Biochimica et Biophysica Acta, 464 (1977) 347—355

BBA 77585

β-ADRENERGIC EFFECT ON Na^+-K^+ TRANSPORT IN RAT SKELETAL MUSCLE

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(Received July 13th, 1976)

Summary

1. Intact rat extensor digitorum longus muscles soaked in L-isoproterenol plus 10^{-5} M ouabain gained less sarcoplasmic Na^+ than did muscles soaked in ouabain alone. Half maximal effect was produced by 10^{-8} M L-isoproterenol.

2. D-Isoproterenol and oxidized L-isoproterenol were only 3 and 1%, respectively, as potent as L-isoproterenol. Other catechols tested had no effect.

3. The effect of L-isoproterenol on sarcoplasmic Na^+ content appears to be a β-adrenergic function in that it was blocked by propranolol, but not by phentolamine, and could be mimicked by dibutyryl cyclic AMP or by caffeine.

4. Reduced gain in sarcoplasmic Na^+ was accompanied by reduced loss of sarcoplasmic K^+.

5. L-Isoproterenol increased loss of sarcoplasmic Na^+ in the absence of ouabain, in muscles recovering from cold treatment.

6. Results suggest that the β-adrenergic system stimulates a coupled Na^+-K^+ pump.

7. A model is proposed in which stimulation of the Na^+-K^+ pump in response to β-adrenergic agents involves a number of intermediate steps, identified tentatively.

Introduction

Cheng et al. [1] reported that catechols enhance (Na^+ + K^+)-ATPase activity in a plasma membrane fraction of rat skeletal muscle. The effect was produced not only by β-adernergic agonists but also by several other compounds having an orthodihydroxybenzene structure. It was of interest to determine whether cation transport in intact skeletal muscle would respond to the broad family of compounds that stimulates (Na^+ + K^+)-ATPase or only to β-adrenergic agents, as do Na^+ and K^+ fluxes in turkey erythrocytes [2,3]. The latter proved to be the case.

Materials and Methods

Male Sprague-Dawley rats, 50—80 g, were obtained from Charles River Breeding Laboratories, Wilmington, Mass. [U-^{14}C]Sucrose was obtained from New England Nuclear, Boston, Mass., D-isoproterenol-D-bitartrate from Winthrop Laboratories, New York, N.Y., and 1,3,5(10)-estratrien-2,3,17β-triol-17-acetate (2-hydroxyestradiol) from Steraloids, Inc., Pawling, N.Y. Ouabain octahydrate, L-isoproterenol-D-bitartrate, L-β-3,4-dihydroxyphenylalanine (L-DOPA) and the sodium salt of N^6,$O^{2'}$-dibutyryl adenosine 3′ : 5′-cyclic monophosphoric acid (dibutyryl cyclic AMP), were from Sigma Chemical Co., St. Louis, Mo.

Glucose/Krebs-Ringer bicarbonate buffer, pH 7.4, gassed with 95% O_2, 5% CO_2, contained (mM): Na^+, 145; K^+, 4.8; Ca^{2+}, 2.5; Mg^{2+}, 1.2; Cl^-, 126; HCO_3^-, 28; $H_2PO_4^-$, 1.2; SO_4^{2-}, 1.2; glucose, 11. No preservatives were added to prevent oxidation of catechols, but solutions of diluted catechols were replaced by fresh ones every hour. Oxidized L-isoproterenol was prepared by exposure to room air of 1 mM catecholamine, either in this buffer or in 100 mM imidazole, pH 7.0, for at least 24 h.

Effects of catechols or other test compounds on Na^+ and K^+ contents of rat extensor digitorum longus muscles were monitored as follows. In most of the experiments contralateral muscles weighing 20—45 mg were affixed at rest length to separate Lucite racks and bathed for 2 h at room temperature, approx. 23°C, in bicarbonate buffer, one muscle without and one with catechol or other test compound. Because it is normally low and detection of a decrease in it difficult, sarcoplasmic Na^+ content was elevated by addition of ouabain, final concentration 10^{-5} M, after the first hour. Trace [^{14}C]sucrose was also present during the second hour. Propranolol, when used, was present during a 30 min preincubation as well as during the 2 h with catecholamine.

