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The Role of Adenosine Triphosphate and Adenosine Triphosphatase in the Release of Catecholamines from the Adrenal Medulla

IV. Adenosine Triphosphate-Activated Uptake of Calcium by Microsomes and Mitochondria

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SUMMARY

Studies were carried out on radioactive calcium uptake by cell fractions isolated from bovine adrenal medullae. Microsomes showed an active uptake of calcium which was dependent on ATP and Mg^{++}, potentiated by an ATP-generating system, and inhibited by agents known to block calcium uptake by muscle microsomes. Mitochondria showed an ATP-activated uptake of calcium which was inhibited by azide, oligomycin, or 2,4-dinitrophenol. These agents did not inhibit active uptake by the microsomes. A semipurified chromaffin granule preparation also showed ATP-activated calcium uptake, but it was largely inhibited by azide. Thiocyanate, which reduced calcium uptake by the microsomes, caused the release of catecholamines from perfused adrenal glands. The regulation of intracellular calcium by the adrenal medulla and its influence on catecholamine secretion are discussed, with emphasis on similarities to muscle physiology.

INTRODUCTION

The important role of calcium in regulating the discharge of secretory products is well documented (1–5). Agents which stimulate secretion from various types of cells also cause the influx of calcium ions (6–8). Release of catecholamines from isolated chromaffin granules can be effected by the addition of ATP and magnesium (9, 10). These facts, together with other information, have led to frequent comparisons between the processes of secretion and contraction (1, 3, 6, 10). In fact, it has been postulated that the secretory discharge process not only resembles muscle contraction but may also involve similar molecular mechanisms (10, 11).

One of the important features of muscle cells is that a stable low level of ionized intracellular calcium is maintained by means of active calcium pumping. The main emphasis in the past has been on ATP-supported accumulation of calcium by elements of the sarcoplasmic reticulum (for references, see refs. 12, 13), but recently the energy-dependent accumulation of calcium by mitochondria has also been considered to be important in regulating levels of calcium in muscle cells (14).

If the secretory process truly resembles contractile phenomena in muscle, a similar control of the calcium concentration in se-

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cretory cells might be expected. This paper reports the occurrence of ATP-activated accumulation of calcium by fractions from the adrenal medulla comparable to those in muscle.[1] Additional experiments have been performed on perfused adrenal glands to assess the role of the microsomal elements in the intact gland. The evidence suggests that the regulation of cellular calcium by mitochondria and microsomes is important in controlling secretory activity.

METHODS

Preparation of cell fractions. Bovine adrenal glands obtained from a local slaughterhouse were transported on ice to the laboratory, and the medullae were dissected away from the cortices. After being minced with scissors, the tissue was homogenized in a glass-Teflon Potter-Elvehjem homogenizer in 5 volumes of 0.28 M sucrose. The homogenate was centrifuged for 5 min at 1000 × *g*, and the residue was discarded. The supernatant fluid was centrifuged for 25 min at 9600 × *g* to yield the crude granule fraction. The resulting supernatant was centrifuged for 60 min at 140,000 × *g* to sediment the microsomal fraction, which was resuspended in a small volume of 0.28 M sucrose with a loosely fitting pestle in a Dounce homogenizer. The crude granule fraction was resuspended in 0.28 M sucrose, layered over 10 ml of 1.4 M sucrose, and centrifuged for 60 min at 140,000 × *g*. This resulted in a pellet and an opaque layer floating on top of the 1.4 M sucrose. The floating layer was removed by aspiration and designated the mitochondrial fraction. This preparation of mitochondria and microsomes is essentially similar to that of Banks (15). To prepare semipurified chromaffin granules, the micropore filtration method was used, which involves filtration through 0.3-μ Millipore filters (10).

