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ADENYL CYCLASE FROM RENAL CORTEX

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SUMMARY

Studies were carried out to characterize the parathyroid hormone-sensitive adenyl cyclase from rat renal cortex. The enzyme activity was tightly associated with the particulate fraction, and was not effectively solubilized by treatment with a variety of surface-active agents. The enzyme showed considerable contamination by ATPase, which was not separated from adenyl cyclase by gel filtration, although cyclic 3',5'-nucleotide phosphodiesterase was removed by this procedure. Filtering the enzyme through a shallow bed of agarose gel gave a preparation with increased specific activity and a linear relationship between activity and concentration of enzyme protein. Adenyl cyclase activity was detected over the pH range 6.0 to 9.0, with optimal parathyroid hormone- and fluoride-stimulated activity at pH 8.0 and 7.5, respectively. Fluoride-stimulated activity was maximal at 8 mM F^-. Incubation of the enzyme at 37° caused progressive loss in activity. A sex difference was observed in the response of the enzyme to monovalent cations. Enzyme from female rats showed inhibition of basal activity by 50 mM Li^+, and no effect of Na^+, K^+, or Rb^+, whereas enzyme from males showed significant activation of basal and hormone-stimulated reactions by 50 mM K^+ and Rb^+. The enzyme required either Mn^{2+} or Mg^{2+} and the latter afforded optimal activation by parathyroid hormone and F^-. The optimal concentration of Mg^{2+} varied with the concentration of substrate used. At higher concentrations of ATP (5, 10, 20 mM), optimal Mg^{2+}/ATP ratios approximated 1.0. At lower levels of ATP (0.5, 1.0 mM) optimum Mg^{2+}/ATP ratios ranged from 0.5 to 3.0. In the presence of optimal Mg^{2+}, Ca^{2+} inhibited adenyl cyclase, with 50% inhibition at approximately 2 mM Ca^{2+}. Mn^{2+} also inhibited the enzyme, but was less effective than Ca^{2+}, giving 50% inhibition of hormone and F^- reactions at 5 and 7.5 mM, respectively.

The apparent affinity of the enzyme for ATP was much lower than has been found with the enzyme from other tissue. Even at concentrations of ATP as high as 20 mM, there was no evidence for saturation of the enzyme with substrate. Parathyroid hormone or F^- affected the enzyme through an increase in v_{max} without a detectable change in affinity for ATP.

Abbreviation: EGTA, ethyleneglycol-bis-(β-aminoethylether)-*N,N'*-tetraacetic acid.

INTRODUCTION

Earlier studies from this laboratory have identified cyclic adenosine monophosphate (cyclic 3′,5′-AMP) as an intermediate in the action of parathyroid hormone[1–5]. The rise in concentration of cyclic 3′,5′-AMP in the physiological receptor organs, kidney[4] and bone[5], is a consequence of the direct activation of the membrane-bound enzyme, adenyl cyclase in these tissues by parathyroid hormone[1–4]. In the kidney the adenyl cyclase specifically activated by parathyroid hormone is located within the tubules of the cortex[4]. It is the purpose of the current study to characterize further the properties of adenyl cyclase from the renal cortex.

MATERIALS AND METHODS

Radioisotopes

[α-^{32}P]ATP (500–1000 mC/mole) and [γ-^{32}P]ATP (10 C/mmole) were purchased from International Chemical and Nuclear Corporation, and tritiated cyclic 3′,5′-AMP (2.35 C/mole) was purchased from Schwarz BioResearch. Other reagents were purchased from standard suppliers.

Parathyroid hormone

Bovine parathyroid hormone, purified from acetone-dried parathyroid glands, was purchased from Wilson Laboratories and diluted in 0.005 M acetic acid to a concentration of 1 mg/ml. The hormone was stored under liquid N_2 until immediately prior to use. Biological activity was 1500 U.S.P. units/mg as determined by bioassay *in vitro*[6].

Enzyme preparation

Female Sprague–Dawley rats at term, 150 to 200 g, maintained under usual housing conditions with standard Purina chow, were killed by decapitation. For some experiments male and non-pregnant female rats were used. The kidneys were quickly removed, placed in chilled 0.25 M sucrose, and the outer renal cortex was dissected taking as little medulla as possible. The tissue was homogenized with 4 vol. of 0.05 M Tris–HCl, pH 7.4, containing 10% v/v dimethylsulfoxide (Tris–dimethylsulfoxide) in a glass tube using a motor-driven Teflon pestle. The particulate fraction was obtained by centrifuging the homogenate at 2000 $\times$ *g* for 10 min, and the precipitate was resuspended in 4 ml of Tris–dimethylsulfoxide and centrifuged again. The particulate enzyme preparation was suspended in an equal volume of Tris–dimethylsulfoxide, and was passed over a 1 cm $\times$ 3 cm bed of agarose gel (Biogel 0.5 M) equilibrated with the same buffer at 4°. In general, 50% of the protein from the prior step was recovered after gel filtration. Effluent fractions containing particles were centrifuged at 2000 $\times$ *g* for 10 min, and the precipitate was resuspended in sufficient Tris–dimethylsulfoxide to provide a protein concentration of approximately 15 mg/ml. The enzyme suspension was divided into small aliquots in glass vials and immersed into liquid N_2. Enzyme prepared in this manner is stable to storage at liquid N_2 temperature at least 12 months without noticeable loss of activity.

