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Effects of Ions on Adrenocorticotropin-Sensitive Adenylate Cyclase of Rat Adrenals[1] (38350)

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Although the essential role of adenylate cyclase activation in the stimulation of the adrenal cortex by adrenocorticotropin (ACTH) is generally accepted (1), information regarding the properties of the enzyme in the normal rat adrenal cortex is remarkably scanty (2). Whereas the effects of ions on the hormone-sensitive adenylate cyclase of broken-cell preparations from several target tissues have been explored in some detail (3–10), the only studies in which influences of ions other than Ca^{2+} on ACTH-sensitive adenylate cyclase of rodent adrenal tissue homogenates were afforded special attention involved the use of an ACTH-responsive mouse adrenal tumor (11, 12).

This study was designed to determine, in homogenates of normal rat adrenals, (a) how Mg^{2+} concentration affects basal, ACTH-stimulated and NaF-activated adenylate cyclase, and (b) how the basal and ACTH-enhanced activity of this enzyme is influenced by monovalent cations (Li^+, Na^+, K^+) at different concentrations of Mg^{2+}.

Materials and Methods. Male Sprague–Dawley rats weighing about 500 g were killed by decapitation, and the adrenal glands immediately cleaned and weighed. The adrenals from groups of four rats each were then pooled. The tissue was hand-homogenized in 8 vol 0.05 *M* Tris–HCl buffer, pH 7.4, in a glass Dounce homogenizer. The crude homogenate was passed through eight layers of gauze and centrifuged at 1400*g* for 20 min at 4°. After washing with buffer and recentrifugation, the pellet was resuspended in 0.7–1.0 ml of 0.05 *M* Tris–HCl, pH 7.4.

The assay of adenylate cyclase used, a modification of the method of Krishna *et al.* (13), measures the conversion of [α-^{32}P]ATP to cyclic 3′,5′[^{32}P]AMP. The final volume of the incubation mixture in each tube was 65 μl. All tubes contained 50 m*M* Tris–HCl (pH 7.4), 0.015% bovine albumin, 4 m*M* theophylline, 3 m*M* phosphoenol pyruvate, 890 milliunits pyruvate kinase, and either 1.25, 2.5, or 10 m*M* $MgCl_2$. The tissue homogenate added (in 25 μl) contained 0.1–0.2 mg protein, determined in pellet samples by the method of Lowry *et al.* (14). When monovalent ions were added, their chloride salts were used. The concentrations of Li^+, Na^+ or K^+ used are given in Fig. 1. When present, synthetic β^{1-24} ACTH (Cortrosyn) was in the concentration of 2×10^{-5} *M* and NaF was 10 m*M*. When ACTH or NaF were omitted, 0.05 *M* Tris–HCl buffer was used to bring the volume to 65 μl. ACTH was added 2 min after the tissue homogenate. The mixture was made up at 0–4°. The 10-min incubation in a water bath at 37° was started with the addition of 1.23 m*M* ATP (containing [α-^{32}P]ATP, New England Nuclear, specific radioactivity 20–30 cpm/pmole). Incubation was terminated by rapid addition of a diluting solution containing 40 m*M* ATP, 15 m*M* cyclic AMP, 50 m*M* Tris–Cl and 20,000 cpm of cyclic [^{3}H]AMP (Schwarz Bio-Research) followed by heating at 110° for 5 min. Cyclic 3′,5′ [^{32}P] AMP was separated from other ^{32}P-labeled compounds by chromatography on Dowex 50 W-X$_2$, 200–400 mesh, and 2 $BaSO_4$ precipitations. ^{3}H and ^{32}P radioactivities of the second $BaSO_4$ supernatant were counted in a Packard Tri-Carb liquid scintillation counter. Total cyclic AMP formed was corrected on the basis of cyclic [^{3}H]AMP recovered (usually between 70

