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CATECHOL, A STRUCTURAL REQUIREMENT FOR ($Na^+ + K^+$)-ATPase STIMULATION IN RAT SKELETAL MUSCLE MEMBRANE

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Summary

1. Catecholamines can nearly double ($Na^+ + K^+$)-ATPase activity of a sarcolemma fraction from skeletal muscle.

2. This effect is not mediated by cyclic AMP and is not β-adrenergic.

3. Orthodihydroxybenzene compounds and their orthoquinone derivatives enhance ($Na^+ + K^+$)-ATPase activity.

4. Enhancement of ($Na^+ + K^+$)-ATPase activity by catechols is not due to increased availability of ATP.

5. It is suggested that catechols and their orthoquinones somehow alter or protect the configuration of the enzyme so that it becomes more active or so that its activity is maintained under conditions in which its activity is otherwise diminished.

Introduction

The hypothesis that led to the studies reported herein seems to have little to do with the results.

We were led to these studies by reports by Gardner and Aurbach and colleagues [1,2] that β-adrenergic catecholamines increased ^{24}Na and ^{42}K fluxes into and out of turkey erythrocytes. They concluded that these effects were specific for β-adrenergic substances and were secondary to activation of adenylate cyclase. Some, but not all, of the effects they observed are consistent with the postulate that there was activation of ($Na^+ + K^+$)-ATPase (EC 3.6.1.3) and

Abbreviations: 2-Hydroestriol, 1,3,5-(10)-estratrien-2,3,16α,17β-tetrol; 2-hydroxyestradiol, 1,3,5-(10)-estratrien-2,3,17β-triol-17-acetate; EGTA, ethyleneglycol-bis(β-aminoethylether)-*N,N'*tetraacetic acid; L-DOPA, L-β-3,4,-dihydroxyphenylalanine; dibutyryl cyclic AMP, $N^6,O^{2'}$-dibutyryl adenosine 3′ : 5′-cyclic monophosphoric acid; cyclic AMP, adenosine 3′ : 5′-cyclic monophosphoric acid.

its Na^+-K^+ transport function. Since cyclic AMP stimulates some phosphokinases and since the phosphoenzyme form of the ATPase is an intermediate in the (Na^+ + K^+)-ATPase reaction, it occurred to us that the increased ^{24}Na efflux and ^{42}K influx observed by Gardner, Aurbach et al. [1,2] in response to β-adrenergic agents might have been due to increased (Na^+ + K^+)-ATPase activity, which in turn might have occurred as a result of increased phosphorylation of the ATPase.

We found no evidence to support these conjectures, but we did find that (Na^+ + K^+)-ATPase activity was enhanced by catecholamines. The effect was not β-adrenergic.

Since 1970 there have been a number of reports of binding of catecholamines and related dihydroxybenzenes to various biomembrane preparations [3—8]. When we found that enhanced (Na^+ + K^+)-ATPase activity produced by L-isoproterenol was not a β-adrenergic effect we then examined the possibility, which proved true, that the effect might be produced by those dihydroxybenzenes that bind to cell membranes.

Materials and Methods

D-Isoproterenol-D-bitartrate and D-epinephrine-L-bitartrate were obtained from Winthrop Laboratories, New York, N.Y. 2-Hydroxyestradiol and 2-hydroestriol were purchased from Steraloids, Inc., Pawling, N.Y. All other chemicals, including L-isoproterenol-D-bitartrate, L-epinephrine bitartrate, and the sodium salts of cyclic AMP and dibutyryl cyclic AMP were purchased from Sigma Chemical Co., St. Louis, Mo.

