The Role of ATP and ATPase in the Release of Catecholamines from the Adrenal Medulla

I. ATP-Evoked Release of Catecholamines, ATP, and Protein from Isolated Chromaffin Granules

A. M. POISNER[1] AND J. M. TRIFARÓ[2]

Department of Pharmacology, Albert Einstein College of Medicine, New York, New York 10461

(Received July 5, 1967)

SUMMARY

Studies were carried out on isolated chromaffin granules from bovine adrenal medullae. The granules were purified by Millipore filtration and incubated at 30°C. ATP caused a concentration-dependent release of catecholamines at concentrations from 0.0625 to 0.5 mM. AMP, cyclic 3',5'-AMP,[3] and CTP were ineffective, while ADP and adenosine tetraphosphate had some releasing activity. ATP-evoked release was observed in the presence of Mg or Mn, but not in that of Ca, Ba, or Sr. Na and K were equally effective in supporting release by ATP + Mg, but 0.3 *M* sucrose abolished the response. AMP and NEM also inhibited ATP-evoked release. There was a correlation between catecholamine release and ATPase activity. Discharge of catecholamines was accompanied by liberation of ATP and protein but not of cholesterol. A model is proposed to explain the present results in relation to catecholamine secretion *in vivo*.

INTRODUCTION

Beginning with the discovery that the catecholamines in the adrenal medulla are stored in membrane-limited particles—the so-called chromaffin granules (1, 2)—studies on catecholamine storage and release *in vitro* have aroused a great deal of attention. This is partly because the chromaffin granules are relatively stable *in vitro* (particularly at lower temperatures) and partly because the isolation of semipurified granules is relatively easy. Although studies on storage mechanisms *in vitro* have provided clues of probable relevance to physiology and pharmacology, such as the elucidation of the Mg + ATP-activated and reserpine-blocked uptake of amines (3–5), studies on catecholamine release by possible physiological agents *in vitro* have been less successful.

For an *in vitro* system to be useful as a model for catecholamine release *in vivo*, it must be consistent with what is known about adrenomedullary secretion. The generally accepted facts are these: Stimulation of the nerves to the adrenal medulla results in the release from the nerve endings of acetylcholine which diffuses across the synaptic cleft and causes depolarization of the chromaffin cell (6). Depolarization is accompanied by an influx of ions including calcium (7, 8). The presence of calcium is critical for the release of catecholamines

[1] Research Career Development Awardee, U.S.P.H.S.

[2] Senior Postdoctoral Fellow, U.S.P.H.S.; present address: Department of Pharmacology, McGill University, Montreal, Canada.

[3] The following abbreviations are used: cyclic 3',5'-AMP, adenosine 3',5'-phosphate; NEM, *N*-ethylmaleimide; CTP, cytidine triphosphate; EGTA, ethylene glycol bis(β-aminoethylether)-*N*,*N*'-tetraacetic acid); TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.

by acetylcholine (9) or by KCl and a host of other agents (10, 11). The next step apparently involves the release of catecholamines, ATP (12, 13) and protein (14, 15) from the chromaffin granules. The granule membrane is retained by the cell, probably as a discrete particle (16–19).

It has been reported by many workers (20) that acetylcholine does not release catecholamines from isolated chromaffin granules. Furthermore, experiments *in vitro* with calcium ions have similarly yielded negative results (21, 22) or revealed only minimal effects (23, 24). Thus a satisfactory model system for studying the release of catecholamines has not been developed.

Since the bulk of recent evidence indicates that catecholamine release occurs from granules at or close to the plasma membrane, it would seem that an alteration in the environment of the granules at this locus must occur to bring about catecholamine release. Therefore it was thought desirable to examine the effect on chromaffin granules of substances (25) which might be expected to be released by the plasma membrane during depolarization.

In the present study we have obtained evidence that one substance with this property, ATP (25), in low concentrations causes release of catecholamines, ATP, and protein from chromaffin granules *in vitro*. In addition we have examined the underlying mechanism for catecholamine release *in vivo*.

METHODS

Preparation of chromaffin granules. Adrenal glands were obtained from the slaughterhouse on ice and the medullae were dissected out. After weighing and mincing, each medulla was homogenized in 5 volumes of ice-cold 0.3 M sucrose (which was adjusted to pH 7.0 with NaOH and sometimes contained 1 mM EDTA or EGTA). After removing a low-speed sediment by centrifuging at 800 *g* for 10 min, the supernatant was passed through a series of Millipore filters (25-mm syringe filter). The order of the filters used was as follows: prefilter, 3 μ, 1.2 μ, 0.65 μ, 0.45 μ, 0.3 μ, and 0.22 μ. After passage through the 0.22 μ filter, the filtrate was centrifuged for 10 min at 20,000 *g*. The supernatant was discarded and the sediment was resuspended in the sucrose medium (or sometimes in the incubation medium to be used), a loose-fitting Dounce homogenizer being used. Usually 1 ml of suspension medium was used for each gram of medulla homogenized. This granule suspension was stored at 4° and was used either immediately or after 24 hours.

Incubation procedure. Two milliliters of an incubation mixture was prewarmed to 30° and at zero time 100 μl of the granule suspension was added. Incubation times varied from 0 to 60 min. Sometimes additions were made to the mixtures at 0 or 10 min in 5–20 μl volumes. These will be described later. The incubation was terminated by rapidly adding 4.0 ml of ice-cold 0.3 M sucrose containing 2 mM EDTA, cooling in an ice-water bath for a few minutes, and centrifuging in the cold at 20,000 *g* for 10 min. Incubations were usually done in duplicate or triplicate.

