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EFFECTS OF CATIONS AND OUABAIN ON THYROID ADENYL CYCLASE

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SUMMARY

The effects of various cations and ouabain on basal thyroid adenyl cyclase activity and on its response to the stimulatory agents, thyroid-stimulating hormone (thyrotropin, TSH) and NaF, were investigated.

1. Basal or F^--induced enzyme activity was not modified by the addition of 0.1 M Na^+ or K^+. However, TSH-stimulated cyclase activity was inhibited ($49 \pm 8\%$) by 0.1 M Na^+ and augmented ($41 \pm 9\%$) by 0.1 M K^+. Simultaneous addition of 0.1 M Na^+ and 0.1 M K^+ to the incubation effected no change in TSH-induced cyclase activation.

2. Li^+ inhibits TSH-induced cyclase activation without affecting basal activity; in the presence of 2.5 mM Mg^{2+}, 50% inhibition occurs at 10–25 mM Li^+ and greater Li^+ concentrations are required with higher Mg^{2+} concentrations.

3. Ouabain, $1 \cdot 10^{-6}$–$1 \cdot 10^{-4}$ M abolished TSH activation of adenyl cyclase but did not alter NaF effects thereon. The degree of inhibition of TSH-induced adenyl cyclase activation produced by ouabain decreased with increasing concentrations of added K^+.

4. Among several divalent cations tested, only Mg^{2+} and Mn^{2+} (5 mM) augmented adenyl cyclase activity ($Mn^{2+} \gg Mg^{2+}$). The stimulatory effect of Mn^{2+} on basal and F^--stimulated activity was not observed in the presence of TSH. Other divalent cations (Ca^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}) markedly reduced or abolished basal, TSH- and F^--induced cyclase activation. None of the monovalent or divalent cation additions modified the assay ATP-regenerating system or phosphodiesterase activity.

5. Conclusions: (a) activity of thyroid adenyl cyclase is modified by cations and ouabain, (b) such alterations may be occasioned by ion binding to the enzyme, and, (c) TSH and F^- activate the same cyclase system but do so by different means.

INTRODUCTION

Adenyl cyclase is bound to membranes in several tissues and is though to be localized in the plasma membrane[1]. A relationship of the enzyme to ion transport

Abbreviation: TSH, thyroid-stimulating hormone (thyrotropin).

has been adduced from observations that hormone-stimulated lipolysis, a reflection of adenyl cyclase activity in fat cells[2], is modified by K^+ concentration and ouabain[3,4]. We have previously demonstrated[5–7] that changes in the ionic composition of the buffer medium modify thyroid slice metabolism, as well as adenyl cyclase activity in thyroid subcellular fractions subsequently prepared from these slices. In the present study, we examined the effects of monovalent and divalent cations, and of ouabain, added directly to the adenyl cyclase assay incubation mixture, on basal, TSH- and NaF-induced enzyme activation.

MATERIALS AND METHODS

Bovine TSH [B4, 2 U.S.P. units/mg] was a gift from the Endocrinology Study Section, National Institutes of Health. NaF was purchased from Matheson Coleman and Bell, and ouabain was obtained from CalBiochem.

[α-^{32}P]ATP, (3–5 C/mmole) was obtained from International Chemical and Nuclear Corp. 3′,5′-Cyclic AMP (cyclic AMP) and cyclic [^{3}H]AMP [1.4 C/mole] were purchased from Schwarz BioResearch and ATP from P-L Biochemicals. Pyruvate kinase and sodium phosphoenolpyruvate were purchased from Sigma.

Sheep thyroid glands were obtained from an abattoir. For adenyl cyclase assay, the glands were trimmed of fat and connective tissue and 300 mg wet weight of slices were homogenized in 1.0 ml of cold 0.25 M sucrose–1 mM EDTA in a Dounce homogenizer (Kontes). Adenyl cyclase activity was measured by a recently detailed method[8] using [α-^{32}P]ATP as substrate under conditions where ATP is maintained at a constant level by a regenerating system. The application of this method to thyroid tissue has been detailed elsewhere[9]. Final concentrations of reaction components under "basal" conditions in a total volume of 0.05 ml were: ATP, 0.1 mM; [α-^{32}P]ATP, 2 μC; theophylline, 8 mM; $MgCl_2$, 5 mM; Tris–HCl, 21 mM; pyruvate kinase, 50 μg/ml; sodium phosphoenolpyruvate, 16 mM; 0.08% albumin, and 2.5 mg/ml thyroid homogenate protein. The pH was 7.5. [α-^{32}P]ATP and cyclic [^{32}P]AMP formed therefrom were isolated by chromatography on precoated polyethyleneimine-impregnated cellulose thin-layer sheets (Brinkman). Only the total counts of the ATP spot and cyclie AMP spot were needed for calculating conversion rates[8,9]. The reproducibility of the values measured was 5% or better.