Membrane fractions enriched in sarcolemma were prepared by a modification of the method of Schapira et al. [9]. Subsequent to dissection, all procedures were at 4—5°C. All sucrose solutions were in 1 mM Tris, pH 7.4, adjusted at 22°C. Male Sprague-Dawley rats (from Charles River Breeding Laboratories, Wilmington, Mass.) weighing 150—200 g were killed by cervical fracture. Muscle from the hind limbs was put into four volumes of cold 0.25 M sucrose, minced and homogenized for 15 s with a Brinkman Polytron PT 10 homogenizer set at maximum speed. The homogenate was filtered through one layer of gauze and the filtrate centrifuged at 2000 × *g* for 10 min. For extraction of contractile proteins the sediment was suspended in 10 volumes (per initial muscle wet weight) of 0.5 M LiBr containing 0.05 mM EGTA and 10 mM Tris, pH 8.5, and magnetically stirred overnight, 12—16 h. The suspension was centrifuged at 2500 × *g* for 15 min; the sediment was discarded and the supernatant centrifuged at 150 000 × *g* for 30 min. The high speed pellet was suspended in three volumes (per initial muscle weight) 0.6 M KCl containing 10 mM Tris, pH 8.0, with 20 strokes in a glass homogenizer, allowed to sit for 10—20 min, and centrifuged at 5000 × *g* for 15 min. The supernatant was centrifuged at 150 000 × *g* for 30 min. The high speed pellet was suspended in 8.5% (w/v) sucrose and layered on top of five-step discontinuous 30—50% sucrose gradients. Gradient tubes were centrifuged at 100 000 × *g* for 2 h. The sarcolemma-enriched fraction was collected at the 8.5—30% sucrose interface, pelleted at high speed, resuspended in 8.5% sucrose and stored at −20°C for at least 1 week. Some

fractions were used over a period of several months with repeated freezing and thawing.

$(Na^+ + K^+)$-ATPase activity was measured, with slight modification, by the method of Schimmel et al. [10]. Membrane suspensions (2—4 μg protein) were incubated in 0.25 ml of a solution containing 30 mM imidazole buffer, pH 7.5, 0.5 mM EGTA, 3 mM ATP (disodium salt), 3 mM $MgCl_2$, 110 mM NaCl, and 15 mM KCl. Duration and temperature of incubation were varied as indicated in Results. The reaction was stopped by addition of an equal volume of 10% $HClO_4$. Inorganic phosphate concentration was determined as described by Bonting et al. [11]. $(Na^+ + K^+)$-ATPase activity was calculated by either or two methods: (a) as the difference between P_i released in the above solution and in paired assays without Na^+ and K^+; or (b) as the ouabain-sensitive component, measured as the decrement in P_i released in paired assays when 1 mM ouabain (as the octahydrate) was added to the system. All determinations were in duplicate.

Protein concentration was determined by the method of Lowry et al. [12], with bovine serum albumin as standard, and with both membranes and albumin diluted in 8.5% sucrose, 1 mM Tris, pH 7.4.

Results

$(Na^+ + K^+)$-ATPase activity of sarcolemma. At 37°C, after 1 h of incubation, $(Na^+ + K^+)$-ATPase activity was 40 μmol P_i released/mg membrane protein, about 2—10 times the rate for skeletal muscle reported by others [9,10,13,14]. Activity was the same whether measured as the increment in ATPase activity upon addition of Na^+ and K^+ to the incubation mixture used for assay of basal Mg^{2+}-ATPase activity or measured as the decrement in ATPase activity upon addition of ouabain, 1 mM, to the full (Na^+, K^+, Mg^{2+})-ATPase incubation mixture (Table I). Since the results were the same and it was simpler to determine activity on the basis of ouabain inhibition, we used the latter method routinely.

Effect of L-isoproterenol. At 37°C in four experiments, 10^{-5} M L-isoproterenol increased $(Na^+ + K^+)$-ATPase activity in 15 min by 7 ± 1.4% (S.E.), a small but significant effect by *t*-test ($P < 0.01$). By 60 min enhancement of

TABLE I

COMPARISON OF OUABAIN-SENSITIVE WITH $(Na^+ + K^+)$-ACTIVATED ATPase

$(Na^+ + K^+)$-ATPase activity was calculated as the ouabain-sensitive portion of ATPase in the presence of Na^+, K^+, and Mg^{2+}, or as $(Na^+ + K^+)$-activated ATPase incremented upon Mg^{2+}-ATPase. Ouabain concentration, 1 mM. Values (±S.E.) are given as percent of total (Na^+, K^+, Mg^{2+})-ATPase. Each row represents values percent of total ATPase activity obtained from a single preparation assayed on different days, the number of which is in parentheses.