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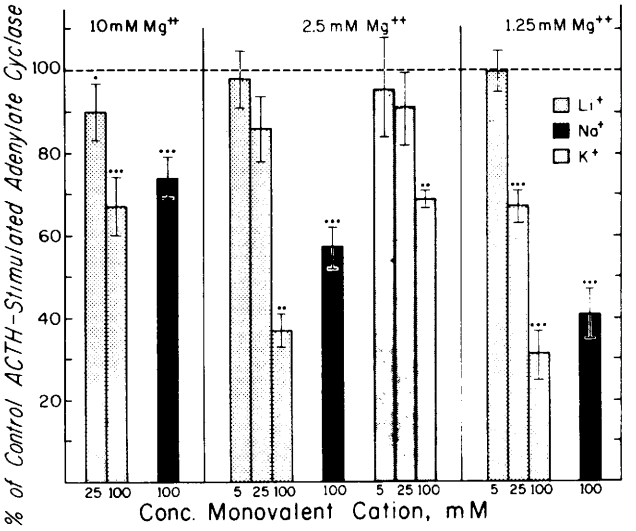

FIG. 1. Effect of monovalent cations on stimulation of adrenal adenylate cyclase by ACTH. Each experiment involved five homogenate pools, in which enzyme activity was determined (a) without addition of a monovalent cation or ACTH (basal level), (b) after addition of the monovalent cation indicated, in the concentration shown, (c) after addition of ACTH, and (d) with (b) and (c) combined. For each homogenate pool, influence of the monovalent cation on stimulation of adenylate cyclase was calculated from the formula (d/b) × 100/(c/a). The bars stand for means of the values thus derived, the vertical brackets for ± 1 SE. Significance of the difference of the cation-influenced ACTH effect from that of ACTH alone (c/a, broken horizontal line) was determined by the paired t test. * = $P < 0.05$; ** = $P < 0.02$; *** = $P < 0.01$. The differences between results obtained in different homogenate pools (represented by different bars) were evaluated by the simple t test.

and 80%). A blank for each experiment was prepared by boiling of the tissue extract before addition of the [α-^{32}P]ATP. This value was then subtracted from each experimental value. All experiments were performed on at least five homogenate pools. Each enzyme assay was done in triplicate. The results of 5–20 experiments done under identical conditions were averaged, and the standard errors of the means were determined. Adenylate cyclase activity was expressed as nmoles cyclic AMP formed per mg protein per 10 min. For statistical analysis, differences between the results of experiments involving the same tissue homogenate pools were evaluated by the paired t test, and the differences between those obtained on different homogenate pools by the simple t test.

Results. Table I demonstrates the dependence of basal adrenal adenylate cyclase activity on the concentration of Mg^{2+} in the medium. Activity was significantly ($P < 0.01$) higher at 2.5 m*M* than at 1.25 m*M* Mg^{2+}, and in turn higher ($P < 0.01$) at 10 m*M* than at 2.5 m*M* Mg^{2+}. The response of the enzyme to ACTH and NaF, as a function of medium Mg^{2+} concentration, showed different patterns. ACTH stimulated adenylate cyclase maximally (over 20-fold) at 1.25 and 2.5 m*M* Mg^{2+} [the absolute level of enzyme activity was significantly ($P < 0.01$) higher at 2.5 m*M* Mg^{2+} because of the greater basal activity]; with Mg^{2+} at 10 m*M*, both the factor of stimulation and the absolute level of ACTH-stimulated enzyme activity declined sharply ($P < 0.01$). Stimulation by NaF at 1.25 m*M* was less than half that by ACTH; at 2.5 m*M* Mg^{2+}, the differences were less striking, but still significantly ($P < 0.01$) in favor of ACTH; at 10 m*M* Mg^{2+}, NaF-stimulated adenyl cyclase was at the same absolute level as at 2.5 m*M* Mg^{2+}, but the factor by which basal activity was raised had declined ($P < 0.01$). Nevertheless, the fall was not as precipitous as in the case of ACTH, and at 10 m*M* Mg^{2+} NaF-activation of adenylate cyclase exceeded that by ACTH significantly ($P < 0.01$).

The effects of various concentrations of monovalent cations on basal adrenal adenylate cyclase activity (Li^+, Na^+, K^+) at the three concentrations of Mg^{2+} (cf. Fig. 1) were erratic. Significant ($P < 0.05$) depressions were achieved by Li^+ at 25 m*M* (24%) and 100 m*M* (32%) when Mg^{2+} was 2.5 m*M*, and by 100 m*M*

TABLE I. Effect of Medium Mg^{2+} Concentration on Basal, ACTH-stimulated and NaF-stimulated Adrenal Adenylate Cyclase Activities.