ATPase activity was assayed using [α-^{32}P]ATP under the same conditions as described above for adenyl cyclase assay. Aliquots of the incubation were co-chromatographed with carrier ATP, ADP and AMP on polyethyleneimine-cellulose thin-layer sheets in 1 M LiCl[8]. The ATP, ADP and AMP spots were cut out, and counted in scintillation vials containing standard toluene scintillation medium.

In determining phosphodiesterase activity, incubation conditions were identical to those described for the adenyl cyclase assay except that ATP, phosphoenolpyruvate and pyruvate kinase were omitted and each reaction mixture contained 104.5 pmoles (approx. 1760 counts/min) cyclic [^{3}H]AMP. The reactions were terminated by boiling for 3 min after the addition of 0.1 ml water. Then 0.4 ml of water was added, the precipitate removed by centrifugation, the supernatant made up to 3.0 ml, treated twice with $ZnSO_4$ and $Ba(OH)_2$, as described by PASTAN AND KATZEN[10] and the radioactivity of an aliquot of the supernatant measured. Since adenosine is not precipitated by treatment with Ba^{2+}–Zn^{2+} (ref. 11) and since

thyroid homogenate likely contains significant phosphatase activity which may convert 5'-AMP to adenosine, aliquots of the supernatant were cochromatographed with carrier cyclic AMP, 5'-AMP and adenosine on polyethyleneimine-cellulose thin-layer sheets in 0.3 M LiCl. This system effectively separates each of these compounds from the other[8]; the respective spots were cut out and counted. The results obtained did not differ significantly from those seen with the technique of PASTAN AND KATZEN[10].

Protein was assayed according to LOWRY *et al.*[12].

The data obtained in adenyl cyclase assays were evaluated by a 1-factorial, 1-nested analysis of variance[13].

RESULTS

Effects of monovalent cations

The effects of chloride salts of Li^+, Na^+ and K^+ on adenyl cyclase activity were determined in the presence or absence of 10 munits/ml TSH or 10 mM NaF. In a total of six experiments, none of the monovalent cations had any significant effect on basal activity. Li^+, at 0.1 M, inhibited F^-- and TSH-stimulated activity 26 ± 6 and $50\pm7\%$, respectively. Na^+ and K^+, at 0.1 M, had no significant effect on F^--stimulated activity ($3\pm11\%$ inhibition and $6\pm9\%$ augmentation, respectively). In contrast, TSH-stimulated cyclase activity was inhibited $49\pm8\%$ and augmented $41\pm9\%$, by 0.1 M concentrations of Na^+ and K^+, respectively. In the presence of both Na^+ and K^+, 0.1 M, basal and stimulated thyroid adenyl cyclase activity was unchanged from that observed with no monovalent cation additions. The results of these studies are depicted graphically in Fig. 1. When incubations were carried out in the presence of both Li^+ and K^+, 0.1 M, the inhibitory effects of Li^+

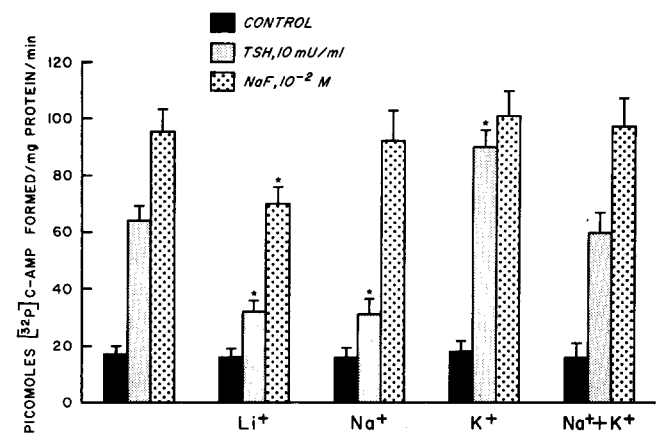

Fig. 1. Effects of monovalent cations on thyroid adenyl cyclase activity in the absence or presence of TSH (10 munits/ml) or NaF (10 mM). Thyroid homogenate was incubated for 5 min at 37° in the presence of an ATP-regenerating system (see MATERIALS AND METHODS). Monovalent cation additions were added at a final concentration of 0.1 M to the incubation medium. The data represent the mean ± S.E. (vertical bars) of six experiments (each experimental determination performed in triplicate). Asterisks indicate statistically significant ($P<0.01$) differences from corresponding effect under standard (no monovalent cation additions) conditions.