For certain experiments enzyme was prepared without dimethylsulfoxide or without filtration on agarose.

Protein was determined using the method of LOWRY *et al.*[7]. Ca^{2+} was determined by atomic absorption spectroscopy.

Enzyme assays

Adenyl cyclase. Formation of ^{32}P labeled cyclic 3',5'-AMP from [α-^{32}P]ATP was measured in an assay similar to that described by KRISHNA *et al.*[8] modified by adding KCl and an ATP-regenerating system. The reaction mixture contained: Tris–HCl, 0.05 M, pH 7.4; 4.5 mM $MgCl_2$; 9 mM theophylline; 0.013% bovine serum albumin; 0.03 M KCl; 1.1 mM [α-^{32}P]ATP (specific activity approximately 25 counts/min per pmole); 10 μg creatine phosphokinase; 4 mM creatine phosphate; approximately 400 μg of enzyme suspension protein; and 5 μg/ml parathyroid hormone, or 7 mM NaF as indicated in a total volume of 70 μl. Incubation was carried out for 10 min at 37° in a Dubnoff metabolic shaker, and the reaction was stopped by adding 100 μl of carrier solution containing 0.04 M ATP, and 125 mM [^3H]cyclic 3',5'-AMP (approximately $3 \cdot 10^4$ counts/min), in 0.05 M Tris–HCl, pH 7.4. Reaction mixtures were heated to boiling in a metal block for 3 min; 0.4 ml water was added, and the precipitated protein removed by centrifuging for 10 min at 700 $\times$ *g*.

The supernatant fluid was applied to a 0.5 cm $\times$ 3.0 cm column of Dowex 50W-X8, 100–200 mesh in the H^+ form, and the column was eluted with water. Cyclic 3',5'-AMP appeared in the fourth through sixth ml of effluent. To this fraction were added 0.2 ml each of 8% $ZnSo_4$ and saturated $Ba(OH)_2$. The mixture was centrifuged and Ba^{2+}–Zn^{2+} precipitation was repeated without disturbing the first precipitate. Radioactive phosphorus in the supernatant fluid represented cyclic 3',5'-AMP[8]. 3 ml of supernatant fluid were added to 15 ml of liquid scintillation solution[9], and ^{32}P and ^3H were measured simultaneously with a liquid scintillation detector. Appropriate corrections were made in the final calculations for recovery of [^3H]cyclic 3',5'-AMP which was usually 30%. For each experiment a series of "blank reaction" control tubes was boiled without prior incubation at 37°, and then processed as described above. The radioactivity recovered from these controls was routinely subtracted from that measured in the experimental tubes. The radioactivity of the reaction blanks never exceeded 20% of the radioactivity specifically attributable to cyclic 3',5'-AMP generated in the basal adenyl cyclase reaction, or 6% of the radioactivity attributable to cyclic 3',5'-AMP in the hormone- or F^--stimulated reactions.

The addition of an ATP-regenerating system to the reaction mixtures has been shown in studies reported earlier[6] to afford near-linear rates of reaction over 15 min of incubation.

Phosphodiesterase. Cyclic nucleotide phosphodiesterase was assayed as described previously[6].

ATPase was measured by the precipitation of radioactive orthophosphate hydrolyzed from [γ-^{32}P]ATP precipitated by the method of SUGINO AND MIYOSHI[10].

RESULTS

Identity of reaction product

In studies reported previously, 95% of the radioactive product of a series of parathyroid hormone-stimulated reactions were converted to 5'-AMP by incubation with purified cyclic 3',5'-nucleotide phosphodiesterase. These results established the

identity of the radioactive product of the adenyl cyclase reaction as cyclic 3',5'-AMP.

Properties of the enzyme suspension

The adenyl cyclase preparation used here showed contamination by ATPase (0.5 μmole P_i formed per min per mg of protein) and phosphodiesterase (11.7 pmoles cyclic 3',5'-AMP hydrolyzed per min per mg of protein). Attempts to solubilize the enzyme by homogenizing fresh tissue with various agents, including urea, *n*-butanol, acetone, glycerol, and detergents, resulted in each case in the complete destruction of hormone-sensitive activity. Two detergents, Triton X-100® and Lubrol PX®, each at 0.5% in the homogenizing medium, allowed some apparent solubilization in that 20% of the F^--sensitive activity remained in the supernatant fraction after centrifugation at 100 000 × *g* for 1 h; 80% of the activity remained in the particulate fraction. Neither higher concentrations of detergent nor exposure to ultrasound with detergent effected any further solubilization.