(A) Total ATPase	(B) Mg^{2+}-ATPase	(C) Total ATPase + ouabain	(A)-(B) $(Na^+ + K^+)$-ATPase	(A)-(C) Ouabain-sensitive ATPase
100	40 ± 0.5 (3)	41 ± 0.7 (5)	60 ± 0.5 (3)	59 ± 0.7 (5)
100	40 ± 1.3 (2)	40 ± 5.0 (7)	60 ± 1.3 (2)	60 ± 5.0 (7)

cumulative (Na^+ + K^+)-ATPase activity by L-isoproterenol was nearly 30% (Fig. 1). The effect of L-isoproterenol was increased still further by raising the temperature of the assay mixture to 50°C. At the end of 1 h at 50°C, 10^{-5} M L-isoproterenol increased cumulative (Na^+ + K^+)-ATPase activity by 93% above control.

The ATPase reaction rate decreased with time, an effect particularly evident at 50°C. L-Isoproterenol reduced this time-dependent rate loss, suggesting that its action might be to protect the enzyme.

Under none of these conditions did L-isoproterenol affect the basal Mg^{2+}-ATPase activity or the portion of (Na^+ + K^+)-ATPase activity not inhibited by ouabain.

On the premise that preincubation of membranes with Na^+, Mg^{2+} and ATP, but in the absence of K^+, might lead to accumulation of a phosphoenzyme, membranes were so preincubated for 10 min and further incubated for 1 h at 37°C after addition of K^+. (Na^+ + K^+)-ATPase activity was increased by L-isoproterenol, 10^{-4} M, to 70% above control (Fig. 2). However, the control (Na^+ + K^+)-ATPase activity was reduced from 43 to 35 $\mu mol \cdot mg^{-1} \cdot h^{-1}$ by preincubation. In the presence of 10^{-4} M L-isoproterenol, (Na^+ + K^+)-ATPase activity was 61 $\mu mol \cdot mg^{-1} \cdot h^{-1}$ without preincubation, and 59 $\mu mol \cdot mg^{-1} \cdot h^{-1}$ with preincubation. These results suggest that no accumulation of phospho-

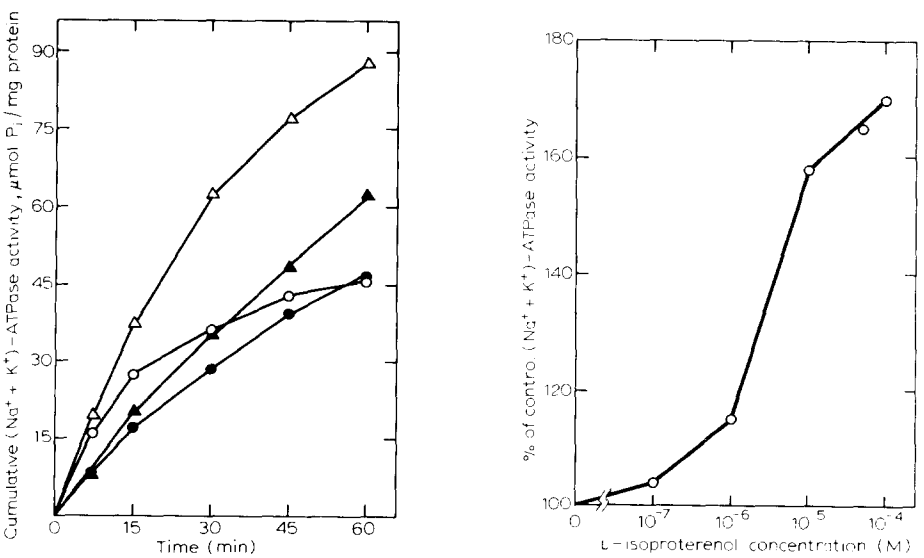

Fig. 1. Time courses of cumulative (Na^+ + K^+)-ATPase activity in a representative experiment. The reaction mixture as described in Materials and Methods was incubated in the presence and absence of 10^{-5} M L-isoproterenol. At 37°C, closed circle (●) and closed triangle (▲) represent absence and presence of L-isoproterenol, respectively. At 50°C, open circle (○) and open triangle (△) represent absence and presence of L-isoproterenol, respectively.