TABLE I

COMBINED EFFECTS OF Li^+ AND K^+ ON THYROID ADENYL CYCLASE

Results are mean ± S.E. of four experiments, each experimental determination performed in triplicate.

Additions	*pmoles cyclic [^{32}P]AMP formed/mg protein per min*		
	Basal	*TSH (10 munits/ml)*	*NaF (10 mM)*
None	16.6 ± 2.5	66.2 ± 7.3*	106.4 ± 9.1*
Li^+, 0.1 M	16.0 ± 2.1	31.9 ± 4.7‡	78.3 ± 8.6‡
K^+, 0.1 M	18.5 ± 3.9	98.7 ± 8.2**	99.3 ± 8.4*
Li^+, 0.1 M + K^+, 0.1 M	18.1 ± 2.7	39.6 ± 7.8‡	80.4 ± 8.3‡

* Significantly ($p < 0.01$) greater than basal.
‡ Significantly ($P < 0.01$) less than with no cation additions.
** Significantly ($P < 0.01$) greater than with no cation additions.

on stimulated adenyl cyclase activity persisted (Table I). In these and all other studies detailed herein, both TSH and NaF effects were studied in the same experiment.

During the course of these studies, WOLFF *et al.*[14] reported 50% inhibition of TSH-stimulated beef thyroid adenyl cyclase at Li^+ concentrations of 4–8 mM when the Mg^{2+} concentration in the incubation medium was 2.5 mM; greater Li^+ concentrations were required for inhibition as the Mg^{2+} concentration was raised. In addition, in their studies, Na^+ had a substantially lesser inhibitory effect on TSH-stimulated cyclase activity than Li^+ (ref. 14). Since the data reported in Fig. 1 (wherein the inhibitory effects of 0.1 M Li^+ and Na^+ on TSH-induced cyclase activation were of the same magnitude) were obtained in the presence of 5 mM Mg^{2+}, the inhibitory effects of Li^+ and Na^+ on TSH- and NaF-augmented adenyl cyclase

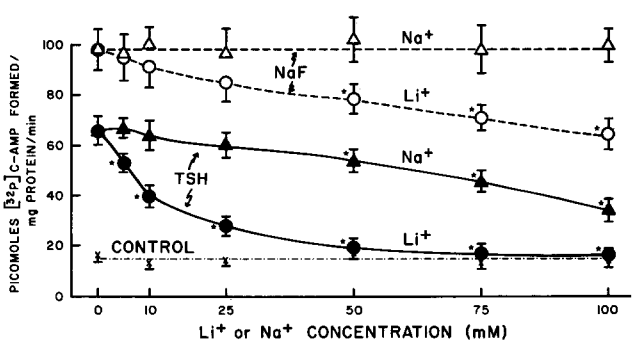

Fig. 2. Effect of Li^+ and Na^+ on thyroid adenyl cyclase at an incubation Mg^{2+} concentration of 2.5 mM. ○ and ● represent Li^+ additions; △ and ▲ are Na^+ additions. Solid lines represent TSH(10 munits/ml)-induced activation and broken lines are NaF (10 mM) experiments. Control = basal conditions without cation additions. Each point represents the mean ± S.E. (vertical bars) of four experiments (each experimental determination performed in triplicate); asterisks indicate statistically significant ($P < 0.05$–0.01) differences from corresponding effect under standard (no monovalent cation additions) conditions.