Incubation medium. The standard incubation mixture contained (mM): KCl, 160; NaCl, 5; TES buffer (pH 7.0), 10; $MgCl_2$, 0.5; EDTA, 0.05. Variations of this mixture will be described under results.

Analytical methods. Catecholamines were assayed using the trihydroxyindole fluorometric method (26). Inorganic phosphate was determined according to Martin and Doty (27). ATP was measured using the firefly luminescence technique (12, 13). Protein was determined using a modified biuret method (28). Cholesterol was measured on chloroform-methanol extracts by a fluorometric method as described previously (17).

Calculation of results. The release of catecholamines and inorganic phosphate (PO_4) was calculated from the increase in concentration of these substances in the supernatant from zero time to the end of the incubation. When additions of nucleotides were made at 10 min, release was calculated from the increment in concentration in the supernatant of these substances above that present at 10 min. Sometimes results have been expressed as a percent of

release under control condition (i.e., with no nucleotide present). Release of ATP and protein were calculated by the difference in quantity of these substances in the pellet before and after the incubation period. In one experiment cholesterol was measured in the supernatant before and after incubation to determine release of this lipid.

Chemicals used. ATP, ADP, AMP, cyclic 3′,5′-AMP, and CTP were obtained from Sigma Chemical Co. and from Boehringer-Mannheim. TES buffer (29) was obtained from Calbiochem. EGTA was obtained from Geigy Pharmaceuticals.

RESULTS

Effect of ATP, Mg, and Ca on Catecholamine Release

Initially experiments were done to test the effect of ATP, Mg, Ca, and combinations of these agents on catecholamine release from isolated chromaffin granules. ATP (0.5 mM) caused release of catecholamines in the presence of Mg or Mg + Ca. ATP alone or in combination with Ca produced only minimal or negligible effects, as did the divalent cations alone or in combination (Fig. 1). The effect of ATP plus Mg (0.5 mM of each) was observed in each of 75 experiments with TES buffer and in each of 10 with PO_4 buffer (also 10 mM, pH 7.0). In addition ATP plus Mg elicited catecholamine release in each of 8 experiments in the presence of 10^{-5} M reserpine, a concentration known to block the uptake of catecholamines by chromaffin granules (4). The rate of catecholamine release in the presence of 0.5 mM ATP plus Mg ranged from 2 to 10 times that in the presence of Mg alone.

In a few experiments, other concentrations of Mg and Ca were tested. With 0.5 mM ATP, catecholamine release was greater at 0.5 than at 0.05 mM Mg, but no further increase was obtained by raising the Mg concentration to 5.0 mM. When Ca alone was tested, a small and variable release of catecholamines was observed with no difference between 0.5 and 2.0 mM Ca. The maximum releasing effect of Ca (2.0 mM) was less than one-eighth of that of 0.5 mM ATP plus Mg.

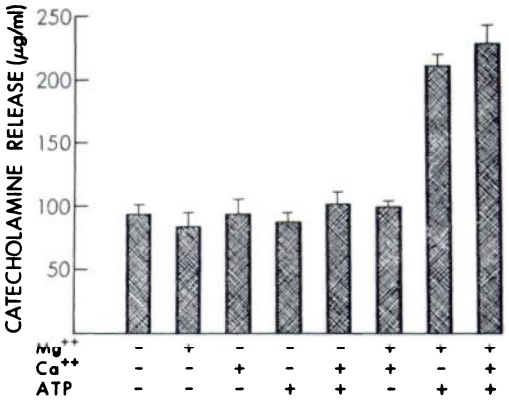

FIG. 1. *Effect of Mg, Ca, ATP, and combinations of these agents on catecholamine release from isolated chromaffin granules*

Isolated chromaffin granules (equivalent to 369 μg/ml bound catecholamine) were incubated for 10 min at 30° in a medium containing (mM): KCl, 160; NaCl, 5; TES buffer, pH 7.0, 10; EGTA, 0.05. When Mg, Ca, or ATP were present, the final concentration was 0.5 mM. The vertical bars represent the mean (±SE) release of catecholamine in micrograms per milliliter.

Effect of Na, K, and Sucrose on Catecholamine Release

Several experiments were done to examine the effects of Na, K, and sucrose on the ATP-induced release of catecholamines. No difference was noted between media containing 160 mM KCl plus 5 mM NaCl and those containing 165 mM NaCl plus no KCl, but the ATP-induced release of catecholamines was completely blocked by 0.3 M sucrose (Fig. 2). This may provide an explanation for the failure of many previous investigators to observe the release phenomenon since incubations have frequently been carried out in 0.25 or 0.3 M sucrose. In one experiment when the KCl concentration was raised to 200 mM, spontaneous and ATP-evoked release of catecholamines were depressed to about one-third of that in 160 mM KCl. This may reflect a stabilizing effect of hypertonicity. When KCl was reduced from 160 mM to 100 mM and sucrose was 105 mM (to preserve tonicity), the spontaneous release was