Fig. 2. Effect of L-isoproterenol on (Na^+ + K^+)-ATPase activity of rat skeletal muscle plasma membrane in a representative experiment. Membrane suspensions (2—4 μg of protein) were preincubated in assay medium containing 30 mM imidazole buffer, pH 7.5, 0.5 mM EGTA, 3 mM ATP, 3 mM $HgCl_2$, 110 mM NaCl and various concentrations of L-isoproterenol as indicated for 10 min at 37°C. KCl was added to a final concentration of 15 mM. Incubation was continued for an additional 60 min at 37°C.

enzyme occurred during preincubation of membrane with isoproterenol. They also suggest that isoproterenol may act by protecting the enzyme from partial deterioration during incubation. With preincubation, stimulation of $(Na^+ + K^+)$-ATPase activity was detectable with 10^{-6} M L-isoproterenol, but not consistently with 10^{-7} M.

Sarcolemma $(Na^+ + K^+)$-ATPase enhancement by L-isoproterenol not a β-adrenergic effect, and not mediated by cyclic AMP. The β-adrenergic blocking agents, propranolol and dichloroisoproterenol, had no effect on the enhancement of $(Na^+ + K^+)$-ATPase activity produced by L-isoproterenol. Table II illustrates results with propranolol. In the absence of L-isoproterenol, propranolol did not affect basal $(Na^+ + K^+)$-ATPase activity until high concentrations of the blocker were used. At 10^{-3} M, propranolol reduced $(Na^+ + K^+)$-ATPase 26 ± 1.3%, but still did not block enhancement of the ATPase activity produced by L-isoproterenol (Table II).

Neither cyclic AMP nor dibutyryl cyclic AMP enhanced $(Na^+ + K^+)$-ATPase activity (Table III).

Orthodihydroxybenzene structure required for enhancement of $(Na^+ + K^+)$-ATPase activity (Table IV). D-Isoproterenol was as active as L-isoproterenol in enhancement of $(Na^+ + K^+)$-ATPase activity. Furthermore, all substances with an orthodihydroxybenzene structure, including pyrocatechol, were active. Substitution for one of the hydroxyls destroyed activity as in the case of 4-hydroxy-3-methoxymandelic acid. The metadihydroxy analog, resorcinol, was inactive. The aromatic structure was required; ethylene glycol was inactive. Substances with multiple ring structures could not be shown to be active, as in the case of two estrogens (2-hydroestriol and 2-hydroxyestradiol).

The α-adrenergic agonist, phenylephrine, was inactive.

Oxidized catecholamines enhance $(Na^+ + K^+)$-ATPase activity, probably not by formation of Schiff bases. Solutions of epinephrine and of isoproterenol in 100 mM imidazole buffer, pH 7.0, were exposed to air at room temperature for 24—72 h. Under these conditions the orthodihydroxy structure became a minor component. The major component was probably the orthoquinone, as indicated by fluorescence spectroscopy. The oxidized forms were as active as the original catecholamine in enhancement of $(Na^+ + K^+)$-ATPase activity (Table V).

TABLE II

LACK OF EFFECT OF PROPRANOLOL ON L-ISOPROTERENOL ENHANCEMENT OF $(Na^+ + K^+)$-ATPase ACTIVITY

The reaction mixture as described in Materials and Methods was incubated at 37°C for 1 h in the presence of 10^{-5} M L-isoproterenol and various concentrations of propranolol as indicated. Results are presented as percent of $(Na^+ + K^+)$-ATPase activity in the absence of isoproterenol, but in the presence of the indicated concentration of propranolol. Each value represents the mean of five preparations ±S.E.