In several experiments the use of ouabain was avoided. In order to elevate Na^+ contents, muscles were soaked at 4°C for 3 h, sometimes with external K^+ concentration reduced from 4.8 to 1.2 mM. Some were then allowed to recover for 1 h, either at 23°C or at 15°C. [^{14}C]Sucrose was present during the last hour of incubation. When there was no recovery period, one muscle of each pair was soaked in 10^{-6} M L-isoproterenol during the entire 3 h at 4°C. When there was a recovery period, 10^{-6} M L-isoproterenol was administered to one muscle of each pair only during the 1 h at 23 or 15°C.

After incubation muscles were blotted, weighed, placed in 5 ml water containing 50 ppm Li^+ and extracted overnight at 4°C. That total extraction of certain electrolytes is achieved by this technique is justified as follows. Extraction of Na^+, K^+ and Cl^- into cold water is as complete as extraction into hot water (Table I) which, in turn, leaves no residual Na^+ or K^+ bound to muscle proteins that is recoverable by ashing [4]. Addition of salts used as doping agent or internal standard in flame photometry does not alter recovery of Na^+ or K^+ in the aqueous extractions. Adrian [5] found no effect of CsCl and we found no effect of Li_2SO_4.

Aliquots of extract were analyzed for [^{14}C]sucrose by liquid scintillation counting and for Na^+ and K^+ concentrations by flame photometry. Sarcoplasmic Na^+ or K^+ content was calculated as total muscle Na^+ or K^+ minus that con-

TABLE I

COMPARISON OF METHODS FOR AQUEOUS EXTRACTION OF IONS FROM MUSCLE

Eight pairs of muscles were incubated in bicarbonate buffer for 1.3—2.5 h at room temperature. Each muscle was blotted and weighed prior to extraction with glass distilled, deionized water. For extraction, one muscle of each pair (A) was placed in a plastic tube with 5 ml water and refrigerated overnight. The contralateral muscle (B) was placed in a Pyrex tube with 5 ml water and the tube placed in a boiling water bath for 1 h. Blank tubes containing 5 ml water were also boiled for 1 h after which there had accumulated a measurable amount of Na^+. Volume was readjusted to 5 ml after the tubes had cooled. Concentrations of Cl^- in the extracts were measured with a Cotlove chloridometer and of Na^+ and K^+ with a Patwin flame photometer with Li^+ as internal standard. Appropriate blanks were subtracted. Results expressed in mequiv/kg wet weight are means ± S.E. of whole muscle ion content uncorrected for extrasarcoplasmic contents. The last column shows means ± S.E. of paired differences, none of which are significantly different from zero.

	Refrigerated (A)	Boiled (B)	Paired Δ (A-B)
Na^+	40.2 ± 1.3	44.9 ± 3.0	−4.7 ± 3.2
K^+	105.3 ± 1.5	103.9 ± 2.8	+1.4 ± 2.5
Cl^-	32.0 ± 0.7	30.4 ± 1.2	+1.6 ± 1.0

tained in the sucrose space, which includes a major part of the volume of sarcoplasmic reticulum as well as extracellular volume [6,7]. Results are given as paired differences between contralateral muscles, treated minus control.

Results

Muscles treated with L-isoproterenol gained less sarcoplasmic Na^+ (Na_s^+) than did their contralateral controls. The maximum difference was 9.5 mequiv/kg wet weight, or 33% of control Na_s^+ (Fig. 1). The response to L-isoproterenol was half maximal at 10^{-8} M and peaked at 10^{-6} M, a sensitivity similar to that found for stimulation of Na^+ and K^+ fluxes in turkey erythrocytes [2,3] and several hundred times greater than that found for enhancement of muscle membrane ($Na^+ + K^+$)-ATPase activity [1].

D-Isoproterenol and oxidized L-isoproterenol, compounds which were equipotent with L-isoproterenol in enhancing membrane ($Na^+ + K^+$)-ATPase activity [1], also reduced the net gain in Na_s^+ (Fig. 1). However, in intact muscle these compounds were only about 3% and 1%, respectively, as potent as L-isoproterenol, which could have occurred if the D-isomer were contaminated with a small amount of L-isomer and if deliberate oxidation of the L-isomer were incomplete.

The β-adrenergic blocker, propranolol, blocked the effect of L-isoproterenol on Na_s^+ and could abolish it (Fig. 2) although relatively high concentrations of blocker, at least equimolar with the concentration of agonist used, were required to do the latter. Similarly high concentrations were needed to abolish isoproterenol-stimulated K^+ influx in turkey erythrocytes [2]. In the absence of agonist, 10^{-5} M propranolol had no effect on Na_s^+.