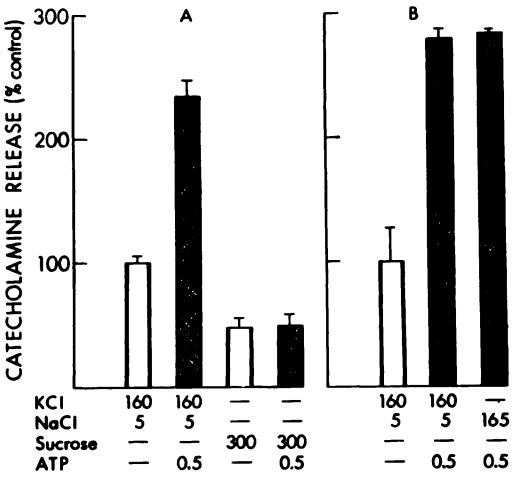

FIG. 2. *Effect of KCl, NaCl, and sucrose on ATP-evoked release of catecholamines from isolated chromaffin granules*

Isolated chromaffin granules were incubated at 30° for 10 min (in A) or for 30 min after 10 min preincubation (in B). The concentration (mM) of KCl, NaCl, sucrose, and ATP in the incubation media is indicated below the vertical bars. In addition the media contained (mM): TES buffer, pH 7.0, 10; $MgCl_2$, 0.5; and EDTA, 0.05. ATP was added at zero time (in A) or after the 10 min preincubation period (in B). Catecholamine release is expressed as a percentage of the release obtained in the absence of ATP in the standard incubation medium.

not affected, but the ATP-evoked release was reduced to about 45% of that in the absence of sucrose.

Time Course of Catecholamine Release

The time course of the spontaneous and ATP-evoked release of catecholamines was examined up to 60 min. In the first few minutes both spontaneous and ATP-evoked release were relatively rapid. However, after about 10 min, the spontaneous release practically stopped while the ATP-evoked release continued (Fig. 3). The rapid initial release may be related to the sudden temperature rise (from 0° to 30°).

Relation of ATP Concentration to Catecholamine Release

When increasing concentrations of ATP were employed (0.0625–0.5 mM), a linear relationship was obtained between the logarithm of the ATP concentration and the release of catecholamines (Fig. 4). The threshold for the response seemed to be about 0.0625 mM ATP.

Effect of Other Nucleotides on Catecholamine Release

When nucleotides other than ATP were tested at 0.5 mM, no release of catecholamines was observed with AMP, cyclic 3′,5′-AMP, or CTP. ADP gave variable results, sometimes producing a small response (much less than that of ATP) and at other times showing no effect. The releasing effect of ADP was more consistent with longer incubation periods. The releasing effect of ADP may be due to the adenylate kinase activity (30) in chromaffin granules as will be discussed later and in the next paper (35). Adenosine tetraphosphate produced a definite release of catecholamines, although it was less potent than ATP (Fig. 5).

Effect of Divalent Metal Ions

The effect of various divalent metal ions at 0.5 mM was studied to see which ones could support the ATP-evoked release of catecholamines. Of the metals tested, only Mg and Mn were effective in this regard while Ca, Ba, and Sr were unable to substitute for Mg (Fig. 6).

Effect of ATP on the Release of ATP and Protein

Since it is known that not only catecholamines but also ATP (12, 13) and soluble protein (14, 15) are released from chromaffin granules when the adrenal medulla is stimulated with acetylcholine, the effect of ATP on the release of granule ATP and protein was examined. ATP (0.5 mM) caused a significant release of ATP and of protein from the isolated granules. Furthermore, the molar ratio of catecholamines:ATP released was 4.0, which is very similar to the ratio found in the granules before incubation (17) (Table 1). While the scatter is somewhat greater for the protein data, the results are consistent with release of catecholamines and soluble protein from the granules in the same propor-

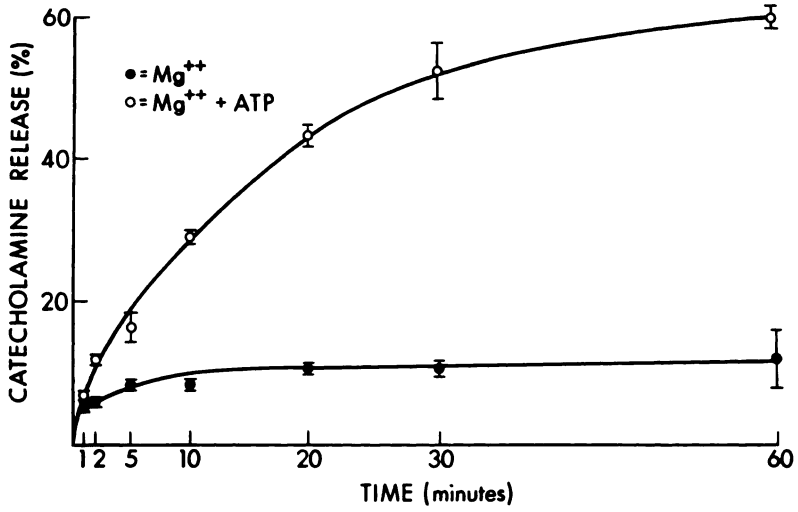

FIG. 3. *Time course of release of catecholamines from isolated chromaffin granules*

Isolated chromaffin granules were incubated at 30° for varying periods of time in the presence or absence of ATP (0.5 mM). The incubation medium contained (mM): KCl, 160; NaCl, 5; TES buffer, pH, 7.0, 10; $MgCl_2$, 0.5; and EDTA, 0.05. Catecholamine release is expressed as a percentage of the bound amines (±SE).