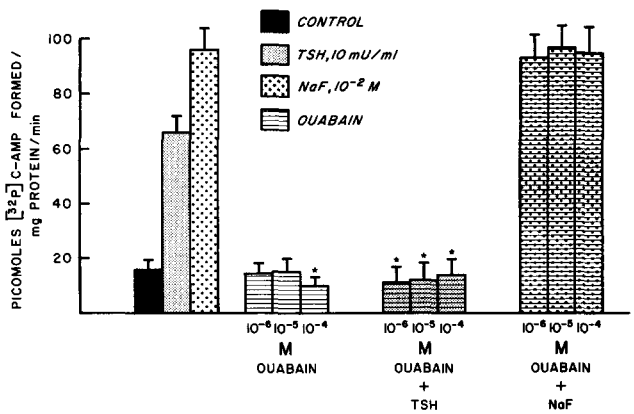

Fig. 3. Effect of ouabain on basal and stimulated adenyl cyclase activity. Incubation conditions were as described under MATERIALS AND METHODS; incubation time = 5 min. Results are mean ± S.E. (vertical bars) of six experiments (each experimental determination performed in triplicate). Asterisks indicate statistically significant ($P < 0.05$–0.01) differences from corresponding effect in absence of ouabain.

activity were compared at an incubation medium Mg^{2+} concentration of 2.5 mM. As can be seen from Fig. 2, under these conditions, 50% inhibition of TSH-induced cyclase activity was observed at 10–25 mM Li^+ but was seen only at 0.1 M Na^+. Maximum Li^+ inhibition (67%) was attained at 50 mM. In contrast to the effects on TSH-induced cyclase activity, Li^+ inhibition of NaF-induced cyclase activation was not appreciably greater when the incubation medium Mg^{2+} concentration was halved (Fig. 2).

Effects of ouabain

Ouabain, $1 \cdot 10^{-6}$–$1 \cdot 10^{-4}$ M, abolished TSH activation of thyroid adenyl cyclase but did not alter NaF effects thereon. Increasing the TSH concentration to

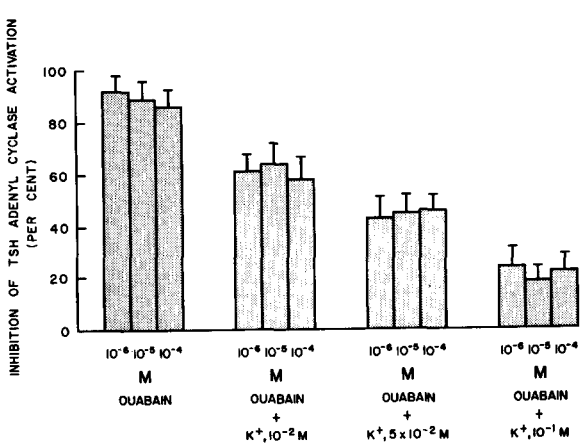

Fig. 4. Modification of ouabain-induced inhibition of TSH cyclase activation by added K^+. Incubation time = 5 min. Results are the mean ± S.E. (vertical bars) of four experiments each experimental determination performed in triplicate).

100 munits/ml did not reverse the inhibitory action of ouabain. Basal cyclase activity was only marginally reduced by ouabain, $1 \cdot 10^{-4}$ M. The results of six experiments are summarized in Fig. 3. As can be seen from Fig. 4, percent inhibition of TSH-induced adenyl cyclase activation produced by ouabain decreased with increasing concentrations of added K^+.

TABLE II

MODIFICATION OF LOW-DOSE OUABAIN-INDUCED INHIBITION OF TSH ADENYL CYCLASE ACTIVATION BY ADDED K^+

Results are mean ± S.E. of three experiments, each experimental determination performed in triplicate.

Ouabain concn. $\times 10^{-8}$ *(M)*	*% Inhibition of TSH adenyl cyclase activation*		
	–	$+5 \cdot 10^{-3}$ *M* K^+	$+1 \cdot 10^{-2}$ *M* K^+
50	70 ± 8	48 ± 5	39 ± 4
10	52 ± 6	26 ± 4	12 ± 3
5	40 ± 5	16 ± 3	0
1	27 ± 5	0	0

It should be noted, however, that, in these experiments, there was no evident relationship between the concentration of ouabain and the counteracting effects of increasing concentrations of K^+.

Because no such relationship was evident in these studies, a series of experiments with K^+ and ouabain was carried out at lower ouabain concentrations, *i.e.* where a dose-related inhibitory effect of ouabain on TSH-induced cyclase activation was demonstrable. Results of three such experiments are summarized in Table II. It can be seen that the lower the ouabain concentration, the more effective is the counteracting effect of K^+.

Although not detailed here, the inhibitory effect of ouabain was not altered when Na^+, 0.01–0.1 M, was added to the incubation medium.

TABLE III

COMPARATIVE EFFECTS OF Mg^{2+} AND Mn^{2+} ON BASAL AND STIMULATED THYROID ADENYL CYCLASE ACTIVITY

Results are the mean ± S.E. of three experiments; each experimental determination performed in triplicate.