Determination of calcium uptake. Calcium uptake was studied, employing ^{45}Ca, by the Millipore filtration method, which has also been used in studies on mitochondria and muscle microsomes (16, 17). The fraction to be studied was incubated at room temperature (24°) in a medium containing (under standard conditions) KCl, 144 mM; $MgCl_2$, 10 mM; *N*-tris(hydroxymethyl)-methyl-2-aminoethanesulfonic acid buffer (pH 7.0), 20 mM; ^{45}Ca, 10^{-5} M (0.01 μCi/ml); and ATP at various concentrations. These conditions were used unless otherwise indicated. The reaction was stopped by rapid filtration (under suction) through 0.3-μ Millipore filters, which yielded perfectly clear filtrates. On some occasions 0.22-μ or 0.45-μ filters were employed and gave similar results, indicating no loss of particles through the filters. Aliquots (0.2 ml) of the filtrates were added to 15 ml of Bray's solution and counted in a Packard Tri-Carb scintillation counter. Controls were always carried through the whole procedure under identical conditions, but with no added cell fractions, and were used to determine the total available radioactivity. Under the conditions employed, the concentration of ^{45}Ca in the incubation medium was not reduced by filtration through Millipore filters in the absence of cell fractions, and averaged about 10,000 cpm/0.2-ml aliquot.

Density gradient centrifugation. For subfractionation of microsomes, the resuspended microsomal fraction was layered over equal volumes of 20%, 25%, and 30% (w/v) sucrose and centrifuged for 60 min at 134,000 × *g*. The isolated subfractions (described under RESULTS) were tested for ATP-activated calcium uptake as described above and assayed for protein.

Perfusion of adrenal glands. Bovine adrenal glands were perfused in a retrograde fashion as described previously (18). The solutions used will be described under RESULTS. The effluent samples were collected in acidified vessels and frozen or stored on ice prior to assay for catecholamines.

Chemical determinations. Catecholamines were assayed fluorometrically (19). Protein was determined by the method of Lowry *et al.* (20), using bovine serum albumin as a standard.

Drugs and chemicals. ATP, cyclic 3′,5′-AMP, and pyruvate kinase were obtained

[1] An account of some of the preliminary findings was presented at the Second International Neurochemistry Meeting in Milan, September 1, 1969.

from Sigma Chemical Company; phosphoenolpyruvate, from Boehringer-Mannheim; and $^{45}CaCl_2$, from Tracerlab (original specific activity, 5.03 mCi/mg).

RESULTS

Effect of ATP on microsomal binding of calcium. To determine whether ATP would affect the binding of calcium, several series of experiments were performed. In one of the first, the experimental conditions were similar to those used for studies on microsomal membranes derived from skeletal muscle (21). In this experiment, 12 aliquots of the microsomal preparation were used for the controls, and 12 other aliquots were used for the ATP-treated samples. ATP (1.6 mM) caused a significant increase (4540 ± 60 cpm/mg of protein) in ^{45}Ca binding during 10 min of incubation at room temperature (24°) (Fig. 1A).

In another experiment, the effect of ATP was examined at 0° to indicate whether an enzymatic mechanism might be involved. ATP-activated uptake did not occur during incubation at this temperature (Fig. 1B). The microsomes were first incubated for 5 min with ^{45}Ca before the addition of ATP.

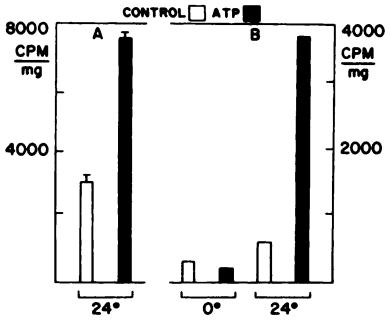


FIG. 1. *Effect of ATP on calcium uptake by adrenal medullary microsomes*

Two separate experiments are illustrated. A. Microsomes were incubated for 10 min in the standard medium in the absence (□) or presence (■) of 1.6 mM ATP. The vertical bars show the mean (± standard error) uptake in counts per minute per milligram of protein. N = 12 for each. B. Duplicate aliquots of microsomes were first incubated for 5 min at 0° or 24°, and the incubation was continued for a further 10 min in the absence (□) or presence (■) of 2.0 mM ATP. The vertical bars show the uptake of calcium during the final 10 min of incubation.