Lower concentrations of propranolol blocked effects of D- and oxidized L-isoproterenol on Na_s^+. For example, 10^{-7} M propranolol abolished the effect of 10^{-6} M and $3 \cdot 10^{-6}$ M, but not 10^{-5} M, D-isoproterenol, and 10^{-8} M propranolol abolished the effect of 10^{-6} M oxidized L-isoproterenol. These results

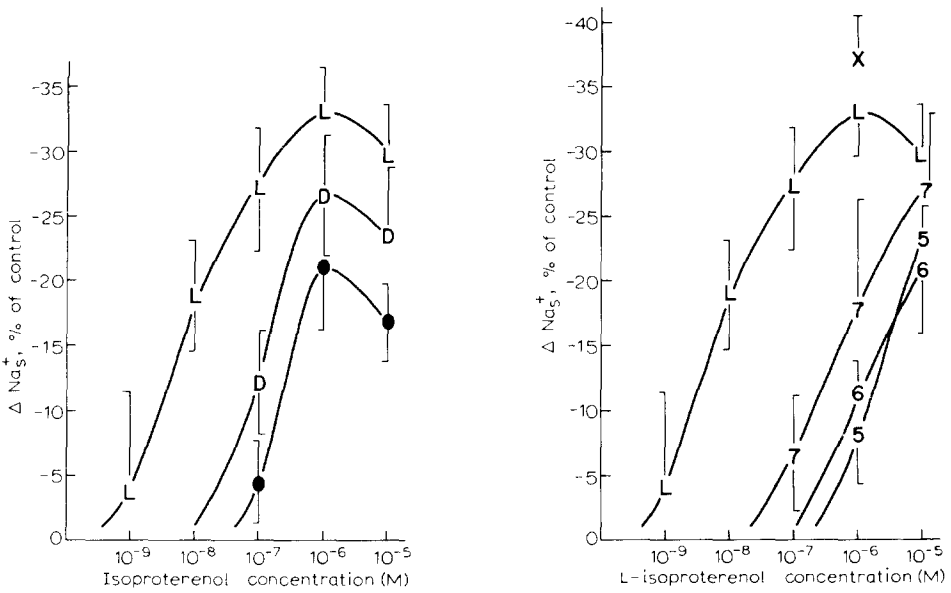

Fig. 1. Effects of L-isoproterenol (L), D-isoproterenol (D) and oxidized L-isoproterenol (•) on sarcoplasmic Na^+ content. Contralateral muscles were soaked for 2 h at 23°C, one without and one with isoproterenol at the concentration indicated. Ouabain, 10^{-5} M, was present during second hour. ΔNa_s^+ is the paired difference between Na_s^+ of contralateral muscles, treated minus control, expressed here as percent of control Na_s^+. Each point is the mean ±S.E. of 7—17 paired differences.

Fig. 2. Blockade of L-isoproterenol effect on sarcoplasmic Na^+ content. Contralateral muscles were soaked for 2.5 h at 23°C, one without and one with L-isoproterenol at the concentration indicated during the last 2 h, both with 10^{-5} M ouabain during the last hour. Blockers were administered to test muscles, but not control muscles, during entire 2.5 h. Blockers were propranolol at 10^{-7} M (7), 10^{-6} M (6), 10^{-5} M (5) and phentolamine at 10^{-5} M (X). Curve with no blocker (L) is shown for comparison. ΔNa_s^+ is the paired difference between Na_s^+ of contralateral muscles, treated minus control, expressed here as percent of control Na_s^+. Each point is the mean ±S.E. of 7—26 paired differences.

are consistent with the interpretation that the agonist properties of these two compounds were due to contamination with L-isoproterenol.

The α-adrenergic blocker, phentolamine, at 10^{-5} M, failed to block the action of 10^{-6} M L-isoproterenol (Fig. 2).

Three other orthodihydroxybenzene compounds, two of which enhanced membrane ($Na^+ + K^+$)-ATPase activity [1], pyrocatechol, L-DOPA and 2-hydroxyestradiol, had no effect on Na_s^+ when used at 10^{-5}, 10^{-5} and 10^{-6} M, respectively (Table II).

