processes in the remaining renal tissue which include changes in the action of PTH on phosphate excretion. Since the adenylate cyclase system plays a major role in PTH action in the kidney, the present studies were undertaken to examine whether the alterations in the renal actions of the hormone in animals with unilateral nephrectomy relate with changes in the activity of the adenylate cyclase system in vitro.

In the present studies unilateral nephrectomy resulted in significant compensatory growth of the contralateral kidney as compared to controls and sham operated animals. This increase in renal mass was associated with a marked increase in DNA content, whereas RNA levels were similar to controls, suggesting that there was predominant hyperplasia.

Glomerular filtration rate in the unilaterally nephrectomized animals was 62% of the controls, indicating renal adaptation in the remaining kidney. Fractional excretion of phosphate was also increased. After PTH infusion, the absolute increment in fractional phosphate excretion and urinary cAMP was similar in both groups, suggesting that secondary hyperparathyroidism did not occur as a consequence of the unilateral nephrectomy.

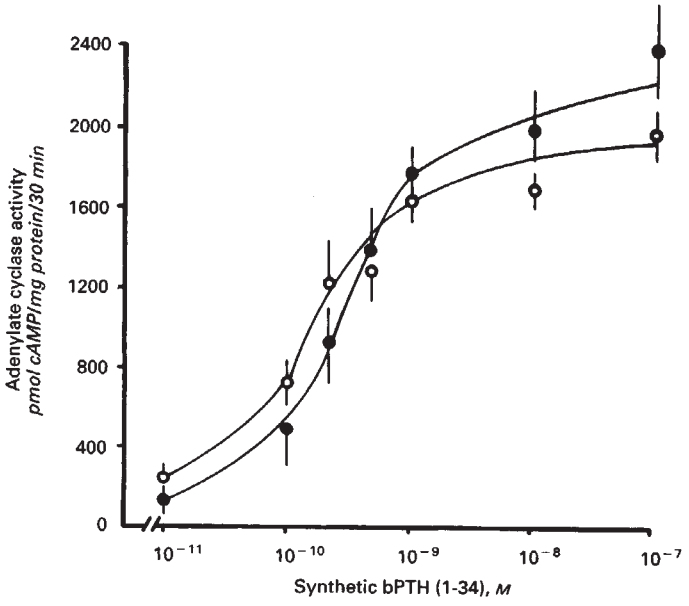

Fig. 4. *Effect of Gpp(NH)p (0.1 mM) on synthetic bPTH (1-34)-stimulated adenylate cyclase activity in membranes from kidneys with compensatory growth* (○) *and control* (●). Basal enzyme activity in presence of Gpp(NH)p and absence of hormone (806 ± 108 pmol cAMP/mg protein/30 minutes and 977 ± 78, respectively) was subtracted from each point ($N = 6$).

Renal growth was associated with alterations in the interaction of PTH with the adenylate cyclase system in renal cortical membranes. Thus, in the absence of added guanine nucleotides there was a decrease in PTH-stimulated adenylate cyclase activity as compared to controls, without changes in the Kact for the hormone.

To elucidate the nature of the altered response of adenylate cyclase to PTH, experiments were performed to examine the catalytic unit of the system at increasing concentrations of the substrate ATP in the medium. The results of these experiments showed that the affinity of the enzyme for ATP and the V_{max} of the reaction were almost identical in membranes from kidneys with compensatory growth and controls, suggesting that the altered response of the enzyme to PTH is not a consequence of

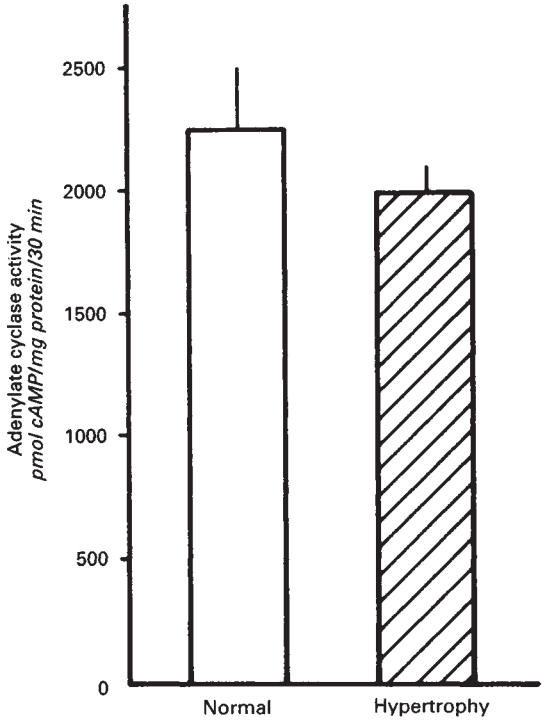

Fig. 5. *Adenylate cyclase activity in the presence of 10 mM NaF in membrane preparations from kidneys with compensatory growth and control.* Basal enzyme activity was subtracted from the points ($N = 6$).