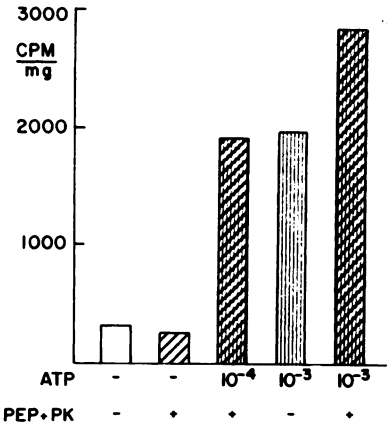


FIG. 2. *Effect of ATP and an ATP-generating system on calcium uptake by adrenal medullary microsomes*

Microsomes were incubated for 5 min following a 5-min preliminary incubation period as described for Fig. 1B. During the final 5 min of incubation, ATP and/or phosphoenolpyruvate plus pyruvate kinase (PEP + PK) were present as indicated below the vertical bars. The concentration of ATP when used was 10^{-4} or 10^{-3} M, as indicated, and the concentrations of phosphoenolpyruvate and pyruvate kinase were 2 mM and 10 μg/ml, respectively. The vertical bars show the mean calcium uptake, in counts per minute per milligram of protein, in duplicate aliquots.

^{45}Ca uptake in the absence of ATP was essentially independent of temperature and was not significantly different from calcium uptake in the presence of ATP at 0°: uptake during 5 min at 24° was 1660 cpm/mg of protein; during 5 min at 0° it was 1480; and with ATP present during 15 min at 0° it was 1590. Uptake at room temperature during 15 min, with ATP present during the final 10 min, was 4510 cpm/mg (Fig. 1B).

Effect of an ATP-generating system on calcium uptake. Since ATP-activated uptake of calcium has been reported to be potentiated by ATP-generating systems (22), one such system was examined using the adrenal medullary microsomes. Phosphoenolpyruvate together with pyruvate kinase had no effect on calcium uptake in the absence of ATP, but potentiated the effect of added ATP (Fig. 2). This experiment also showed that 10^{-4} M ATP could activate calcium uptake in the presence of phosphoenolpyruvate plus pyruvate kinase.

Requirement for magnesium. The ATP-activated uptake of calcium by microsomes from muscle displays a requirement for magnesium, as does its ATPase activity (12). The effect of magnesium on adrenal medullary microsomes proved to be similar: ATP did not increase calcium uptake in the absence of magnesium (Fig. 3).

Time course of calcium uptake. Preliminary studies had shown that passive binding (binding at 0° or at room temperature in the absence of ATP) was very rapid. Some 80–100% of the equilibrium value was attained in 5 min. Uptake in the presence of ATP was also very rapid; two-thirds of the final value was observed in 5 min (the shortest time period measured) (Fig. 4).

ATP-activated uptake of calcium by mitochondria. The uptake of calcium by mitochondria, which can be supported by respiratory substrates or by exogenous ATP, is well documented, particularly for liver mitochondria (23). Mitochondria prepared from the adrenal medulla also exhibited ATP-activated calcium uptake (Fig. 5). Just as with liver mitochondria, the ATP-activated calcium uptake was inhibited by azide, oligomycin, 2,4-dinitrophenol; however, the ATP-activated uptake of calcium by microsomes was not affected by these agents (Fig. 5). These results indicate that the ATP-activated uptake of calcium by the microsomal fractions is not due to the presence of small quantities of mitochondria. Results reported in a later section show that the microsomes also have physical properties quite different from mitochondria.

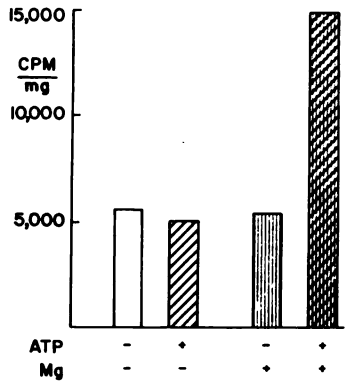


FIG. 3. *Effect of magnesium and ATP on uptake of calcium by adrenal medullary microsomes*

Microsomes were incubated for 20 min in the standard medium or in the standard medium minus magnesium. When ATP was added, its concentration was 2.0 mM. The vertical bars show the mean calcium uptake, in counts per minute per milligram of protein, in duplicate aliquots.

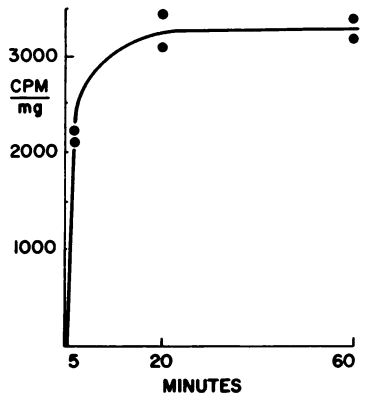


FIG. 4. *Time course of calcium uptake by adrenal medullary microsomes in the presence of ATP*

Microsomes were first incubated in the standard medium for 5 min, and incubation was continued for various times in the presence of 1.0 mM ATP. The curved line shows the calcium uptake, in counts per minute per milligram of protein, after the addition of ATP. The solid circles show the results obtained from duplicate aliquots.