Divalent cation (5 mM)	*pmoles cyclic [^{32}P]AMP formed/mg protein per min*			
	Basal	*TSH (10 munits/ml)*	*TSH (100 munits/ml)*	*NaF (10 mM)*
None	0.7 ± 0.4*	0.6 ± 0.5*	0.8 ± 0.6*	0.9 ± 0.4*
Mg^{2+}	17.3 ± 3.1‡	64.9 ± 7.6**	62.1 ± 8.2**	101.5 ± 8.6**
Mn^{2+}	25.9 ± 4.2‡	27.3 ± 4.8‡	26.7 ± 4.5‡	111.6 ± 12.2**

* Not significantly different from blank (no tissue or boiled tissue) values.
‡ Significantly ($P < 0.01$) greater than blank values.
** Significantly ($P < 0.01$) greater than basal values.

Effects of divalent cations

Since Mg^{2+} has been shown to be involved in the activation of adenyl cyclase[15], the effects of several other divalent cations were tested in the presence of Mg^{2+} (5 mM), *i.e.* under otherwise standard incubation conditions (virtually no enzyme activity, whether basal or with TSH or NaF, was observed in the absence of Mg^{2+} (ref. 10, Table III)). The results of five experiments are summarized in Fig. 5. Doubling the Mg^{2+} concentration increased basal adenyl cyclase activity but did

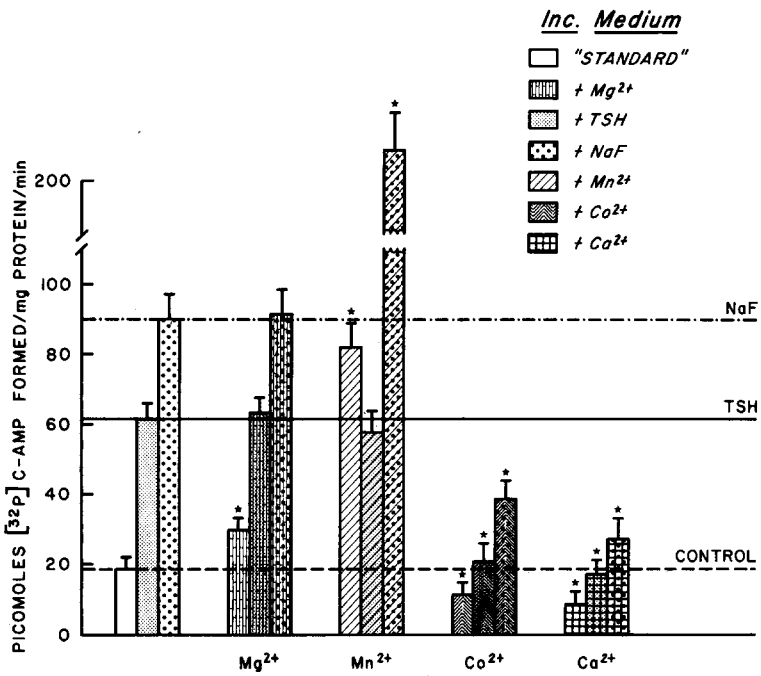

Fig. 5. Effects of divalent cations on thyroid adenyl cyclase activity in the absence or presence of TSH (10 munits/ml) or NaF (10 mM). Thyroid homogenate was incubated for 5 min at 37° in the presence of an ATP-regenerating system (see MATERIALS AND METHODS). Divalent cations were added at a final concentration of 5 mM to the incubation medium. Note that the effects of Mg^{2+} were measured at 5 and 10 mM. Horizontal lines represent basal (— — —), TSH (———) and F^- (—·—) activities measured in the presence of 5 mM $MgCl_2$ alone. Results are the mean ± S.E. (vertical bars) of six experiments (each experimental determination performed in triplicate). Asterisks indicate statistically significant ($P<0.05$–0.01) differences from corresponding effect under standard conditions (*i.e.* in presence of 5 mM $MgCl_2$ only).

not affect F^-- or TSH-stimulated activity. Mn^{2+}, at 5 mM, increased basal activity far more than did 10 mM Mg^{2+}. While this was also true for F^--stimulated activity, the combination of Mn^{2+} and TSH resulted in lesser cyclase activation than was obtained with Mn^{2+} alone. As can be seen from Table III, 5 mM Mn^{2+} alone (*i.e.* in the absence of Mg^{2+}) could support basal and F^--stimulated cyclase activity, but under these conditions no TSH effect could be demonstrated. The blank values (no tissue or boiled tissue) for cyclic [^{32}P]AMP formation in the presence of Mn^{2+} (0.5 ± 0.4 pmole cyclic [^{32}P]AMP formed/mg protein per min) were no higher than those obtained without divalent cation addition (0.7 ± 0.4 pmole) or in the presence

TABLE IV

LACK OF EFFECT OF CATIONS OR OUABAIN ON ATP-REGENERATING SYSTEM

Results are mean ± S.E. of six experiments, each experimental determination performed in triplicate.