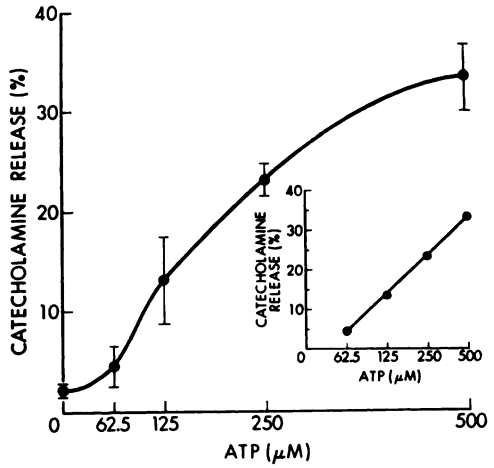

FIG. 4. *Effect of ATP concentration on the release of catecholamines from isolated chromaffin granules*

The larger curve shows the release of bound catecholamine from isolated chromaffin granules by increasing concentrations of ATP during 30 min incubation at 30° (following a 10 min preincubation without ATP). The incubation medium contained (mM): KCl, 160; NaCl, 5; TES buffer, pH 7.0, 10; $MgCl_2$, 0.5; and EDTA, 0.05. The inset shows the results with ATP plotted logarithmically.

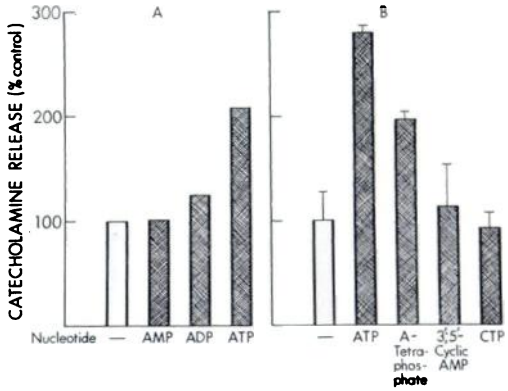

FIG. 5. *Effect of ATP and other nucleotides on release of catecholamines from isolated chromaffin granules*

Isolated chromaffin granules were incubated at 30° for 10 min (in A) or for 30 min after 10 min preincubation (in B) as described in Fig. 2. The incubation medium contained (mM): KCl, 160; NaCl, 5; TES buffer, pH 7.0, 10; $MgCl_2$, 0.5; and EDTA, 0.05. The nucleotides used (indicated below the vertical bars) were present at a concentration of 0.5 mM). Catecholamine release is expressed as a percentage of the release in the absence of nucleotide.

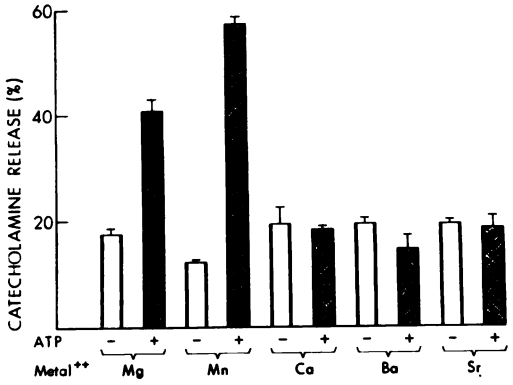

FIG. 6. *Ability of various divalent metal ions to support ATP-evoked release of catecholamines from isolated chromaffin granules*

Isolated chromaffin granules were incubated for 10 min at 30° in an incubation medium containing (mM): KCl, 160; NaCl, 5; TES buffer, pH 7.0, 10; EDTA, 0.05; and various divalent metal ions (0.5 mM) as indicated at the bottom of the figure. Catecholamine release in the presence (+) or absence (−) of ATP (0.5 mM) is expressed as a percentage of the bound catecholamines.

tion as that which exists in the granules (15).

Effect of ATP, Mg, and Ca on the Release of Inorganic Phosphate

Since the chromaffin granules are known to contain an active ATPase (31, 32) which can act on externally applied ATP, it was considered of interest to study the release of inorganic phosphate (PO_4) under the present experimental conditions. It was found that conditions leading to catecholamine release (that is ATP plus Mg but not ATP plus Ca or Ca alone) led to a

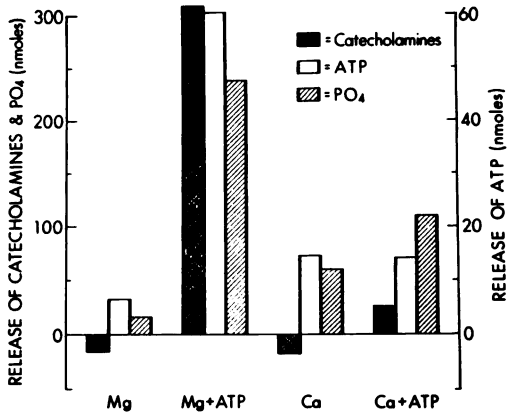

FIG. 7. *Effect of ATP on the release of catecholamines, ATP, and inorganic phosphate from isolated chromaffin granules*