Propanolol concentration (M)	Percent of activity without L-isoproterenol
0	124 ± 2.0
10^{-6}	123 ± 2.9
10^{-5}	119 ± 2.2
10^{-4}	122 ± 2.4
10^{-3}	119 ± 3.0

TABLE III

LACK OF EFFECT OF CYCLIC AMP AND DIBUTYRYL CYCLIC AMP ON $(Na^+ + K^+)$-ATPase ACTIVITY

The reaction mixture as described in Materials and Methods was incubated at 37° C for 1 h in the presence of various concentrations of cyclic AMP and dibutyryl cyclic AMP as indicated. Results are presented as percent of control $(Na^+ + K^+)$-ATPase activity. Each value represents the mean of four preparations ±S.E.

	$(Na^+ + K^+)$-ATPase Activity (percent of control)
Cyclic AMP (10^{-4} M)	103 ± 2.2
Cyclic AMP (10^{-3} M)	103 ± 1.4
Dibutyryl cyclic AMP (10^{-4} M)	101 ± 2.1
Dibutyryl cyclic AMP (10^{-3} M)	99 ± 2.5

It was suggested to us by Dr. Paul Talalay that the orthoquinones might be the active agents and that they might exert their effects by formation of Schiff bases with the enzyme. [^{14}C]Epinephrine was oxidized by exposure to 95% O_2 and 5% CO_2 for 1 h. The product was incubated with membrane preparations and binding of [^{14}C]epinephrine to membrane was measured by a Millipore filtration method. Protein-bound radioactivity was not increased by treatment with $NaBH_4$. Hence there was no evidence that there was Schiff base formation between protein and epinephrine.

Effect of ATP concentration. If the $(Na^+ + K^+)$-ATPase penetrates the full thickness of the membrane and if ouabain binds to the ATPase at its outer surface and if the site of ATP association is at the inner surface, then, if membrane vesicles in our preparation are oriented normally, ATP must be translocated to the interior of the vesicles, or, if the membrane vesicles are inverted, ouabain must be translocated to the interior.

TABLE IV

EFFECT OF VARIOUS AGENTS ON $(Na^+ + K^+)$-ATPase ACTIVITY

The agents, $5 \cdot 10^{-5}$ M, except where indicated otherwise, were incubated with the assay mixture for 1 h at 37° C. Each value represents the mean (±S.E.) of three preparations.

	Percent of control
L-Isoproterenol	131 ± 5
D-Isoproterenol	132 ± 6
L-Epinephrine	131 ± 6
D-Epinephrine	131 ± 4
L-DOPA	130 ± 7
DL-Dihydroxymandelic acid	126 ± 5
Pyrocatechol *	122 ± 4
DL-4-hydroxy, 3-methoxymandelic acid	102 ± 1
Resorcinol	103 ± 1
Tyrosine	99 ± 0.5
Phenylalanine	101 ± 2
Phenylephrine	100 ± 2
Ethylene glycol	99 ± 4
2-Hydroxyestradiol **	105 ± 3
2-Hydroestriol **	102 ± 2

* Six preparations

** Five preparations, concentration $5 \cdot 10^{-6}$ M.

TABLE V

COMPARISON OF THE EFFECT OF L-ISOPROTERENOL AND OXIDIZED L-ISOPROTERENOL ON $(Na^+ + K^+)$-ATPase ACTIVITY

The reaction mixture as described in Materials and Methods was incubated at 37 or 50°C for 1 h in the presence of various concentrations of L-isoproterenol as indicated. Results are presented as percent of control $(Na^+ + K^+)$-ATPase activity. Each value represents the mean of five preparations ±S.E.