Effect of various agents on ATP-activated calcium uptake by microsomes. Various agents were tested to determine their influence on the ATP-activated uptake of calcium by adrenal medullary microsomes. Some of these have been examined previously in studies on muscle microsomes and on mitochondria. The results are shown in Table 1. Agents which inhibited the ATP-activated calcium uptake included a sulfhydryl inhibitor (*p*-hydroxymercuribenzoate), drugs known to block calcium uptake by muscle microsomes (quinidine, Amytal, and thiocyanate), and the alkaline earths strontium and barium. Agents which did not affect calcium uptake included caffeine and cyclic 3′,5′-AMP. Replacing KCl with NaCl also did not affect calcium uptake.

Calcium uptake by chromaffin granules. Chromaffin granules prepared by Millipore filtration also displayed ATP-activated calcium uptake. However, since granules prepared in this fashion are contaminated by mitochondria (24, 25), it was considered

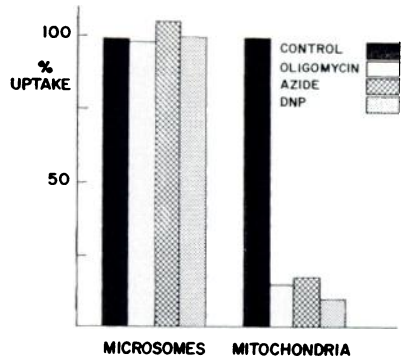


FIG. 5. *Effect of various metabolic inhibitors on ATP-activated calcium uptake by adrenal medullary microsomes and mitochondria*

Microsomes and mitochondria were incubated in the standard medium for 10 min in the absence or presence of 2.0 mM ATP. When metabolic inhibitors were present, their concentrations were: oligomycin, 1 μg/ml; sodium azide, 1.0 mM; and 2,4-dinitrophenol (DNP), 5×10^{-5} M. The vertical bars show the mean ATP-activated calcium uptake of duplicate samples as a percentage of the control. For the microsomes the control value was 8160 cpm/mg, and for the mitochondria it was 12,800 cpm/mg.

TABLE 1

Effect of various agents on ATP-activated uptake of calcium by adrenal medullary microsomes

Microsomes were incubated for 10 min under standard conditions with or without 1.0 mM ATP. The concentrations of the agents tested are shown in the second column. In the experiments with KSCN and NaCl, the KCl in the standard medium was replaced by the test substance. In the experiment with barium, the calcium concentration was 5×10^{-6} M. Inhibition of the ATP-activated calcium uptake (uptake with ATP minus uptake without ATP) is shown in the column on the right.

Agent	Concentration	Inhibition
	M	%
p-Hydroxymercuribenzoate	10^{-3}	100
Quinidine	5×10^{-3}	60
Sodium Amytal	5×10^{-3}	57
KSCN	144×10^{-3}	50
$BaCl_2$	5×10^{-3}	40
$SrCl_2$	2×10^{-4}	26
NaCl	144×10^{-3}	0
Caffeine	8×10^{-3}	0
Cyclic 3′,5′-AMP	10^{-3}	0

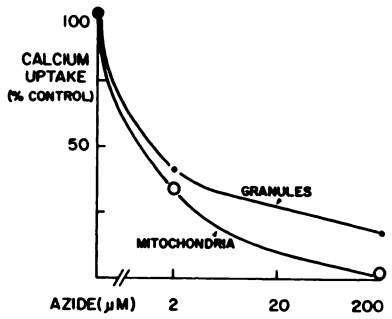
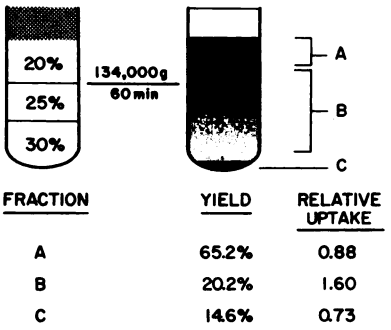