The foregoing results indicate that the effect of L-isoproterenol on Na_s^+ is a β-adrenergic function. To determine whether it might be mediated by cyclic AMP we treated muscles with dibutyryl cyclic AMP or with caffeine. Either agent at 10^{-3} M mimicked the effect of L-isoproterenol on Na_s^+. Table III shows that muscles soaked in 10^{-3} M, but not 10^{-4} M, dibutyryl cyclic AMP or in 10^{-3} M caffeine gained significantly less Na_s^+ than did their contralateral controls.

In general, compounds which reduced the net gain in sarcoplasmic Na^+ con-

TABLE II

LACK OF EFFECT OF OTHER CATECHOLS ON Na_s^+

Contralateral muscles were soaked for 2 h at 23°C without or with catechol. During the second hour 10^{-5} M ouabain was present. Data are means ±S.E. of paired differences, treated minus control, expressed as percent of control Na_s^+. The number of muscle pairs is n.

	ΔNa_s^+ (percent of control)	n
Pyrocatechol (10^{-5} M)	−6 ± 8	7
L-DOPA (10^{-5} M)	+4 ± 4	17
2-Hydroxyestradiol (10^{-6} M)	+7 ± 10	8

tent also reduced the net loss in sarcoplasmic K^+ content (K_s^+) suffered by muscles exposed to ouabain. The greatest mean ΔK_s^+ found was 10 mequiv/kg wet weight, or 14% of control K_s^+.

In particular, at the four highest concentrations of L-isoproterenol tested, 10^{-8}—10^{-5} M, ΔNa_s^+ and ΔK_s^+ were of opposite sign and both were significantly different from zero. No difference in K_s^+ (or Na_s^+) was found when sufficiently high concentrations of propranolol were also present, or at 10^{-9} M L-isoproterenol or with the catechols listed in Table II. A coupled effect was produced by 10^{-3} M dibutyryl cyclic AMP ($\Delta Na_s^+ = -6 \pm 1$ mequiv/kg, $\Delta K_s^+ = +4 \pm 1$ mequiv/kg) but not by 10^{-3} M caffeine ($\Delta Na_s^+ = -3 \pm 1$ mequiv/kg, $\Delta K_s^+ = -0.4 \pm 2.0$ mequiv/kg).

The relationship between ΔK_s^+ and ΔNa_s^+ under all conditions tested is illustrated in Fig. 3, a scattergram of the mean of paired differences between K_s^+ of contralateral muscles (treated minus control) versus mean of paired differences between Na_s^+. Each point represents one of the experimental conditions cited earlier in the text, including catecholamines with or without blockers, catechols, dibutyryl cyclic AMP and caffeine but excluding propranolol alone. The regression line for these 29 points is $\Delta K_s^+ = -0.79\ \Delta Na_s^+ - 0.76$. Virtually the same line is obtained when one plots individually the data from 61 pairs of muscles used in the five experiments with 10^{-9}—10^{-5} M L-isoproterenol, $\Delta K_s^+ = -0.80\ \Delta Na_s^+ - 0.75$.

Data in Fig. 3 lead to the conclusion that the β-adrenergic effect of L-iso-

TABLE III

EFFECT OF DIBUTYRYL CYCLIC AMP AND CAFFEINE ON Na_s^+

Contralateral muscles were soaked for 2 h at 23°C without or with agent indicated. During the second hour 10^{-5} M ouabain was present. Data are means ±S.E. of paired differences, treated minus control, expressed as percent of control Na_s^+. n is the number of muscle pairs. P is the probability that the differences occurred by chance.

	ΔNa_s^+ (percent of control)	n
Dibutyryl cyclic AMP (10^{-4} M)	−4 ± 2	17
Dibutyryl cyclic AMP (10^{-3} M)	−24 ± 4	7 $P < 0.01$
Caffeine (10^{-3} M)	−11 ± 3	7 $P < 0.02$