*Additions**	*% of total radioactivity remaining as [α-^{32}P]ATP*		
	Basal	*TSH (10 munits/ml)*	*NaF (10 mM)*
None**	92 ± 5	89 ± 6	94 ± 4
Li^+	93 ± 6	91 ± 7	89 ± 5
Na^+	91 ± 4	93 ± 6	95 ± 7
K^+	94 ± 5	92 ± 6	93 ± 5
Mg^{2+}	91 ± 4	90 ± 5	91 ± 6
Mn^{2+}	90 ± 7	92 ± 5	91 ± 4
Co^{2+}	88 ± 6	91 ± 5	93 ± 7
Ca^{2+}	88 ± 7	90 ± 6	90 ± 5
Ouabain, 0.1 mM	92 ± 6	91 ± 4	94 ± 7

* Monovalent cation additions at 0.1 M; divalent cation additions, 5 mM.
** Standard incubation medium without cation additions.

of Mg^{2+} (0.7 ± 0.5 pmole) thus excluding the possibility of non-enzymatic cyclic AMP formation in the presence of Mn^{2+}.

Marked diminution in basal and stimulated adenyl cyclase activity was observed in the presence of either Co^{2+} or Ca^{2+} at 5 mM concentration. Although not detailed here, concentrations of added Ca^{2+} as low as 1.0 mM, caused a 40–60% reduction in cyclase activity. No significant adenyl cyclase activity was obtained in the presence of either Cu^{2+} or Zn^{2+} (5 mM).

In the absence of an ATP-regenerating system, <5% of total radioactivity was recovered as [α-^{32}P]ATP following a 1-min incubation period; with phosphoenol-

TABLE V

LACK OF EFFECT OF CATIONS OR OUABAIN ON CYCLIC-AMP HYDROLYSIS

Results are mean ± S.E. of four experiments, each experimental determination performed in triplicate.

*Additions**	*Cyclic [^{3}H]AMP hydrolyzed (pmoles/5 min)*		
	Basal	*TSH (10 munits/ml)*	*NaF (10 mM)*
None**	20.4 ± 1.2	19.1 ± 1.5	18.9 ± 1.3
Li^+	20.8 ± 1.4	19.9 ± 1.7	21.0 ± 1.8
Na^+	19.8 ± 1.6	20.3 ± 1.5	19.9 ± 1.9
K^+	20.6 ± 1.7	21.1 ± 1.9	20.6 ± 1.3
Mg^{2+}	21.1 ± 2.0	19.9 ± 1.7	20.1 ± 1.2
Mn^{2+}	19.8 ± 1.9	20.8 ± 1.4	20.7 ± 1.6
Co^{2+}	20.4 ± 1.3	20.0 ± 1.3	19.7 ± 1.9
Ca^{2+}	20.9 ± 1.1	20.9 ± 1.6	21.2 ± 1.8
Ouabain, 0.1 mM	20.7 ± 1.5	20.5 ± 1.4	19.7 ± 1.9

* Monovalent cations, 0.1 M; divalent cations, 5 mM.
** Standard incubation medium without cation additions.

pyruvate, 16 mM, and pyruvate kinase, 50 μg/ml, <10% breakdown of [α-^{32}P]ATP to [α-^{32}P]ADP and 5′-[^{32}P]AMP was seen during incubations up to 30 min[9]. In the absence of theophylline, >85% of cyclic [^{3}H]AMP was hydrolyzed to 5′-[^{3}H]AMP during a 5-min incubation with thyroid homogenate; in the presence of 8 mM theophylline in the incubation medium, <20% hydrolysis of cyclic [^{3}H]AMP occurred during incubation periods varying from 5 to 15 min (G. BURKE: unpublished data).