FIG. 6. *Effect of sodium azide on ATP-activated uptake of calcium by adrenal medullary mitochondria and chromaffin granules*

Mitochondria prepared by density gradient centrifugation and chromaffin granules prepared by Millipore filtration were incubated for 20 min in the standard medium after a 3-min preliminary incubation. At zero time, 1.0 mM ATP was added with or without sodium azide at 2×10^{-6} or 2×10^{-4} M. ATP-activated calcium uptake is expressed as a percentage of the control uptake in the absence of azide. The ATP-activated uptake of calcium was 3170 cpm for the granule preparation and 2730 cpm for the mitochondrial preparation.

likely that a significant portion of this active calcium uptake was due to mitochondria. Therefore, studies were conducted with sodium azide, which inhibits ATP-activated calcium uptake by mitochondria. A large portion of the active calcium uptake by a chromaffin granule fraction prepared by Millipore filtration was inhibited by sodium azide (Fig. 6). Parallel experiments with a mitochondrial fraction showed that the chromaffin granule fraction was somewhat less sensitive to the inhibitory effect of azide than was the mitochondrial fraction (Fig. 6). Whether the residual activity in the presence of azide is due to chromaffin granules remains to be determined.

Fractionation of microsomes on a density gradient. Some muscle microsome membranes seem much more active for calcium uptake than others obtained from the same muscle (26). To examine the possible heterogeneity of the microsomal fraction from adrenal medulla, several preparations were fractionated further on sucrose density gradients. Three fractions were obtained after centrifugation over a discontinuous sucrose gradient of 20%, 25%, and 30% (w/v). The microsomes proved to have a



FRACTION	YIELD	RELATIVE UPTAKE
A	65.2%	0.88
B	20.2%	1.60
C	14.6%	0.73

FIG. 7. *ATP-activated uptake of calcium by subfractions of adrenal medullary microsomes*

Microsomes were subfractionated by density gradient centrifugation over 20%, 25%, and 30% (w/v) sucrose as shown. After centrifugation, aliquots of the three fractions (and of the original suspension) were examined for protein and for ATP-activated calcium uptake. The distribution on the gradient in terms of protein is indicated, under the column labeled "yield," as a percentage of protein recovered. Total protein recovered equaled 98.9% of that applied. The column on the right indicates the relative ATP-activated calcium uptake compared to the original suspension, which was given a value of 1.0 (the actual value was 3070 cpm/mg of protein).

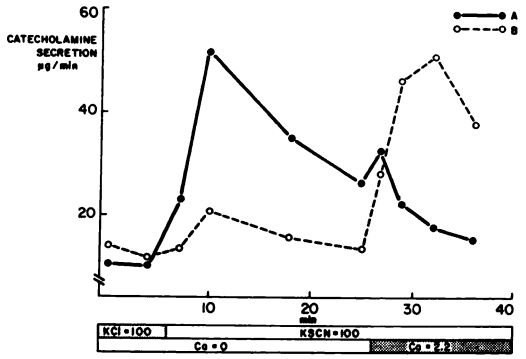


FIG. 8. *Effect of thiocyanate on catecholamine secretion from isolated, perfused bovine adrenal gland*

Experiments on two different adrenal glands (*A* and *B*) perfused from the same perfusion bottles are illustrated. The glands were perfused with a calcium-free, high-potassium medium for 60 min before these data were obtained. The composition of the medium was: KCl, 100 mM; NaCl, 60 mM; $NaHCO_3$, 6 mM; dextrose, 10 mM; and ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid, 0.1 mM. The horizontal bars below the graph indicate where changes in the composition of the perfusion medium were made. At 5 min, 100 mM KCl was replaced by KSCN; at 25 min, 2.2 mM calcium was added.

very low specific gravity: about two-thirds had a density less than that of 25% (w/v) sucrose (0.73 M sucrose). This may be compared with the specific gravity of mitochondria and lysosomes from the adrenal medulla, which reach equilibrium in 1.35 and 1.6 M sucrose, respectively (27). Only 15% of the microsomal protein was sedimented through 30% (w/v) sucrose (0.88 M). The relative ATP-activated calcium uptake in the densest fraction was less than that of the original microsomal fraction, and the intermediate layer showed the highest relative uptake (Fig. 7).