Isolated chromaffin granules were incubated for 10 min at 30° in a medium containing (mM): KCl, 160; NaCl, 5; TES buffer, pH 7.0, 10; and EDTA, 0.05. When Mg, Ca, or ATP was added, the final concentration of each was 0.5 mM. Release of catecholamines and inorganic phosphate (PO_4) (left ordinate) and of ATP (right ordinate) are expressed in nanomoles per milliliter above (or below) that released with no additions. In the case of PO_4, release represents the increase of this substance in the incubation mixture, some coming from hydrolysis of exogenous ATP and some from hydrolysis of granule ATP.

marked rise in PO_4 in the incubation medium (Fig. 7). Some of this probably represents PO_4 liberated from exogenous ATP and some from ATP released from the granules. This seems reasonable since even in the absence of ATP release (that is in the presence of ATP alone) there was a rise of PO_4. And studies in our laboratory as well as elsewhere (33) have shown

TABLE 1

Effect of ATP on the release of catecholamines, ATP, and protein from isolated chromaffin granules

Isolated chromaffin granules were incubated for 10 min at 30°C in a medium containing (mM): KCl, 160; NaCl, 5; TES buffer, pH 7.0, 10; $MgCl_2$, 0.5; EDTA, 0.05; with or without ATP, 0.5. The total volume was 2.1 ml. Each value is the mean (±SE) of 7–9 observations.

Substance released	Control	ATP treated	Significance treated *vs* control
Catecholamines (nmoles/ml)	39.9 ± 2.2	93.4 ± 5.1	$p < 0.001$
ATP (nmoles/ml)	14.9 ± 1.9	23.1 ± 1.0	$p < 0.005$
Ratio: catecholamines: ATP	2.7	4.0	—
Protein (μg/ml)	21.4 ± 14.7	65.7 ± 6.8	$p < 0.02$

that upon incubation ATP lost from chromaffin granules is largely dephosphorylated in the incubation medium.

Effect of ATP on the Release of Cholesterol

Since ATP was shown to release the major water-soluble constituents of the granules, that is catecholamines, ATP, and protein, we next looked at the release of a membrane constituent, cholesterol. In six experiments 0.5 mM ATP did not release granule cholesterol. This suggests that membranes are not broken down during catecholamine release, a view consistent with chemical (16–18) and electron microscopic (19) studies on acetylcholine-evoked release of catecholamines from the adrenal medulla.

Effect of Inhibitors of ATPase on ATP-Induced Release of Catecholamines

To study the possible role of granule ATPase in the ATP-induced release of catecholamines, two types of ATPase inhibitors were tested. Both AMP (0.5 mM) and NEM (0.2 mM) blocked the ATP-induced release of catecholamines (Fig. 8). The blockade of ATP action by AMP and NEM was shown in other experiments to be accompanied by a block of PO_4 release; NEM has also been shown elsewhere (34) to block granule ATPase. Information on the nature of the blockade by these agents (competitive *vs.* noncompetitive) will be presented in the next paper (35). NEM itself, in higher concentrations, caused the release of catecholamines.

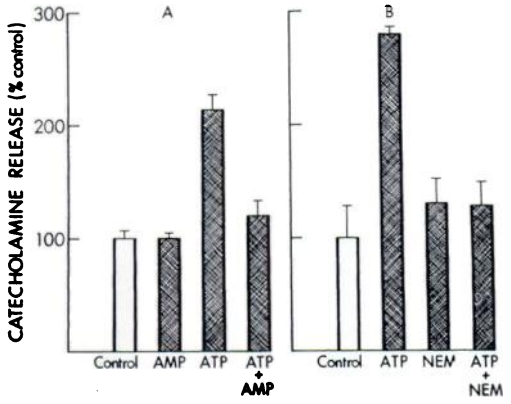

FIG. 8. *Inhibition of ATP-evoked release of catecholamines from isolated chromaffin granules by AMP and by NEM*

Isolated chromaffin granules were incubated for 10 min at 30° (in A) or for 30 min after 10 min preincubation period (in B) as described in Fig. 2. The incubation medium contained (mM): KCl, 160; NaCl, 5; TES buffer, pH 7.0, 10; $MgCl_2$ 0.5; and EDTA, 0.05. When AMP (0.5 mM) or NEM (0.2 mM) was used, it was present throughout the total incubation period. When ATP (0.5 mM) was used, it was added at zero time (in A) or after the 10 min preincubation period (in B). Catecholamine release is expressed as a percentage of the release in the control medium without ATP.

DISCUSSION

The present study has clearly demonstrated that ATP releases catecholamines *in vitro* from isolated chromaffin granules at concentrations lower than those used to show active uptake of amines. It seems remarkable that this phenomenon was not fully described earlier since numerous studies on isolated chromaffin granules have been carried out (cf. 3–5). Although ATP has been employed in many of these studies, there have been at least two important differences. First, uptake of labeled exogenous catecholamines has usually been examined and, when only radioactive amine accumulation is measured, no analysis of release is possible. Secondly, most studies with isolated granules have used 0.25–0.3 M sucrose as the incubation medium, and the present results show that sucrose prevents ATP-evoked release. It is of interest that sucrose also prevents ATP-induced mitochondrial contraction (36). Further parallels between these phenomena will be mentioned later. The only indications of an effect of ATP on catecholamine release have come in preliminary studies using high concentrations of ATP (37, 38).