Dissociation of phosphodiesterase activity was achieved by filtration of the enzyme suspension on agarose gel. After filtration of the suspension over a shallow bed of agarose, the particles were suspended in 2 ml of Tris–dimethylsulfoxide, and applied to a column, 2 cm × 50 cm, of agarose gel (Biogel 0.5 M). 1-ml fractions were collected, and tested for adenyl cyclase, ATPase, and phosphodiesterase (Fig. 1).

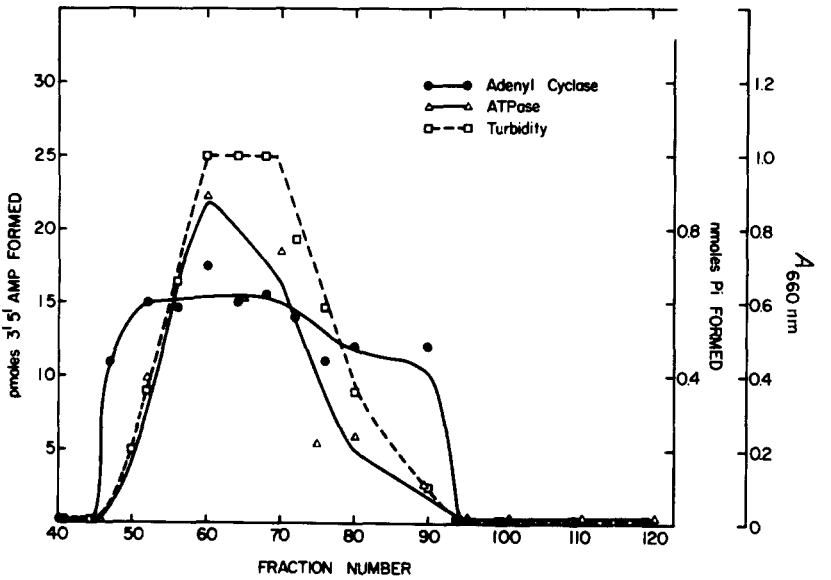

Fig. 1. One lot of fresh tissue was prepared as described in the text, and passed over a column of agarose gel, 2 cm × 50 cm (Biogel 0.5 M). 1-ml fractions were collected, and tested for F^--stimulated adenyl cyclase, ATPase, and phosphodiesterase as described in the text. Included volume of the column, 160 ml; void volume, 45 ml. Adenyl cyclase and ATPase appeared in Fractions 46–90, corresponding to the calculated void volume and the appearance of turbidity. Phosphodiesterase was not detected in any fraction. The starting material showed F^--stimulated adenyl cyclase activity of 1000 pmoles cyclic 3',5'-AMP per 10 min per mg of protein, ATPase activity of 0.5 μmoles P_i formed per mg of protein per min and phosphodiesterase activity of 11.7 pmoles cyclic 3',5'-AMP hydrolyzed per min per mg of protein. Results are presented as the mean of three determinations.

Adenyl cyclase and ATP appeared near the calculated void volume for the column (approximately 1/3 of the column volume, or 50 ml), and corresponded to fractions that were turbid in appearance. Phosphodiesterase was not detectable in these fractions, whereas activity equivalent to 11.7 pmoles of cyclic 3',5'-AMP hydrolyzed per min per mg of protein was found prior to gel filtration.

Enzyme activity was proportional to the amount of enzyme added when the content of protein in the reaction mixture was less than 100 μg. Filtration of the suspension over a shallow bed of agarose gel provided a significant increase in the specific activity of adenyl cyclase.

Effects of pH and F^- and stability at 37°

One batch of enzyme was prepared from fresh tissue and filtered through agarose gel. The filtered suspension was divided into several aliquots and centrifuged at 1000 × *g* for 10 min. Each aliquot was resuspended in Tris–HCl, 0.05 M, at the appropriate pH. The control reaction was easily detectable at pH 6, and showed little change over the pH range 6 to 9 (Fig. 2). Parathyroid hormone was without effect on the reaction at pH 6, but caused an increase in activity at pH 7 and a maximum effect at pH 8. The F^--stimulated reaction was greater than the control at pH 6 and was maximal at pH 7.5. F^- activated adenyl cyclase over a narrow concentration range with a maximal effect at 8 mM.

Preincubation of the enzyme suspension at 37° was carried out for 8 min, and the enzyme was then diluted into adenyl cyclase reaction mixtures for assay. Basal activity was reduced to 54% of the initial activity by the preincubation. Parathyroid hormone- and F^--stimulated reactions were reduced to 70% and 80% of initial activities, respectively.