Effect of thiocyanate on catecholamine release from perfused adrenal glands. Various anions potentiate muscle contraction, and one of the most potent is thiocyanate (28). This effect is thought to be due partly to an influence on the electrical properties of the cell membrane and partly to an effect on the sarcoplasmic reticulum (29). Several experiments were performed on glands already depolarized by perfusion with high potassium media.

The effect of replacing 100 mM KCl with 100 mM potassium thiocyanate on catecholamine secretion from two adrenal glands in the absence of calcium and the effect of subsequent addition of calcium are shown in Fig. 8. In the absence of calcium [and after prolonged perfusion with 0.1 mM ethylene glycol bis(β-aminoethyl ether)-N,N′-tetraacetic acid (EGTA)], thiocyanate caused the release of catecholamines. The addition of 2.2 mM calcium caused a further increment in catecholamine release. Of the two glands used in this experiment, one was very sensitive to the effects of thiocyanate, with calcium causing only a small increment (curve *A*, Fig. 8), while the other was less sensitive to thiocyanate, with calcium causing a larger increment in catecholamine release (curve *B*). Ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid at a low concentration (0.1 mM) was used in this experiment in order to ensure that the response on addition of thiocyanate would not

be due to traces of calcium possibly present in the thiocyanate. In two other experiments, catecholamine secretion was increased 2-fold and 8-fold when perfusion was switched from regular Locke's solution to a solution in which 80 mM NaCl was replaced with 80 mM sodium thiocyanate.

DISCUSSION

The present experiments were carried out to obtain information about the handling of calcium by fractions of the adrenal medulla in a manner analogous to similar studies on muscle. The microsomal fraction from the adrenal medulla exhibits ATP-activated uptake of calcium, just as do similar fractions from muscle. Furthermore, this capacity to take up calcium bears much similarity to the ATP-activated process in muscle; there is a requirement for magnesium, potentiation by phosphoenolpyruvate and pyruvate kinase, and inhibition by various drugs such as Amytal and quinidine and by *p*-hydroxymercuribenzoate, a sulfhydryl reagent (12, 17, 22).

The uptake by the microsomal fraction is clearly not due to the presence of mitochondria. Both the density and sedimentation properties of mitochondria are different from those of microsomes. Furthermore, the ATP-activated uptake of calcium by mitochondria was inhibited by azide, oligomycin, and 2,4-dinitrophenol, which did not inhibit the uptake by microsomes.

It should be noted that there is a negative report concerning active uptake of calcium by adrenal medullary microsomes (30); however, in that study the microsomes were stored at −20° before use. We have found[2] that the ability to accumulate calcium is rapidly lost on storage, and there has been similar experience with muscle microsomes (31). The ATPase activity of adrenal medullary microsomes is apparently not lost on storage (30).

These findings on the ATP-activated uptake of calcium by mitochondria and microsomes from the adrenal medulla may help to explain the observations that the release of catecholamines from the intact adrenal medulla is increased by anoxia and metabolic inhibitors (32). Such conditions should reduce the levels of available ATP and, consequently, the binding of intracellular calcium.

Rubin (32) has shown that moderate depletion of energy stores (by anoxia) increases the spontaneous and calcium-evoked release of catecholamines. The increase in spontaneous release caused by anoxia and glucose deprivation was depressed by more than 80% by removal of calcium from the perfusion medium, and therefore (32) is probably due largely to calcium influx. The potentiation of catecholamine release by nitrogen and cyanide (32) in response to low concentrations of calcium may also reflect the decreased capacity of the medulla to bind calcium. This could be related to diminished mitochondrial or microsomal calcium uptake, or both.

A further reason for supposing that microsomal calcium uptake may be physiologically important comes from the results obtained with thiocyanate. Thiocyanate is known to potentiate muscular contraction, and some of this effect is believed to be due to its known ability to block calcium uptake by muscle microsomes (29). Thiocyanate reduced the ability of adrenal medullary microsomes to take up calcium *in vitro* and increased catecholamine secretion from the intact gland. If thiocyanate increases the intracellular levels of free calcium by preventing uptake by the endoplasmic reticulum, this could lead to catecholamine release. In fact, thiocyanate can now be added to the small list of agents which cause adrenal medullary discharge in the absence of extracellular calcium, and since it is unlikely that thiocyanate breaks down the chromaffin cells, the increased availability of intracellular calcium seems a reasonable explanation. The most effective agents known to cause adrenal medullary secretion in the absence of extracellular calcium are strontium and barium (33). Since these agents also interfere with calcium binding by adrenal medullary microsomes, it seems possible that part of their action may be mediated through the release of intracellular calcium. The action of barium on smooth muscle may be related to intracellular calcium stores (34), and we have obtained similar results on perfused

[2] Unpublished experiments.

adrenal glands,[2] indicating a possible indirect action of barium.