In previous work the releasing effect of ATP may also have been obscured by other factors, such as absence of an optimal concentration of magnesium or perhaps contamination with nongranule ATPase, which would have effectively lowered the concentration of ATP. It is virtually impossible solely by differential centrifugation to ob-

tain granule preparations which are free from all other subcellular particles, particularly mitochondria (39). The best techniques for obtaining pure chromaffin granules using centrifugation have employed density gradients (32) which segregate the granules in a layer of sucrose of very high molarity and thus preclude the demonstration of the releasing effect of ATP. The porous membrane filter method (38), which yields granules of high chemical (38, and unpublished observations) and morphological (S. Malamed, personal communication) purity, permits separation in isotonic medium, which is necessary to demonstrate the full effect of ATP.

The specificity of the releasing effect by nucleotides is relatively high. AMP, cyclic 3′,5′-AMP, and CTP were without effect. Adenosine tetraphosphate (which is probably hydrolyzed rapidly to ATP) and ADP also caused catecholamine release, although less than that caused by ATP. The effect of ADP, which was small and variable, may well be due to the adenylate kinase activity in chromaffin granules (30). This conjecture is strengthened by the evidence presented in the following paper (35) that, when an effect of ADP on chromaffin granules is seen, it is delayed in onset and then resembles that of ATP.

The divalent metal ion requirements are also relatively specific. Of the metals tested, only Mg and Mn were able to support ATP-evoked release of catecholamines. Ca, Sr, and Ba, all of which support acetylcholine-evoked release *in situ*, were ineffective. This division of metal ion requirements is similar to that found for ATP-evoked mitochondrial contraction (40). In contrast to the divalent ions, there was no difference between the major physiological monovalent cations, Na and K. It is possible that the ATP-evoked release of catecholamines has no monovalent cation specificity, a phenomenon analogous to ATP-induced mitochondrial contraction (40) and histamine release from mast cells (41). Preliminary experiments suggest that lithium and choline can substitute for Na or K and further studies will be necessary to test this possibility.

ATP releases two other major components of the chromaffin granule, ATP and protein, which are known to be released when the adrenal gland is stimulated with acetylcholine. Furthermore, the ratio of catecholamines:ATP:protein released *in vitro* is comparable to the loss of these substances from the intact adrenal medulla stimulated with acetylcholine (12, 14). In contrast, ATP caused no loss of cholesterol, a component of the granule membrane. There is also no increase in the efflux of cholesterol (17) or loss of cellular content of this lipid (18) when catecholamine secretion from the adrenal gland is evoked with acetylcholine.

Very little of the total phosphate present in the chromaffin granules is present as inorganic phosphate (PO_4)—most is present as nucleotide phosphate—yet PO_4 accumulates in the incubation medium during catecholamine release. This appearance of PO_4 reflects hydrolysis of ATP. It is known that both exogenously added (32) and endogenously released (33) ATP is dephosphorylated by isolated chromaffin granules. The splitting of ATP in different media correlated with release of catecholamines.

The release of catecholamines by ATP was inhibited by two known inhibitors of ATPase, NEM and AMP. This fact, plus the metal and nucleotide specificity, suggests that the release of granule constituents is a consequence of ATPase activity. This view is supported by the correlation between catecholamine and PO_4 release.

The release of catecholamines (and other granule constituents) from isolated chromaffin granules by ATP can be accommodated into a scheme for the physiological release of catecholamines *in vivo* by utilizing a concept for molecular events associated with membrane depolarization described by Abood (25) and by invoking known properties of calcium ions. In this view (25), the membrane is considered to contain complexes of protein, phospholipid, ATP, and Ca. On stimulation (neural or chemical) the Ca and ATP are freed (42) permitting hydrolysis of ATP and influx of ions with subsequent depolarization. Re-

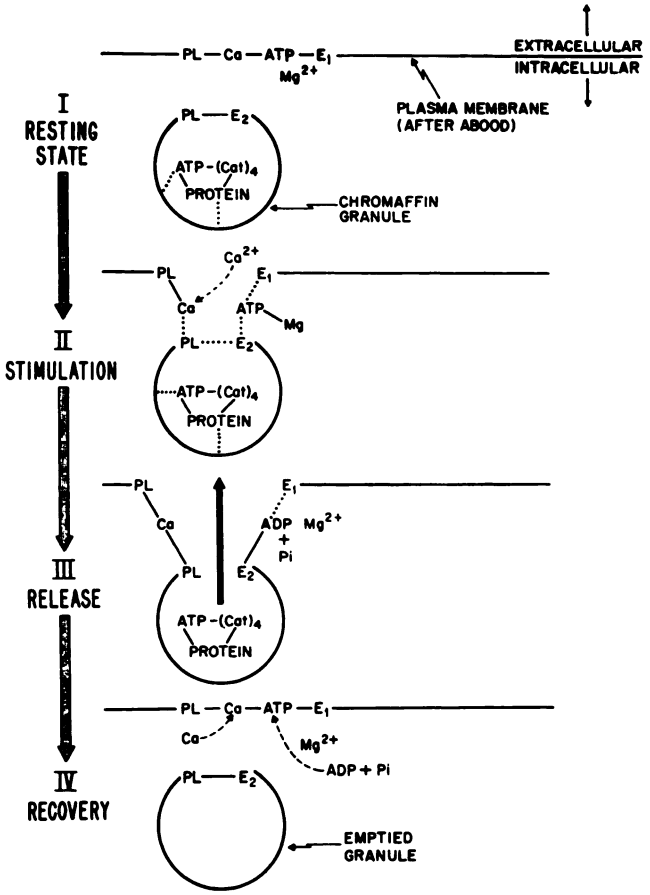

FIG. 9. *A proposed model for release of catecholamines from the adrenal medulla*

The above scheme shows a proposed sequence of events in the adrenal chromaffin cell during release of catecholamines. The initial stimulus could be acetylcholine, KCl, or a host of other secretogogues which cause depolarization and calcium influx. For a full explanation see text.

polarization is associated with rephosphorylation of ADP to ATP and binding with Ca in the membrane. A consequence of the liberation of membrane Ca and ATP (and influx of Ca) would be a temporary localized increase in concentration of these substances in the vicinity of the plasma membrane (25). A proposed model for the molecular events associated with catecholamine secretion from the adrenal medulla based on these concepts is shown in Fig. 9.