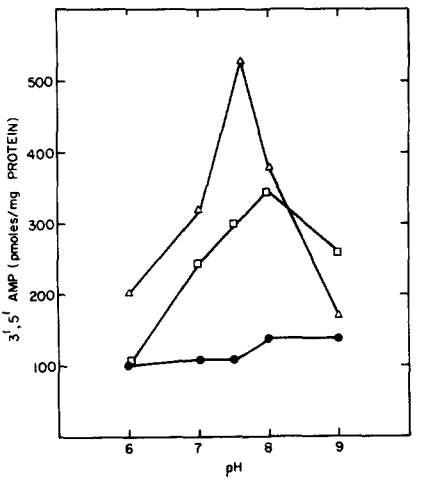

Fig. 2. Effect of pH. Enzyme from one lot of fresh tissue was divided into separate aliquots and centrifuged at 1000 × *g* for 10 min. Each aliquot was resuspended in Tris–dimethylsulfoxide at the indicated pH. Adenyl cyclase was measured as described in the text. Each point represents the mean of four determinations. ●—●, basal reaction; □—□, parathyroid hormone (PTH) (5 μg/ml); △—△, +F^- (7 mM).

TABLE I

EFFECT OF MONOVALENT CATIONS ON ADENYL CYCLASE ACTIVITY

Adenyl cyclase activity was measured as described in the text. Cations were added as the Cl^- salts to a final concentration of 50 mM. Results are expressed as the mean ± standard error of 4 determinations. PTH, parathyroid hormone.

Additions	*Cyclic 3',5'-AMP (pmoles/mg protein)*					
	Females			*Males*		
	Basal	*PTH*	*F⁻*	*Basal*	*PTH*	*F⁻*
None	92 ± 10	316 ± 17	466 ± 42	184 ± 35	605 ± 10	1400 ± 23
Na^+ (50 mM)	101 ± 6	291 ± 33	444 ± 58	154 ± 3	558 ± 33	1360 ± 44
K^+	137 ± 75	276 ± 32	690 ± 190	236 ± 9*	800 ± 40*	1370 ± 55
Li^+	47 ± 8*	286 ± 16	435 ± 25	207 ± 8	527 ± 6**	1380 ± 35
Rb^+	69 ± 30	375 ± 48	422 ± 41	293 ± 16*	743 ± 28*	1290 ± 47

* $P < 0.05$.
** $P < 0.01$, compared to activity determined without added cation.

Monovalent cations

Na^+, K^+, Li^+, and Rb^+ as the Cl^- salts (50 mM) were each tested on enzyme from female rats (Table I). Li^+ caused significant inhibition of basal activity ($P < 0.05$), but did not affect hormone- or F^--stimulated activity. None of the other monovalent cations tested showed significant effects on adenyl cyclase activity. These results contrasted with those reported earlier[6] wherein K^+ caused significant increases in basal and hormone-stimulated activity. The earlier results were obtained with

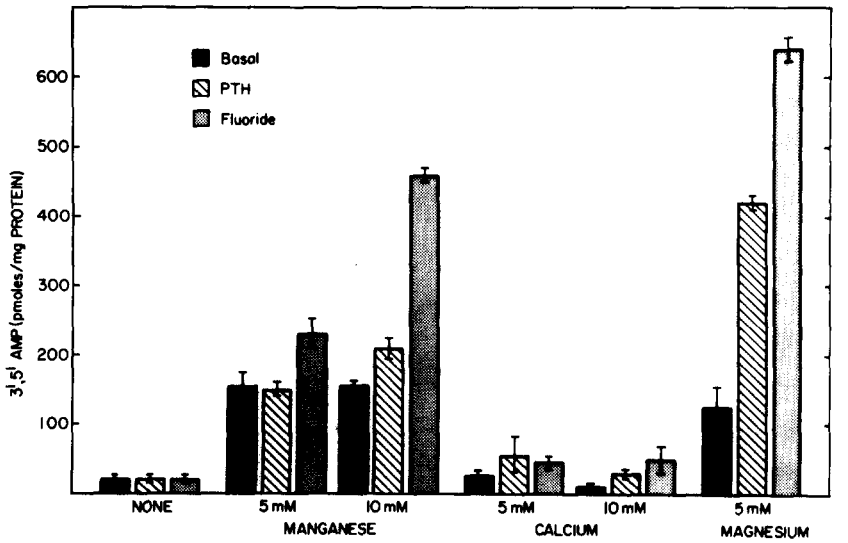

Fig. 3. Effects of divalent cations. Adenyl cyclase was measured as described in the text. Mg^{2+} was omitted except as indicated. Divalent cations were added as the Cl^- salts. Results represent the mean ± standard error of 4 determinations. Parathyroid hormone (PTH), 5 μg/ml; F^-, 7 mM.

enzyme from male rats; the current experiments show that there is a sex difference in sensitivity to certain monovalent cations. In male rats K^+ and Rb^+ each caused an increase in basal and hormone-stimulated reaction rates ($P < 0.05$), but were without effect on the F^- reaction. Li^+ produced significant depression of the hormone-stimulated reaction ($P < 0.01$), but the inhibition of basal activity observed in the females was not found. There was no effect of sodium with either enzyme preparation.