Neither ouabain, $1 \cdot 10^{-6}$–$1 \cdot 10^{-4}$ M, nor any of the monovalent or divalent cation additions (*i.e.* added to the "standard" 5 mM Mg^{2+}) modified the assay ATP-regenerating system or thyroid phosphodiesterase activity* (Tables IV, V).

DISCUSSION

Many, if not all, of the properties of the thyroid adenyl cyclase enzyme probably stem from its being a component of the plasma membrane[7,9]. A relationship of the enzyme to ion transport has been suggested by the observations that variations in the ionic composition of the buffer medium modify intermediary metabolism in thyroid slices[5,6] as well as the adenyl cyclase activity in subcellular fractions prepared therefrom[7]. Although the results of the present study, wherein the ionic composition of the adenyl cyclase assay medium was altered directly, support such a relationship, it may not be appropriate to consider membrane transport as a mediator of cation effects in a broken cell preparation. Thus, the stimulatory effects of K^+ or the inhibitory effects of Na^+, respectively, on TSH activation of adenyl cyclase could be related to ion binding to the enzyme rather than to ion transport. The lack of alteration of TSH-induced cyclase activation in the presence of Na^+ and K^+ combined further suggests that the two monovalent cations may be competitive in their actions on the hormone-activated adenyl cyclase system in thyroid membranes. In this regard it should be noted that WOLFF *et al.*[14] also reported Na^+-inhibition of TSH-induced cyclase activation and, in 3/4 experiments, K^+ augmentation of basal and TSH-stimulated adenyl cyclase activity. Related observations have been made in other tissues since LUNDHOLM *et al.*[18] reported that K^+ increased basal and adrenaline-induced cyclic AMP content in rat diaphragm.

The direct inhibitory effect of ouabain on TSH activation of thyroid adenyl cyclase and the reversal of this inhibition in the presence of added K^+ are compatible with either the transport or binding thesis, since it could be concluded from these observations that K^+ and ouabain compete for TSH-responsive cyclase receptor sites in the membrane. Analogous observations and conclusions have previously been recorded[19] in studies of ouabain and K^+ effects on kidney slice O_2 consumption.

Although, as noted earlier, there was no relationship in the adenyl cyclase experiments reported herein between high concentrations of ouabain (*i.e.* "supra-maximal" inhibitory concentrations, $1 \cdot 10^{-6}$–$1 \cdot 10^{-4}$ M) and the counteracting effects of increasing concentrations of K^+, such a relationship was evident at lower (dose-

* Although none of the cations tested affected thyroid phosphodiesterase activity, these studies were carried out in the presence of theophylline, a phosphodiesterase inhibitor. Although not directly relevant to the thrust of the present studies, it should be noted that in the absence of phosphodiesterase inhibitors such as theophylline, other workers have observed that rabbit brain[16] and dog heart[17] phosphodiesterase activity is markedly influenced by divalent cations.

related inhibitory) ouabain concentrations. The latter findings support the thesis that ouabain and K^+ are competitive in their effects on TSH-activated adenyl cyclase and militate against the possibility that ouabain is acting non-specifically on the TSH-stimulated component of the adenyl cyclase system. It should be noted, moreover, that the concentrations of K^+ necessary to counteract the inhibitory effects of ouabain on adenyl cyclase are of the same order of magnitude as those required with the (Na^+-K^+)-ATPase system in thyroid[20]. Although the precise relationships between adenyl cyclase activation and augmentation of thyroidal intermediary metabolism remain to be defined[7], ouabain has also been shown to inhibit TSH effects on thyroid slice glucose oxidation (ref. 21 and G. BURKE: unpublished data) and phospholipogenesis[22].

Since the detergent, Lubrol-PX, has recently been shown[23] to effect solubilization of myocardial adenyl cyclase with loss of hormone but not of NaF responsiveness, the possibility arises that ouabain might exert a non-specific detergent-like effect on thyroid adenyl cyclase. Preliminary studies in our laboratory with this compound as well as digitonin, a neutral detergent having some structural similarity to ouabain, militate against this possibility too, however. Thus, homogenization of thyroid tissue with 0.02 M Lubrol-PX or addition of digitonin, $1 \cdot 10^{-6}$–$1 \cdot 10^{-4}$ M, to thyroid homogenate resulted in abolition of basal as well as TSH- and F^--induced cyclase activity (G. BURKE: unpublished data) in contrast to the selective *(vide infra)* inhibition of hormonal cyclase activation by ouabain.