1. The plasma membrane contains (among other components) phospholipid (PL), ATP, Ca, Mg, and protein which in the scheme of Abood is a Na + K activated ATPase (E_1). The granule membrane contains phospholipid and a Mg-activated ATPase (E_2). Catecholamines are stored within the granule in a complex with ATP and protein which is weakly bound to the granule membrane.

2. Upon stimulation, Ca is freed from the plasma membrane (extracellular Ca also enters the cell) (7, 8), thus increasing the local concentration of Ca which then forms a link between anionic groups (possibly PL) in the plasma membrane and those in the membranes of chromaffin granules in the vicinity of the plasma membrane. The plasma membrane ATP which is also freed upon stimulation can be acted upon by the granule ATPase (E_2) (or by

plasma membrane ATPase, E_1). Movement of Na, K, and Ca down their electrochemical gradients takes place while the membrane is in its altered state.

3. The interaction between E_2 and ATP, which causes hydrolysis of ATP, produces some conformational change in the granule membrane (see later discussion) which breaks the weak bonds holding the catecholamine–ATP–protein complex to the chromaffin granule membrane. This permits egress of these substances at the area of increased membrane permeability. It is known that complexes of catecholamines with ATP and protein in the granules are easily broken, e.g., by hypotonicity (2).

4. Restoration of the initial condition (repolarization) involves resynthesis of ATP, rebinding of Ca by the plasma membrane, and return of the granule membrane to its original state.

This sequence is an attempt to provide a molecular description of exocytosis. It fits many of the facts known about the release of catecholamines from the adrenal medulla:

a. Catecholamine release *in vivo* is dependent on extracellular Ca (9, 11).

b. Catecholamine release *in vivo* is blocked by inhibitors of ATP synthesis (43) and of ATPase (unpublished observations).

c. Catecholamine release *in vitro* is dependent on Mg, a good cofactor for granule ATPase (32), but not on Ca, a poor cofactor for granule ATPase (32). The Mg-activated ATPase in granules is probably different from the Ca-activated enzyme (34).

d. The granule membrane appears to be retained in the cell as an intact structure after catecholamine release (16–19).

e. It is known that chromaffin granules have a net negative charge at physiological pH and that Ca can cause aggregation of the granules by combining with anionic groups, while Mg is much less active (22). Moreover, Ca, which permits release of the contents of secretory granules from leukocidin-treated leukocytes, has been shown to cause attachment of these granules to the plasma membrane (44).

f. Release of catecholamines *in vitro* is blocked by inhibitors of the granule Mg-activated ATPase.

The interesting finding that extragranular ATP releases granule ATP permits the speculation of a positive feedback system, as has been proposed for the action of ADP released from platelets during agglutination (45), in which the released ATP may act in turn on the granule membrane to amplify the effects of the initially released ATP.

Work in other fields also provides a possible explanation for the mode of action of ATP in altering the chromaffin granule membrane. One example is in the action of ATP in producing mitochondrial contraction (36). Since an actomyosin-like protein, which contracts in response to ATP, has been extracted from mitochondria (46), a truly contractile event in mitochondrial "contraction" has been proposed (46). It is interesting that mitochondrial contraction (a) is induced by ATP (36), (b) requires Mg or Mn but not Ca (40), and (c) is inhibited by sucrose (40). All these conditions parallel the effects of ATP on release of catecholamines from isolated chromaffin granules.

The remarkable parallels *in vivo* between excitation-contraction coupling in muscle and stimulation-secretion coupling in the adrenal medulla have been commented on previously (9–11). The parallels described here between release of catecholamines *in vitro* from chromaffin granules and contraction *in vitro* of mitochondria (for comparison to actomyosin, see 47) suggest that the granule membrane ATPase may function as a mechanochemical transducer—a phenomenon ascribed to the mitochondrial membrane (48). This possibility is presently under investigation in our laboratory.

ACKNOWLEDGMENTS

We wish to acknowledge the support and encouragement of Dr. W. W. Douglas and the technical assistance of Mr. A. Hooper. Supported by U.S.P.H.S. grants 5TI-GM-65-09, 5K3-GM-25304, and 5RO1-NB-04006.