Effect of divalent cations

Enzyme tested without divalent cation showed little activity and did not respond to either parathyroid hormone or F^- (Fig. 3). 5 mM Mn^{2+} caused a marked increase in basal activity and a further increment was produced by F^-: activation by parathyroid hormone was not observed. Mn^{2+} at 10 mM caused no further rise in basal activity, but produced an additional increase in F^--stimulated activity. At this concentration of Mn^{2+} a small but significant stimulation was found with parathyroid hormone. Ca^{2+} at 5 and 10 mM did not alter the activity of the enzyme without addition of another cation. 5 mM Mg^{2+} was similar to Mn^{2+} in stimulating basal activity, but was significantly more effective in enhancing the response to hormone and F^-. The optimum concentration of Mg^{2+} varied unpredictably with the concentration of ATP. In separate experiments, carried out over a range of ATP concentration from 0.5 to 20 mM, Mg^{2+} was added at Mg^{2+}/ATP ratios of 0.5 to 2.0 (Fig. 4). At 0.5 mM ATP basal activity was greatest at Mg^{2+}/ATP = 1.3; hormone- and F^--stimulated activities were maximal at Mg^{2+}/ATP ratios of 0.5 and 1.3, respectively. At Mg^{2+} concentrations in excess of these ratios, there was no further increase in the basal or hormonally-activated reactions and the F^- reaction showed some inhibition. When

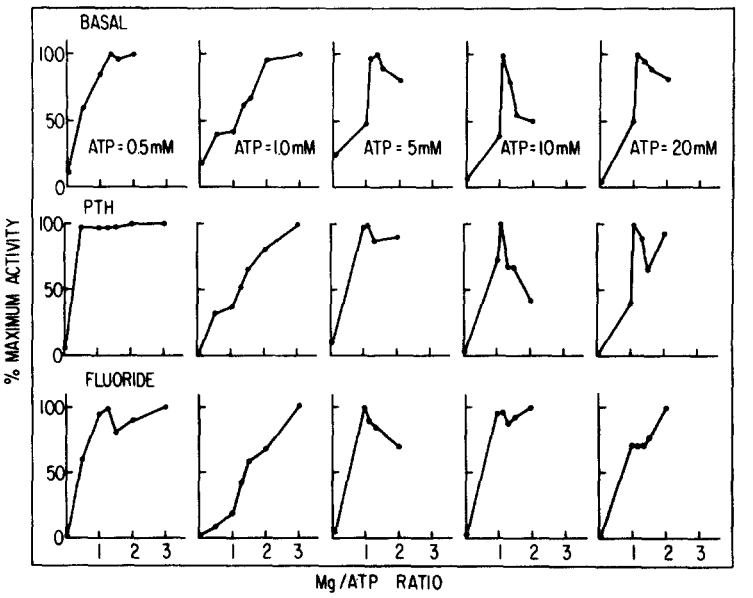

Fig. 4. Effects of magnesium. Adenyl cyclase was measured as described in the text except Mg^{2+} was added to give Mg^{2+}/ATP ratios of 0.5–2 with ATP concentrations as indicated. Results are presented as the mean of three determinations. Parathyroid hormone (PTH), 5 μg/ml; F^-, 7 mM.

ATP was 1 mM, basal activity became maximal at $Mg^{2+}/ATP = 2$; hormone- and F^--stimulated reaction rates, however, continued to increase with Mg^{2+}/ATP ratios as high as 3. For higher ATP concentrations (5, 10, 20 mM), basal or hormone-activated reactions were maximal at $Mg^{2+}/ATP = 1.1$, and showed significant inhibition at higher Mg^{2+} concentrations. F^- reactions at 5 and 10 mM ATP showed maximum activity at $Mg^{2+}/ATP = 1.0$, but at 20 mM ATP there was significant further increase in activity when Mg^{2+}/ATP ratio rose to 2.

In one experiment fresh tissue was obtained and divided into two fractions prior to homogenization. One fraction was homogenized in Tris–dimethylsulfoxide as described previously. The remainder was homogenized in Tris–dimethylsulfoxide containing 0.1 mM ethyleneglycol-bis-(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid (EGTA). The enzyme suspension was centrifuged at 1000 × *g* for 10 min, and the precipitate was resuspended in Tris–dimethylsulfoxide–EGTA. The suspension was centrifuged and washed twice again with Tris–dimethylsulfoxide–EGTA, and finally resuspended in Tris–dimethylsulfoxide. Enzyme prepared in the presence of the Ca^{2+}-chelator showed significantly greater activity in the basal and F^--stimulated reactions (Table II); the hormone-stimulated reaction was not altered by the addition of EGTA. The initial Ca^{2+} content of the suspension was 0.45 mg/mg of protein and after extraction of the preparation with EGTA it was 0.19 mg/mg of protein. Approximately 50% of the membrane-associated Ca^{2+}, therefore, remains tightly bound to the plasma membrane fragments, and is not accessible to extraction by chelator.