Table 3. Cholera toxin-dependent [32-P]-ADP-ribosylation of Gs alpha subunits in cortical membranes from control and kidneys with compensatory growth

	Alpha 42,000 Mr	Alpha 52,000 Mr
Control $N = 5$	240.3 ± 12.2	192.3 ± 18.0
Compensatory renal growth $N = 5$	122.2 ± 25.0	95.6 ± 26.2
P	< 0.01	< 0.05

The relative content of the alpha subunits of Gs was estimated by densitometry from autoradiograms and expressed in arbitrary area units/100 μg of membrane protein.

Table 4. Pertussis toxin-dependent [32-P]-ADP ribosylation of Gi alpha subunits in cortical membranes from control and kidneys with compensatory growth

	Alpha 41,000 Mr
Control $N = 5$	466.6 ± 52.8
Compensatory renal growth $N = 5$	544.8 ± 40.5
P	NS

The relative content of the alpha subunits of Gi was estimated by densitometry from autoradiograms and expressed in arbitrary area units/100 μg membrane protein.

defects in the catalytic unit of the system. Furthermore, basal adenylate cyclase activity in the presence of high concentrations of manganese and in the absence of magnesium was similar in both groups of membrane preparations. Since experiments under these conditions yielded an index of isolated catalytic activity [20, 21], our results rule out an intrinsic defect of the catalytic unit as the cause of altered response of the enzyme to PTH. In addition, whereas the activity of Na-K-ATPase was fivefold higher in membrane preparations of kidneys with compensatory growth as described by others [25, 26], the enrichment in this enzyme marker was almost identical in these membrane preparations and controls, suggesting that similar membrane populations were isolated. These results together with the observation on normal basal adenylate cyclase activity are consistent with specific alterations in the regulation of the adenylate cyclase system in compensatory renal growth.

According to current theories the activation of adenylate cyclase by hormones is regulated by guanine nucleotide-binding regulatory proteins (G), which are responsible for the stimulation (Gs) or inhibition (Gi) of the catalytic unit of the enzyme complex [24, 27]. Therefore in the next series of experiments we examined the effect of Gpp(NH)p on PTH-dependent activation of adenylate cyclase. Gpp(NH)p augmented to a similar extent both basal and PTH-dependent enzyme activity, thus reversing the apparent defective response of the enzyme to PTH in membrane from kidneys with compensatory renal growth. This change was mainly a consequence of increase in V_{max}, although a minor increase in the Kact for PTH was also observed. Likewise, in the presence of NaF, a hormone independent stimulatory agent also related with the actions of Gs [28], enzyme activity was also similar in kidney with compensatory growth and controls. These results suggest that the altered response of adenylate cyclase to PTH may relate to an abnormal regulation of the system by Gs, which may be overcome in the presence of high concentrations of guanine nucleotides or maximal stimulatory concentrations of NaF.

Divalent cations play a key role in the regulation of basal and hormone-dependent adenylate cyclase activity [20, 22, 23]. Previous studies have shown that magnesium interacts with an allosteric metal site closely related to the nucleotide binding regulatory proteins [20, 21]. Moreover, recent studies have demonstrated that magnesium is an essential requirement for the activation of G proteins [18, 29]. It has been proposed that activation of Gs involves binding of guanine nucleotides to form an inactive complex and a magnesium-dependent activation step, resulting in dissociation of Gs into an inhibitory subunit, leaving the guanine nucleotide bound to the activated alpha subunit of Gs [18, 30, 31]. Therefore, experiments were performed to examine the interaction of magnesium with the enzyme system. It was found that under basal conditions (no hormone or guanine nucleotide added) magnesium-dependent adenylate cyclase activity was lower in compensatory renal growth. The addition of Gpp(NH)p resulted in correction of these differences. Thus, the curves of magnesium-dependent activity were shifted to the left, and maximal response to the cation was similarly increased in both groups of membrane preparations. Since there appear to be no alterations at the level of the catalytic unit of the system, the present results also favor the possibility of alterations of the alpha subunit of Gs during renal growth.