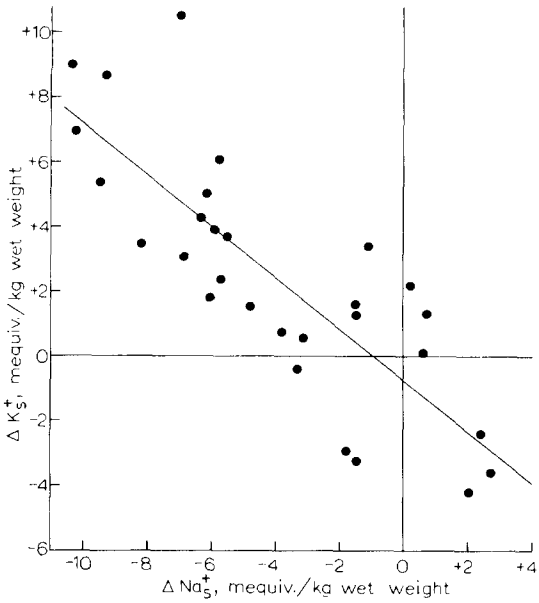

Fig. 3. Coupled changes in sarcoplasmic Na^+ and K^+ contents. Mean of paired differences between K_s^+ of contralateral muscles, treated minus control, is plotted against mean of paired differences between Na_s^+ of same muscles. Each point was obtained from 7 to 26 pairs of muscles and represents a different test compound or combination thereof (i.e. catecholamine with or without blocker, catechol, dibutyryl cyclic AMP or caffeine).

proterenol on Na^+ transport in intact muscle is coupled to an effect in the opposite direction on K^+ transport, which is consistent with stimulation of a coupled Na^+-K^+ pump by the β-adrenergic system.

In all of the experiments reported above muscles were incubated during the last hour with 10^{-5} M ouabain, a concentration which inhibits 70% of (Na^+ + K^+)-ATPase activity in isolated sarcolemma. The possibility exists, therefore, that the action of L-isoproterenol may have been to diminish the inhibition of (Na^+ + K^+)-ATPase exerted specifically by ouabain rather than to stimulate residual (Na^+ + K^+)-ATPase independently of ouabain. To eliminate this possibility we used muscles in which ion contents were perturbed by exposure to cold rather than by exposure to ouabain. In two of the experiments external [K^+] was reduced in an effort to inhibit further the efflux of Na^+ during exposure to cold.

Results of these experiments are presented in Table IV. In two of them (Expts. A and B) contralateral muscles were soaked for 3 h at 4°C, one without and one with 10^{-6} M L-isoproterenol. Inhibition of active Na^+-K^+ transport by cold treatment caused these muscles to lose about 20 mequiv/kg of K_s^+ and gain at least 15 mequiv/kg of Na_s^+ compared to muscles soaked for several hours at room temperature. Reduction of external [K^+] did not add to this inhibition of Na^+-K^+ transport. No effect of L-isoproterenol on Na^+ efflux was expected under these conditions in which there was little or no (Na^+ + K^+)-ATPase activity. The effect of L-isoproterenol in the cold was, if anything, to increase the

TABLE IV

EFFECT OF 10^{-1} M L-ISOPROTERENOL ON Na_s^+ AND K_s^+ IN THE COLD AND DURING RECOVERY FROM COLD TREATMENT

In Expts. A and B contralateral muscles were soaked for 3 h at 4° C at the external K^+ concentration, $[K^+]_0$, indicated, one without and one with 10^{-6} M L-isoproterenol. There was no recovery period. In Expts. C, D, and E contralateral muscles were soaked for 3 h at 4°C at the $[K^+]_0$ indicated without isoproterenol, then for 1 h with $[K^+]_0$ = 4.8 mM at the temeprature indicated without or with 10^{-6} M L-isoproterenol. *n* is the number of muscle pairs. Na_s^+ and K_s^+ are contents of control muscles. ΔNa_s^+ and ΔK_s^+ are paired differences (treated minus control) due to isoproterenol. Data expressed as mequiv/kg wet weight are means ± S.E. *P* is the probability that ΔNa_s^+ occurred by chance.

Expt.	$[K^+]_0$ at 4°C (mM)	Recovery temperature	*n*	Na_s^+	ΔNa_s^+		K_s^+	ΔK_s^+
A	4.8	—	16	22.2 ± 0.9	+4.6 ± 2.5		78.9 ± 1.4	−4.2 ± 2.9
B	1.2	—	20	20.5 ± 0.7	+2.2 ± 0.5	$P << 0.01$	78.4 ± 1.3	−1.5 ± 1.5
C	4.8	15° C	17	15.1 ± 1.2	+1.5 ± 1.0		92.5 ± 1.3	+0.3 ± 1.5
D	4.8	23° C	23	12.4 ± 1.0	−2.2 ± 0.9	$P < 0.02$	92.5 ± 1.7	−0.3 ± 1.6
E	1.2	23° C	20	11.9 ± 0.7	−1.7 ± 0.6	$P < 0.02$	87.3 ± 1.3	−0.7 ± 1.2

gain of Na_s^+. That is, L-isoproterenol may have increased passive influx of Na^+, at least in Expt. B. There was no significant effect of L-isoproterenol on K_s^+.