TABLE II

EFFECT OF EGTA ON ADENYL CYCLASE ACTIVITY

Fresh tissue was divided into two lots. One-half was homogenized in Tris–dimethylsulfoxide; the remainder was homogenized in Tris–dimethylsulfoxide containing 0.1 mM EGTA. Adenyl cyclase activity was measured as described in the text. Each result represents the mean ± standard error of four determinations. PTH, parathyroid hormone.

Medium	*Cyclic 3′,5′-AMP (pmoles/mg protein)*		
	Basal	*PTH*	*F^-*
Tris–dimethylsulfoxide	74 ± 1	590 ± 24	997 ± 29
Tris–dimethylsulfoxide–EGTA	156 ± 7*	557 ± 21	2000 ± 95*

* $P < 0.01$, compared with activity observed without EGTA.

In the presence of optimum concentrations of Mg^{2+}, the addition of Ca^{2+} caused marked inhibition of adenyl cyclase activity (Fig. 5). Half-maximal inhibition for the basal and hormone-stimulated reactions was achieved at a Ca^{2+} concentration of 1.6 mM; half-maximal inhibition of the F^- reaction obtained at 2.5 mM Ca^{2+}. Mn^{2+} also caused inhibition of adenyl cyclase activity in the presence of optimal Mg^{2+}, although this cation was less effective than Ca^{2+}. Half-maximal inhibition was approached with 5 and 7 mM Mn^{2+} for the hormone- and F^--stimulated reactions, and at 10 mM Mn^{2+} the basal reaction was inhibited only 20% (Fig. 5).

Effect of substrate concentration

Since one Mg^{2+} is bound to one ATP at the pH of the reaction medium used

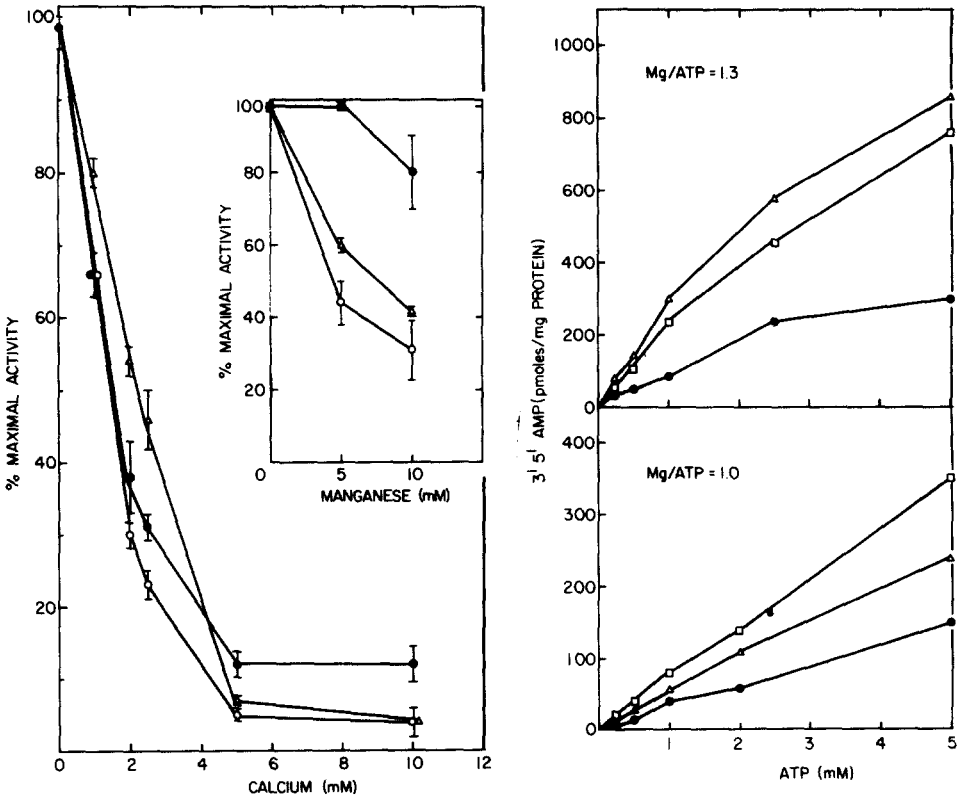

Fig. 5. Inhibitory effects of Ca^{2+} and Mn^{2+}. Adenyl cyclase was measured as described in the text. Ca^{2+} or Mn^{2+} was added as the Cl^- salt. ●—●, basal reaction; ○—○, ¦+ parathyroid hormone (PTH) (5 μg/ml); △—△, +F^- (7 mM). Results are presented as the mean ± standard error of 4 determinations.