A quantitative deficiency of the alpha subunit of Gs was directly demonstrated by cholera toxin-dependent ADP ribosylation of membrane proteins from kidneys with compensatory

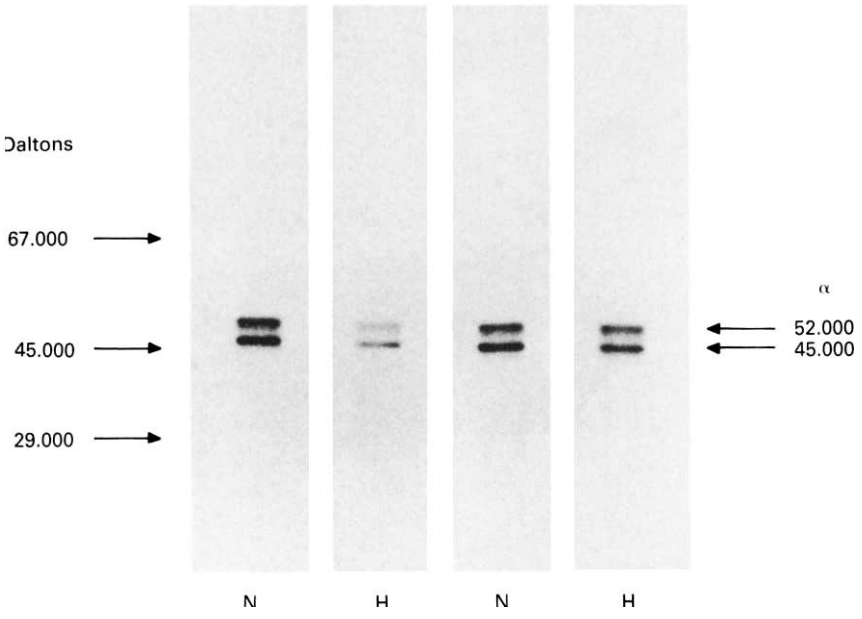

Fig. 6. *Cholera toxin-dependent [32-P]-ADP-ribosylation of proteins in two representative membrane preparations from normal controls (N) and two preparations from kidneys with compensatory growth (H).* Membranes were treated as described in **Methods.** Arrows on the left side indicate molecular weight markers. The arrows on the right indicate the position of the 42,000 and 52,000 Mr alpha subunits of Gs.

growth as compared to controls. Since the transduction of the hormone signal from the receptor to the catalytic unit is mediated by Gs, the decrease in the content of the alpha subunit could account for the impaired response of adenylate cyclase to PTH and magnesium in these membrane preparations. However, the fact that the response of the enzyme to Gpp(NH)p and NaF is apparently normal also suggest that in addition to the quantitative defect, there is a qualitative alteration in the interaction of Gs with the hormone receptor in the absence of saturating concentration of Gpp(NH)p. The addition of the nucleotide may compensate the altered response to PTH by favoring the interaction between Gs and the receptor. The mechanisms whereby this occurs cannot be answered from the present studies. It seems that the defect in Gs may involve other alterations as an increase in the affinity of this protein for guanine nucleotides. Of interest, the renal resistance to PTH observed in patients with pseudohypoparathyroidism is also associated with an important decrease in both the content and the activity of Gs [16, 32, 33]. Furthermore, the resistance of adenylate cyclase observed in renal cortical membranes from those patients was also corrected in the presence of guanine nucleotides [33].

An alternative explanation for the observed changes in adenylate cyclase activity in membranes from kidneys with compensatory growth could be enhanced inhibition of the system by the inhibitory Gi. However, this possibility seems unlikely since the relative content of Gi was similar in these membranes and controls.

We have previously shown that the interaction of renal cortical adenylate cyclase with PTH may be altered during phosphate depletion [34], dietary-induced hyperparathyroidism [35] and metabolic acidosis [15]. However, those studies differ from our present observations in that the defective response of adenylate cyclase to PTH was not corrected in the presence of exogenous guanine nucleotides.

The mechanisms whereby the alterations of PTH-stimulated adenylate cyclase after unilateral nephrectomy relate with the changes in phosphate transport in vivo cannot be fully explained at the present time. The experiments performed demonstrated that enzyme activation processes closely related with the actions of Gs were altered during compensatory renal growth. However, other alterations in the plasma membrane or within the cell which modulates enzyme activity may be involved in the impaired response of adenylate cyclase to PTH in vitro. Thus, it is possible that in spite of a decrease in the relative content of Gs in the membrane, an excess of intracellular GTP or an increased affinity for the nucleotide would allow a normal production of cAMP in response to PTH in vivo. In addition, the elevation in cell number in kidneys with compensatory growth may determine an absolute increase in the content of adenylate cyclase in the whole organ, thus increasing the overall response of the enzyme to the hormone in vivo.

Acknowledgments

The present studies were supported by Grant S1-1309 of the Consejo Nacional de Investigaciones Cientificas y Tecnologicas de Venezuela (CONICIT).

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