Mg^{2+} con, m*M*	N[a]	Basal	ACTH-stimulated	NaF-stimulated
		adenylate cyclase activity, in nmoles cyclic AMP formed per mg protein/10 min		
1.25	(9)	0.045 ± 0.005[b]	0.88 ± 0.07 [×20.9 ± 2.0]	0.42 ± 0.10 [× 9.8 ± 2.2]
2.5	(20)	0.098 ± 0.008	2.34 ± 0.19 [×23.8 ± 1.2]	1.90 ± 0.17 [×19.6 ± 1.1]
10.0	(10)	0.146 ± 0.009	1.23 ± 0.09 [×8.4 ± 0.36]	2.12 ± 0.15 [×14.6 ± 0.5]

[a] N = number of homogenate pools.

[b] Means ± SE In brackets: factor whereby ACTH or NaF increased adenylate cyclase activity (means of results for individual homogenate pools ± SE). For assessment of the statistical significance of differences among values (*P* values in text), those in the same horizontal line were evaluated by the paired *t* test and those in the same vertical column by the simple *t* test.

Na^+ (8%) when Mg^{2+} was 10 m*M*. Interference by the monovalent cations with the adenylate cyclase response to ACTH (Fig. 1) showed a more consistent pattern. The inhibition in general increased as Mg^{2+} concentration decreased. Li^+ at 5 m*M* was ineffective. At 25 m*M*, Li^+ significantly interfered with ACTH at Mg^{2+} levels of 1.25 and 10 m*M*, and at 100 m*M*, at all concentrations of Mg^{2+} used. Na^+ was tested only at the concentration of 100 m*M*. The effects of 100 m*M* Li^+ vs. 100 m*M* Na^+ were compared[2] by plotting the log concentration of Mg^{2+} on the abscissa and the individual values for the depression of the ACTH effect on adrenal adenylate cyclase by 100 m*M* Li^+ and 100 m*M* Na^+ (means shown in Fig. 1) on the ordinate. The regression lines for the two cations in such a plot did not deviate significantly from parellelism, and the potency of Li^+ as a depressor of the ACTH effect was greater by a factor of 2.23 (95% fiducial limits 1.41–3.97) than that of Na^+. Thus, regardless of the concentration of Mg^{2+} (within the range tested), 100 m*M* Li^+ is more effective than equimolar Na^+ in diminishing the stimulatory action of ACTH on adrenal adenylate cyclase. K^+, with Mg^{2+} at 2.5 m*M*, had no effect at 5 and 25 m*M*, and interfered with ACTH action, but significantly ($P < 0.01$) less effectively than equimolar Li^+, at 100 m*M*.

Discussion. The rise in basal adrenal adenylate cyclase activity with increasing concentrations of Mg^{2+} in the medium (Table I) is similar to findings in fat cells (8), thyroid membranes (4), liver homogenates (9) and liver cell membranes (10). Mg^{2+} concentrations were not raised high enough to reveal possible depressing effects on basal adenylate cyclase activity similar to those caused by excess Mg^{2+} in some other tissues (3, 4, 10).

The usual observation (*e.g.* 2, 4, 11, 12) is that hormone-responsive adenylate cyclase is more markedly stimulated by NaF in broken-cell preparations than by the appropriate hormone. There are a few exceptions (8, 10, 15). In this study, ACTH had more than double the effect of NaF on adrenal adenylate cyclase activity at a Mg^{2+} concentration of 1.25 m*M* ($P < 0.01$) (Table I). The difference was slighter but still significantly in favor of ACTH at 2.5 m*M* Mg^{2+}, but when Mg^{2+} concentration was increased to 10 m*M*, NaF activation of adenylate cyclase exceeded that by ACTH ($P < 0.01$). Taunton *et al.* (11), in contrast, found in mouse adrenal tumor homogenate that for both ACTH- and NaF-stimulated adenylate cyclase activity the optimal Mg^{2+}:ATP ratio was 1.5. The curves of ACTH- and NaF-stimulated enzyme activity, plotted against the Mg^{2+}:ATP ratio, were parallel, and stimulation by NaF was always greater than that by ACTH.