Fig. 6. Effect of substrate concentration. Adenyl cyclase was measured as described in the text except that ATP and Mg^{2+} were added separately to achieve the final concentrations and Mg^{2+}/ATP ratios indicated. Results are presented as the mean of 3 determinations. ●—●, basal reaction; □—□, +Parathyroid hormone (PTH) (5 μg/ml); △—△, +F^- (7 mM).

(7.4), it is likely that the substrate for adenyl cyclase is ATP–Mg. Accordingly, the effect of varying substrate concentration was tested at a fixed Mg^{2+}/ATP ratio of 1.0 (Fig. 6). Neither the basal, hormone-, nor F^--stimulated reactions showed saturation by ATP over a range of concentrations from 0.25 to 5.0 mM. This result was confirmed at Mg^{2+}/ATP ratio of 1.3 over the same concentration range. In other experiments using fixed Mg^{2+}/ATP ratios of 1.0, 1.3 and 4.0, the enzyme was not saturated by substrate concentrations as high as 20 mM. At all concentrations of substrate the product formed increased linearly with time over 5–15 min incubation, and the apparent non-saturation of the enzyme could not be attributed to changes in the linearity of the reaction rates with different substrate concentrations.

The relative responses to parathyroid hormone or F^- appeared to depend on the presence of Mg^{2+} in excess of ATP. When ATP/Mg^{2+} = 1.0, the response to F^- was always less than that to parathyroid hormone, regardless of the substrate concentration (Fig. 6). With excess Mg^{2+}, maximal stimulation was always produced by F^-.

DISCUSSION

Many properties of adenyl cyclase from the renal cortex are similar to those found for the enzyme in other tissues[11–15]. The enzyme is tightly associated with the plasma membrane, and treatment of the suspension with a variety of agents did not allow solubilization of hormone-sensitive activity. This result parallels the experience with adenyl cyclase from most mammalian tissues, although KLAINER *et al.*[13] were able to show epinephrine-sensitive activity in preparations from bovine brain extracted in Triton X-100®. Gel filtration did afford separation of some contaminating proteins as evidenced by the removal of phosphodiesterase and increased specific activity of the enzyme. The pH optima, response to F^-, dependence on Mg^{2+} and inhibition by Ca^{2+} found in the present study correspond closely to data obtained with the enzyme from adipocytes and myocardium[11,12].

Substitution of Mn^{2+} (5 mM) for Mg^{2+} provided complete hormone responsiveness with enzyme from fat cells[11], but this cation was considerably less efficient with the renal enzyme. Although Mn^{2+} was as effective as Mg^{2+} for basal activity, it was less effective in the F^--sensitive reaction, and regardless of the concentration of Mn^{2+}, activation of the enzyme by parathyroid hormone did not reach the degree found with Mg^{2+}.

The renal cortical enzyme shows greater stability at 37° than the enzyme from fat cells[11]. Whereas the latter retained less than 30% of initial activity following incubation at 37° for 8 min, the renal enzyme retained 54% of initial baseline activity and 70 and 80% of the initial hormone- and F^--stimulated activity, respectively.

Adenyl cyclase varies in sensitivity to monovalent cations depending on the tissue of origin. The enzyme from fat cells showed 30% stimulation of basal activity by 0.1 M Na^+, K^+, Li^+, and Rb^+, and 35% inhibition of hormone-stimulated activity by Li^+ (ref. 11). Hormonal activation of the enzyme from thyroid was inhibited 50% with as little as 8 mM Li^+ (ref. 14). The renal cortical enzyme appears more like that from fat cells in this regard. The enhancement of hormone-stimulated activity reported earlier in male rats was confirmed in the present experiments. Further, it was found that Rb^+, which can substitute for K^+ in a number of biological systems, was equally effective in stimulating adenyl cyclase. An ancillary finding in the current study indicates a sex difference in responsiveness of the enzyme to K^+. Enzyme obtained from renal cortex of female rats, whether virgin or pregnant, is insensitive to potassium *in vitro*.

A striking feature of the renal cortical enzyme was the lack of apparent saturation by ATP. Tested at fixed ratios of Mg^{2+}/ATP, the enzyme activity was not saturated at an ATP concentration of 20 mM. This result differs sharply from results with myocardium (K_m = 0.08), fat cell (K_m = 0.25–0.50), or amphibian erythrocyte (K_m = 0.16 mM)[11,12,15]. The reason for this difference is not apparent, but did not seem to be caused by a physical change produced by the addition of dimethylsulfoxide to the enzyme, or by changes in the linearity of the reaction rates with different concentrations of substrate. Possibly a true difference in affinity for substrate exists from one tissue to another, or perhaps some property intrinsic to membranes from renal tubules after fragmentation renders the enzyme less accessible to substrate. Alternatively, the observed inability to saturate the enzyme with substrate may be due to competition for ATP and/or Mg^{2+} by contaminating enzymes.