Some agents which did not influence calcium uptake by the microsomes were caffeine, cyclic 3′,5′-AMP, and NaCl. Similar negative results have been obtained with muscle microsomes (35–38). An effect of caffeine on calcium mobilization from microsomes from skeletal muscle has been reported (38), and similar studies on the adrenal medulla are now in progress in our laboratory.

The ATP-activated uptake of calcium by a chromaffin granule fraction was largely blocked by azide and presumably was due to the presence of mitochondria. A purer granule preparation (25) will be needed to determine whether the chromaffin granules possess the capacity for ATP-activated calcium uptake.

In order to compare the present results *in vitro* with those on the intact gland, one may examine the data of Borowitz (39) on ^{45}Ca distribution in adrenal medullary fractions from glands perfused with Locke's solution or Locke's solution plus acetylcholine and then washed out with a nonradioactive solution. In those experiments, the ^{45}Ca content (counts per minute per milligram of protein) was increased by acetylcholine in all cell fractions, especially in the mitochondria and chromaffin granules. However, the increase in the microsomal fraction was not statistically significant. Borowitz concluded, "The calcium of endoplasmic reticulum apparently exchanges readily with extracellular calcium and ^{45}Ca is rapidly lost from this subcellular structure during the 53-min washout period" (39). This conclusion seems plausible; however, it might be possible to examine the distribution of calcium without a prolonged washout period if the free calcium were precipitated with oxalate during the initial centrifugation. The chromaffin granule fractions, although not showing the highest absolute specific calcium-binding ability (counts per minute per milligram of protein), nevertheless showed the greatest increase in ^{45}Ca content relative to the total ^{45}Ca in the gland. In view of the known active and passive binding of calcium to adrenal medullary fractions and biological membranes in general, it would seem that most cell components participate in regulating the intracellular calcium concentration.

Current theories on muscle contraction consider that the sarcoplasmic reticulum is important as an intracellular source of calcium and in promoting relaxation by removing calcium from contractile proteins. However, as mentioned earlier, recent work suggests that control of intracellular calcium levels by mitochondria may be of vital importance in muscle (14). Future experiments similar to those performed by Carafoli *et al.* (14) may reveal the relative importance of mitochondria in the regulation of ionized calcium in the adrenal medulla.

Drugs and agents which release intracellular calcium or block its uptake have profound effects on muscle by initiating contraction or delaying relaxation. In an analogous sense, it would be expected that agents which increase the intracellular calcium ion concentration in the adrenal medulla could initiate the release of catecholamines or, alternatively, potentiate or prolong an evoked secretory response. From the close parallel between muscle and the adrenal medulla in calcium binding as described in this paper, it might be predicted that those drugs and agents which affect muscular function by influencing calcium mobility (12, 28) would have the same type of effect on adrenal medullary secretion: catecholamine release would be analogous to contraction, and termination of the secretory process, to relaxation.

Finally, as the literature on the relation between adrenal medullary secretion and sympathetic transmitter release has grown, it has become apparent that the influence of drugs and ions (especially calcium) on the one process is remarkably similar to its effect on the other (1, 40, 41), and this seems consistent with their similar embryological development. Therefore, it would also be expected that norepinephrine release from sympathetic nerve endings would be regulated in part by the same type of cellular elements. This proposal is consistent with reports that ATP-activated uptake of calcium occurs in microsomal elements from brain and peripheral nerve (42–44).

There thus appears to be a common feature in microsomal elements from muscle,

adrenal medulla, and nerves: ATPase activity and active accumulation of calcium. In muscle, ATP and ATPase are important both in initiating contraction, through their interaction with calcium, and in causing relaxation, by removing calcium. There is now evidence to suggest that ATP and ATPase are also involved in the initiation and termination of the secretory process.

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