In the remaining experiments in Table IV pairs of muscles were soaked for 3 h at 4°C in the absence of isoproterenol, then for 1 h at higher temperature without and with 10^{-6} M L-isoproterenol. During the 1 h recovery period active transport was resumed and muscles gained K_s^+ and lost Na_s^+. L-Isoproterenol had no significant effect on Na_s^+ in muscles allowed to recover at 15°C (Expt. C). However, during recovery at 23°C (Expts. D and E) L-isoproterenol further decreased Na_s^+, presumably by increasing the rate of Na^+ pumping. Equal and opposite effects on K_s^+, if they occurred, would have been about 2% of control K_s^+ and not detectable by our method.

Data in Table IV show that the effect of L-isoproterenol on Na_s^+ occurs at least in part by stimulation of active Na^+ efflux, and not necessarily by interaction with ouabain. The data neither confirm nor deny that this Na^+ efflux is coupled to K^+ influx.

Discussion

The effect of L-isoproterenol on net transport of Na^+ and K^+ in intact muscle is compatible with stimulation of a coupled Na^+-K^+ pump. On the basis of sensitivity, specificity and blockade by propranolol this effect appears to be typically β-adrenergic. Yet stimulation by catechols of (Na^+ + K^+)-ATPase activity in isolated sarcolemma is not [1].

Stimulation of active Na^+-K^+ transport in intact muscle is not a result of direct stimulation of (Na^+ + K^+)-ATPase by L-isoproterenol. Although (Na^+ + K^+)-ATPase activity in isolated sarcolemma is enhanced by L-isoproterenol, this enhancement is also produced by several orthodihydroxybenzene compounds which are not β-adrenergic agonists and requires high (10^{-5} M) concentrations of activator [1]. Thus it appears, at least superficially, to be unrelated to the mechanism in intact muscle.

Apart from the direct stimulation of adenylate cyclase activity, β-adrenergic effects are mediated by intracellular cyclic AMP. Exogenous dibutyryl cyclic AMP stimulated Na^+-K^+ transport in intact muscle but not (Na^+ + K^+)-ATPase activity in isolated sarcolemma. Thus the transport enzyme does not appear to be affected directly by cyclic AMP.

Ho and Sutherland [8] have shown that elevation of cyclic AMP levels in fat cells leads to production of a substance which inhibits adenylate cyclase activity, and which they have named feedback regulator, FR. It may be that this substance, or another produced in the sequence of events following β-adrenergic stimulation, acts directly on (Na^+ + K^+)-ATPase. Support for this conjecture comes from preliminary experiments in which the dialysate of medium in which adipocytes had been incubated with L-isoproterenol, and which presumably contains albumin-bound FR, stimulated coupled Na^+-K^+ transport in intact muscle. If β-adrenergic stimulation of Na^+-K^+ transport requires an intracellular synthesis, this provides a ready explanation for the lack of response in isolated membranes. It may also explain why in Expt. C, Table IV, in which muscles recovered at 15°C from prior incubation at 4°C, there was no effect of isoproterenol.

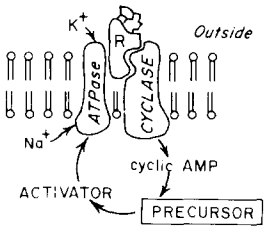

Fig. 4. Proposed mechanism of β-adrenergic stimulation of $(Na^+ + K^+)$-ATPase. R is receptor. Box labeled precursor could, alternatively, represent net synthesis.

Taken together, the results reported herein and by Cheng et al. [1] and by Ho and Sutherland [8] suggest the following model (Fig. 4). In intact muscle, β-adrenergic agonists associate specifically with a receptor on the external surface of the membrane, altering it in such a way that the receptor can couple with adenylate cyclase. Binding of catechols which are not β-adrenergic is unproductive. This coupling of receptor to cyclase stimulates production of cyclic AMP. Cyclic AMP causes release of, or net synthesis of, some intracellular agent, perhaps the FR factor, which acts directly on $(Na^+ + K^+)$-ATPase, enhancing its activity.

Acknowledgements

This project was supported by N.I.H. research grant number AM 17574, awarded by the National Institute of Arthritis, Metabolism and Digestive Diseases, PHS/DHEW, and by a grant from the Muscular Dystrophy Associations of America, Inc.

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