Inhibition of adenyl cyclase activity by Mg^{2+} concentrations in excess of 4–6 mM has been reported for several tissues. On the other hand, BIRNBAUMER *et al.*[11] found that the basal reaction rate for the enzyme from fat could not be saturated by Mg^{2+} even at concentrations as high as 80 mM, tested over a range of ATP concentrations of 0.7 to 5.9 mM. However, when the enzyme was activated by hormone or F^- there was a maximum for Mg^{2+} at 10 mM. The current study shows that the effect of Mg^{2+} on the kidney enzyme is complex, and depends in large measure on the ATP concentration used. At ATP concentrations from 5 to 20 mM basal, hormone- and F^--activated reactions were maximal at Mg^{2+}/ATP ratios near unity, and showed inhibition with higher Mg^{2+} concentrations. At lower levels of ATP no consistent pattern was apparent. At 0.5 mM ATP, basal activity was greatest at Mg^{2+}/ATP = 1.3, with maximal parathyroid hormone- and F^--stimulated activities at Mg^{2+}/ATP ratios of 0.5 and 1.3; whereas at ATP = 1 mM, basal activity became maximal at Mg^{2+}/ATP = 2 and hormone- and F^--stimulated reaction rates increased with Mg^{2+}/ATP ratios as high as 3.0. Interpretation of these results is complicated further by the problem of nonspecific adsorption of Mg^{2+} onto the membrane fragments, which would increase the apparent K_m for the metal, particularly at low concentrations of ATP.

Some authors have concluded that Mg^{2+} interacts with adenyl cyclase at a second "allosteric" site in addition to the catalytic site. In the current study one could not exclude the possibility of a second site for magnesium, although support for this interpretation remains incomplete. In addition to the difficulties encountered in interpreting the effects of Mg^{2+}, the presence of contaminating ATPase complicates adenyl cyclase systems by necessitating the use of ATP-regenerating systems. The regenerating systems used, *e.g.* pyruvate kinase or creatine phosphokinase, require Mg^{2+} in the same concentration range needed for adenyl cyclase, and the alterations in the apparent activity of the latter with changes in Mg^{2+} concentration may merely reflect changes in the activity of the regenerating system. These several problems indicate that there must be further resolution of the adenyl cyclase–receptor complex before conclusions can be drawn about the kinetics of the system. The primary action of parathyroid hormone appears to be activation of adenyl cyclase specifically in renal cortex and bone, recognized as the two physiological receptor tissues for the hormone. The nature of the interrelationships between receptor and enzyme remains to be defined. Were it possible to separate and reconstitute these two functions, further understanding of the problem would be possible.

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REFERENCES

1 L. R. CHASE AND G. D. AURBACH, *Science*, 159 (1968) 545.
2 L. R. CHASE AND G. D. AURBACH, in R. V. TALMAGE AND L. F. BELANGER, *Parathyroid Hormone and Thyrocalcitonin (Calcitonin)*, Excerpta Medica Foundation, Amsterdam, 1968, p. 247.

3 L. R. CHASE, S. A. FEDAK AND G. D. AURBACH, *Endocrinology*, 84 (1969) 761.
4 G. L. MELSON, L. R. CHASE AND G. D. AURBACH, *Endocrinology*, 86 (1970) 511.
5 L. R. CHASE AND G. D. AURBACH, *J. Biol. Chem.*, 245 (1970) 1520.
6 R. MARCUS AND G. D. AURBACH, *Endocrinology*, 85 (1969) 801.
7 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
8 G. KRISHNA, B. WEISS AND B. BRODIE, *J. Pharmacol. Exptl. Ther.*, 163 (1968) 379.
9 G. A. BRAY, *Anal. Biochem.*, 1 (1960) 279.
10 Y. SUGINO AND Y. MIYOSHI, *J. Biol. Chem.*, 239 (1964) 2360.
11 L. BIRNBAUMER, S. L. POHL AND M. RODBELL, *J. Biol. Chem.*, 244 (1969) 3468.
12 G. I. DRUMMOND AND L. DUNCAN, *J. Biol. Chem.*, 245 (1970) 976.
13 L. M. KLAINER, Y. M. CHI, S. L. FREIDBERG, T. W. RALL AND E. W. SUTHERLAND, *J. Biol. Chem.*, 237 (1962) 1239.
14 J. WOLFF, S. C. BERENS AND A. B. JONES, *Biochem. Biophys. Res. Commun.*, 39 (1970) 77.
15 O. M. ROSEN AND S. M. ROSEN, *Arch. Biochem. Biophys.*, 131 (1969) 449.