	$(Na^+ + K^+)$-ATPase activity (percent of control)	
	37°C	50°C
L-Isoproterenol		
$1 \cdot 10^{-6}$ M	109 ± 1.2	—
$1 \cdot 10^{-5}$ M	123 ± 2.5	167 ± 5.4
Oxidized L-isoproterenol		
$1 \cdot 10^{-6}$ M	111 ± 1.7	—
$1 \cdot 10^{-5}$ M	124 ± 4.0	174 ± 9.3

We have no strong evidence that either orientation predominates in our vesicle preparations. However, they do bind insulin and ouabain. Hence it is likely that at least some of the vesicles are oriented normally. If this is so, then ATP, to which normal plasma membranes are relatively impermeable, must cross the wall of the vesicle. It was conceivable that catecholamines might exert their effect by increasing the rate at which ATP enters the interior of the vesicles.

Accordingly, experiments were performed in which ATP concentration was varied in the presence and absence of isoproterenol. $(Na^+ + K^+)$-ATPase activity increased with increasing ATP concentration to a maximum from which it de-

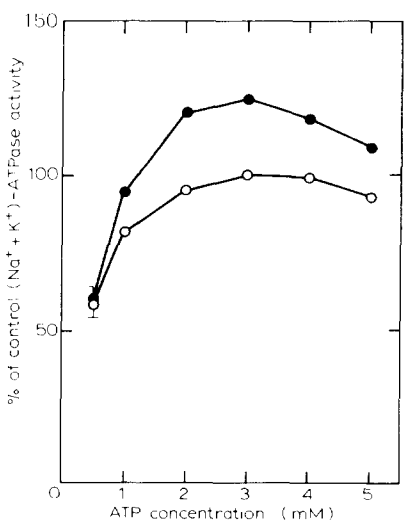

Fig. 3. Effect of ATP concentration. The reaction mixture as described in Materials and Methods and various ATP concentrations as indicated were incubated for 1 h at 37°C in the presence (●———●) or absence (○———○) of 10^{-5} M L-isoproterenol. Each point represents the mean from three preparations. The vertical lines show the S.E. when it exceeds the size of the symbols. The activity of $(Na^+ + K^+)$-ATPase at 3 mM ATP in the absence of L-isoproterenol was taken as 100%.

clined with further increases in ATP concentration. If isoproterenol increases $(Na^+ + K^+)$-ATPase activity by making more ATP available to the vesicle interior, the curve of activity vs. ATP concentration should simply be shifted to the left (toward lower ATP concentration) with no increase in maximum. In fact, however, in the presence of isoproterenol $(Na^+ + K^+)$-ATPase activity was increased at all but the smallest concentration of ATP and there was no shift to the left (Fig. 3). Thus, enhancement of $(Na^+ + K^+)$-ATPase activity by catechols was not due to increased availability of ATP.

Discussion

The effect of β-adrenergic agonists on $(Na^+ + K^+)$-ATPase activity or on Na^+ pumping out of cells may be different for different tissues. We noted in Introduction that Aurbach and colleagues [1,2] found β-adrenergic stimulation of Na^+ efflux from turkey erythrocytes, probably mediated by cyclic AMP. $(Na^+ + K^+)$-ATPase was not assayed in their studies, but some aspects of the results suggest that increased Na^+ efflux may have been due in part to enhanced activity of this enzyme.

A similar effect may occur in the heart. Hegyvary [15] reported that when propranolol was administered to rats for 6 days, myocardial $(Na^+ + K^+)$-ATPase activity was then increased by cyclic AMP.

On the other hand, Luly et al. [16] found that epinephrine, glucagon, or cyclic AMP each reduced $(Na^+ + K^+)$-ATPase activity in preparations of liver.

Our own studies, reported herein, demonstrate enhanced $(Na^+ + K^+)$-ATPase activity in sarcolemma in response to catechols, or their orthoquinones, not mediated by cyclic AMP, and not specifically β-adrenergic.

Although the response of $(Na^+ + K^+)$-ATPase to catechols is far less sensitive and less specific than is typical of β-adrenergic responses, it does require a certain molecular structure, reminiscent of the structures for which binding to membrane preparations has been reported [3—8].