In our study, Li^+, at concentrations of 25 m*M* and 100 m*M*, depressed basal adenylate cyclase when Mg^{2+} was 2.5 m*M*. The minimal or lack-

[2] This was kindly performed by Dr. Paul Leaverton, Professor of Biostatistics, University of Iowa.

ing depression of basal adenyl cyclase by Na^+ found in our system agrees with similar findings on a functional adrenal tumor of mice (11).

We found Li^+ to be the most potent inhibitor of the action of ACTH on adrenal adenylate cyclase among the univalent cations tested. However, the potency of Li^+ in this system was not as great as in the case of brain slices, in which stimulation of adenylate cyclase by norepinephrine was significantly less when as little as 1 m*M* Li^+ was added (7) (Mg^{2+}:3.3 m*M*), or as in the thyroid, where 50% inhibition of the thyrotropin (TSH) effect was achieved with only 4–8 m*M* Li^+ at 2.5 m*M* Mg^{2+} (3, 4). Diminution of the Li^+ effect at the highest (10 m*M*) Mg^{2+} concentration (Fig. 1) is consonant with findings on purified bovine thyroid membranes (4, 5).

The relative effectiveness of Li^+ and Na^+ with regard to their potency to inhibit the action of ACTH on adrenal adenylate cyclase was similar to that reported for the thyroid and TSH (4, 5). A slight interference of 25 m*M* Na^+ and a marked one of 125 m*M* Na^+ with the stimulation of fat cell adenylate cyclase by ACTH was observed by Birnbaumer *et al.* (8) (Mg^{2+}:5 m*M*). Wolff and Jones (4) and Burke (5) found enhancement of the effect of TSH on thyroid membrane adenylate cyclase by 100 m*M* K^+ (Mg^{2+}:5 m*M*). A similar increase of the ACTH action on fat cell adenylate cyclase by 10 m*M* K^+ at 5 m*M* Mg^{2+} has been described (8). We found that only 100 m*M* K^+ had an (inhibitory) influence on activation of adrenal adenylate cyclase by ACTH (Fig. 1). Taunton *et al.* (11) reported a more marked inhibition of the stimulation of this enzyme by ACTH in adrenal tumors at 125 m*M* K^+, but an inconstant potentiating effect of 5–15 m*M* K^+ (Mg^{2+}:2 m*M*).

We have thus found that none of the monovalent cations tested increased stimulation of adenylate cyclase activity by ACTH, and under appropriate conditions each of them depressed it. The effects of these cations were not entirely due to increase in ionic strength, since Li^+ was a more potent inhibitor than equimolar Na^+ or K^+, and raising ionic strength by elevating Mg^{2+} concentration blunted the effects of Li^{2+} and Na^+ instead of accentuating them. This is compatible with the suggestion by Wolff *et al.*, based on studies of thyroid adenylate cyclase (3), that Li^+ competes with Mg^{2+}. Na^+ appears to have a similar action, since a rise in Mg^{2+} concentration diminished its effect on the ACTH-adenylate cyclase interaction, as it did in the case of Li^+ (Fig. 1).

Summary. The basal activity of adenylate cyclase in homogenates of normal rat adrenals increased with the concentration of Mg^{2+} (1.25–10 m*M*) in the medium. The response of the enzyme to adrenocorticotropin (2×10^{-5}*M* synthetic β^{1-24}ACTH) and NaF (10 m*M*) was also determined by the concentration of Mg^{2+} in the incubation mixture: ACTH was more effective than NaF at low Mg^{2+} levels (1.25 and 2.5 m*M*) and less effective at 10 m*M* Mg^{2+}. Of the monovalent cations tested, Li^+ was more potent than Na^+ in inhibiting the response of adrenal adenylate cyclase to ACTH. These cation effects were generally greater when medium Mg^{2+} was lower. The lowest Li^+ concentration found to inhibit ACTH action significantly was 25 m*M*. K^+ did not enhance the effect of ACTH on adrenal adenylate cyclase at 5 or 25 m*M* and depressed it at 100 m*M* (Mg^{2+}:2.5 m*M*).

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