In contrast to the findings of HO *et al.*[24] in adipose cells, ouabain was without effect on F^--induced cyclase activation in thyroid homogenate. We have recently presented data[9] which indicate that while the adenyl cyclase systems in thyroid that respond to TSH and NaF have many many characteristics in common, the sites on the enzyme at which these compounds interact to elicit their effects are distinctly different. (Similar conclusions have been drawn from studies in other laboratories[25,26].) The divergent effects of ouabain, added Na^+, K^+ or Mn^{2+} on enzyme activation induced by F^- and TSH, respectively, in the present study, support this premise.

Li^+, which inhibited both F^-- and TSH-stimulated adenyl cyclase activity, albeit to a considerably greater extent in the latter instance, appears to interfere with thyroid hormone synthesis and/or release in man[27]. WOLFF *et al.*[14] recently reported that Li^+ administration to rats acutely blocks several steps in thyroidal iodine metabolism and that Li^+ inhibits TSH-induced stimulation of adenyl cyclase activity in beef thyroid membranes without affecting basal activity. Although, in our studies, in the presence of 5 mM Mg^{2+}, Na^+ and Li^+ (0.1 M) were equally effective inhibitors of TSH-induced cyclase activation, when the Mg^{2+} concentration was halved, Li^+ inhibition was considerably greater than that achieved with Na^+ (Fig. 2). Thus, although TSH-activated beef thyroid adenyl cyclase appears to be more sensitive to the inhibitory effects of Li^+ (ref. 14) than that of sheep thyroid, our findings (including the lack of K^+ effect on Li^+ inhibition) support the conclusion of WOLFF *et al.*[14] that the Li^+ effect is not merely one of ionic strength. In addition, our data confirm the observations of these workers that the Mg^{2+} concentration influences the ability of Li^+ to block TSH-induced adenyl cyclase activation[14]. The lack of effect of Li^+ on the ATP-regenerating system employed in the adenyl cyclase assay (Table IV) also parallels the findings of WOLFF *et al.*[14] and supports their

contention that Li^+ acts on Mg^{2+} bound to cyclase rather than that bound to ATP.

A Ca^{2+} dependency has been reported for ACTH-stimulated adenyl cyclase activity in fat cells[28,29] and in adrenal cortex[29]. The present study reveals that Ca^{2+}, at concentrations as low as 1.0 mM, inhibits both TSH- and F^--stimulated adenyl cyclase activity. Since a divalent cation chelator, EDTA, is routinely used in the preparation of thyroid homogenates for adenyl cyclase assay[9], it would appear that TSH does not require even tracer amounts of Ca^{2+} for interaction with its thyroid receptor.

Although thyroid adenyl cyclase activation is dependent on Mg^{2+} (ref. 10) and, as has been found by others[14,28], basal adenyl cyclase activity is increased by raising Mg^{2+} concentration in the medium, neither TSH- nor F^--induced enzyme activation was enhanced under these conditions. Mn^{2+}, on the other hand, could replace Mg^{2+} in supporting basal and F^--induced thyroid adenyl cyclase activity. SUTHERLAND *et al.*[15] reported similar findings in brain preparations.

In the presence of Mg^{2+}, the addition of 5 mM Mn^{2+} greatly augmented basal and NaF-induced enzyme activation (Fig. 5); however, Mn^{2+} could not support TSH activation of thyroid adenyl cyclase (Table III) and in the combined presence of 5 mM Mg^{2+} and Mn^{2+}, the addition of TSH to the medium actually caused a diminution in adenyl cyclase activity (Fig. 5). Although these findings suggest that TSH and Mn^{2+} compete for a Mg^{2+}–adenyl cyclase receptor site, the present studies permit no definitive conclusions. The physiologic significance of the inhibitory effects of other divalent cations (Ca^{2+}, Co^{2+}, Zn^{2+}) on basal and stimulated enzyme activity is not presently known. Studies are currently in progress to determine whether these divalent cations compete with Mg^{2+}, a cofactor of the enzyme[15]. NAMM *et al.*[30] reported that excess Ca^{2+} decreased the formation of cyclic AMP in rat heart and speculated that inhibition of cardiac adenyl cyclase by this cation might account for the decrease in cyclic nucleotide.

In summary, thyroid adenyl cyclase, a membrane-bound enzyme, is subject to alterations in activity by monovalent and divalent cations; such alterations may be a consequence of ion binding to the enzyme. TSH and F^- activate the same enzyme system but do so by different means.

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