It is now generally agreed that binding of ^3H-labeled catecholamines to membranes does not titrate true β-adrenergic sites. All substances having the catechol ring, whether D- or L-isomer, can inhibit ^3H-labeled catecholamine binding, and affinity of most ^3H-labeled catecholamine binding sites for β-adrenergic antagonists is relatively low [3—8]. Pairault and Laudat [17] performed binding studies in the presence of high concentrations of pyrocatechol, which appeared to block 98% of the binding of [^3H]norepinephrine. The small residual amount of bound norepinephrine was displaced by low concentrations of propranolol. It seems likely that catecholamines can bind to heterogeneous sites on the plasma membrane. No functional effects of this catechol binding have been substantiated. It appears from our study that one of those binding sites is, or is closely related to, the $(Na^+ + K^+)$-ATPase.

We do not know how this binding of catechols to preparations of muscle membrane causes increased $(Na^+ + K^+)$-ATPase activity. We have demonstrated that it is not a β-adrenergic effect, not mediated by cyclic AMP, not due to increased availability of ATP, and probably not due to formation of Schiff bases or to reduction of enzyme by catechols. We seem to be left with the possibility that catechols and their orthoquinones somehow alter or protect the

configuration of the enzyme so that, in the membrane, it becomes more active or remains active.

The question arose as to whether those substances that enhance ($Na^+ + K^+$)-ATPase activity also enhance Na^+ pumping out of muscle. Accordingly in experiments reported elsewhere [18], we examined the effect of catecholamines on Na^+ content of intact rat muscle. We found a sensitive and specific β-adrenergic stimulation of net outward Na^+ movement and net inward K^+ movement, probably mediated by cyclic AMP.

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References

1 Gardner, J.D., Klaeveman, H.L., Bilezikian, J.P. and Aurbach, G.D. (1973) J. Biol. Chem. 248, 5590–5597
2 Gardner, J.D., Mensh, R.S., Kiino, D.R. and Aurbach, G.D. (1975) J. Biol. Chem. 250, 1155–1163
3 Tomasi, V., Koretz, S., Ray, T.K., Dunnick, J. and Marinetti, G.V. (1970) Biochim. Biophys. Acta 211, 31–42
4 Lefkowitz, R.J. and Haber, E. (1971) Proc. Natl. Acad. Sci. U.S. 68, 1773–1777
5 Schramm, M., Feinstein, H., Naim, E., Long, M. and Lasser, M. (1972) Proc. Natl. Acad. Sci. U.S. 69, 523–527
6 Bilezikian, J.P. and Aurbach, G.D. (1973) J. Biol. Chem. 248, 5577–5583
7 Cuatrecasas, P., Tell, G.P.E., Sica, V., Parikh, I. and Chang, K.J. (1974) Nature 247, 92–97
8 Marinetti, G.V., Lesko, L. and Koretz, S. (1974) Perspectives in Membrane Biology (Gitler, S., ed.), pp. 455–493, Academic Press, New York
9 Schapira, G., Dobocz, I., Piau, J.P. and Delain, E. (1974) Biochim. Biophys. Acta 345, 348–358
10 Schimmel, S.D., Kent, C., Bischoff, R. and Vagelos, P.R. (1973) Proc. Natl. Acad. Sci. U.S. 70, 3195–3199
11 Bonting, S.L., Simon, K.A. and Hawkins, N.M. (1961) Arch. Biochem. Biophys. 95, 416–423
12 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
13 Andrew, C.G. and Appel, S.H. (1973) J. Biol. Chem. 248, 5156–5636
14 Severson, D.L., Drummond, G.I. and Sulakhe, P.V. (1972) J. Biol. Chem. 247, 2949–2958
15 Hegyvary, C. (1975) Fed. Proc. 35, 835
16 Luly, P., Barnabei, O. and Tria, E. (1972) Biochim. Biophys. Acta 282, 447–452
17 Pairault, J. and Laudat, M.H. (1975) FEBS Lett. 50, 61–65
18 Rogus, E.M., Cheng, L.C. and Zierler, K. (1976) Biochim. Biophys. Acta 464, 347–355