REFERENCES

1. H. Blaschko and A. D. Welch, *Arch. Exptl. Pathol. Pharmakol.* **219**, 27 (1953).
2. N.-Å. Hillarp, S. Lagerstedt and B. Nilson, *Acta Physiol. Scand.* **29**, 251 (1953).
3. N. Kirshner, *J. Biol. Chem.* **237**, 2311 (1962).
4. A. Carlsson, N.-Å. Hillarp and B. Waldeck, *Acta Physiol. Scand.* **59**, Suppl. 215 (1963).
5. L. Stjärne, *Acta Physiol. Scand.* **62**, Suppl. 228 (1964).
6. W. W. Douglas, T. Kanno and S. R. Sampson, *J. Physiol. (London)* **188**, 107 (1967).
7. W. W. Douglas and A. M. Poisner, *J. Physiol. (London)* **162**, 385 (1962).
8. W. W. Douglas, T. Kanno and S. R. Sampson, *J. Physiol. (London)* **191**, 107 (1967).
9. W. W. Douglas and R. P. Rubin, *J. Physiol. (London)* **159**, 40 (1961).
10. W. W. Douglas and R. P. Rubin, *Nature* **192**, 1087 (1961).
11. A. M. Poisner and W. W. Douglas, *Proc. Soc. Exptl. Biol. Med.* **123**, 62 (1966).
12. W. W. Douglas, A. M. Poisner and R. P. Rubin, *J. Physiol. (London)* **179**, 130 (1966).
13. W. W. Douglas and A. M. Poisner, *J. Physiol. (London)* **183**, 236 (1966).
14. P. Banks and K. Helle, *Biochem. J.* **97**, 40C (1965).
15. N. Kirshner, H. J. Sage, W. J. Smith and A. G. Kirshner, *Science* **154**, 529 (1966).
16. J. M. Trifaró and A. M. Poisner, *Federation Proc.* **26**, 294 (1967).
17. J. M. Trifaró, A. M. Poisner and W. W. Douglas, *Biochem. Pharmacol.*, in press.
18. A. M. Poisner, J. M. Trifaró and W. W. Douglas, *Biochem. Pharmacol.*, in press.
19. S. Malamed, A. M. Poisner and J. M. Trifaró, *Anat. Record* **157**, 282 (1967).
20. H. Weil-Malherbe and H. S. Posner, *J. Pharmacol. Exptl. Therap.* **140**, 93 (1963).
21. N.-Å. Hillarp, *Acta Physiol. Scand.* **43**, 292 (1958).
22. P. Banks, *Biochem. J.* **101**, 18C (1966).
23. A. Philippu and H. J. Schümann, *Experientia* **18**, 138 (1962).
24. M. Oka, T. Ohuchi, H. Yoshida and R. Imaizumi, *Japan. J. Pharmacol.* **15**, 348 (1965).
25. L. G. Abood, *Intern. Rev. Neurobiol.* **9**, 223 (1966).
26. A. H. Anton and D. F. Sayre, *J. Pharmacol. Exptl. Therap.* **138**, 360 (1962).
27. J. B. Martin and D. M. Doty, *Anal. Chem.* **21**, 965 (1949).
28. A. G. Gornal, C. J. Bardawill and M. M. David, *J. Biol. Chem.* **177**, 751 (1949).
29. N. E. Good, G. D. Winget, W. Winter, T. N. Connolly, S. Izawa and R. M. Singh, *Biochemistry* **5**, 467 (1966).
30. N.-Å. Hillarp, *Acta Physiol. Scand.* **42**, 144 (1958).
31. P. Banks, *Biochem. J.* **95**, 490 (1965).
32. N. Kirshner, A. G. Kirshner and D. L. Kamin, *Biochim. Biophys. Acta* **113**, 332 (1966).
33. A. D'Iorio, *Can. J. Biochem. Physiol.* **35**, 395 (1957).
34. N. Kirshner, C. Holloway, W. J. Smith and A. G. Kirshner, *in* "Mechanisms of Release of Biogenic Amines" (U. S. von Euler and B. Uvnäs, eds.), p. 109. Pergamon, London, 1966.
35. J. M. Trifaró and A. M. Poisner, *Mol. Pharmacol.* **3**, 572 (1967).
36. A. L. Lehninger, B. C. Ray and M. Schneider, *J. Biophys. Biochem. Cytol.* **5**, 97 (1959).
37. A. D'Iorio and J. G. Lague, *in* "Major Problems in Neuroendocrinology" (E. Bajusz and G. Jasmin, eds.), p. 112. S. Karger, Basel, 1964.
38. M. Oka, T. Ohuchi, H. Yoshida and R. Imaizumi, *Life Sci.* **5**, 433 (1966).
39. A. D. Smith and H. Winkler, *J. Physiol. (London)* **183**, 179 (1966).
40. A. L. Lehninger, *Physiol. Rev.* **42**, 467 (1962).
41. B. Uvnäs and I. L. Thon, *in* "Mechanisms of Release of Biogenic Amines" (U. S. von Euler and B. Uvnäs, eds.), p. 361. Pergamon, London, 1966.
42. L. G. Abood, K. Koketsu and S. Miyamoto, *Am. J. Physiol.* **202**, 469 (1962).
43. N. Kirshner and W. J. Smith, *Science* **154**, 422 (1966).
44. A. M. Woodin, J. E. French and V. T. Marchesi, *Biochem. J.* **87**, 567 (1963).
45. G. V. R. Born, *Ann. Roy. Coll. Surg. Engl.* **36**, 200 (1965).
46. T. Ohnishi and T. Ohnishi, *J. Biochem. (Tokyo)* **52**, 230 (1962).
47. A. L. Lehninger, *in* "Horizons in Biochemistry" (M. Kasha and B. Pullman, eds.), p. 421. Academic Press, New York, 1962.
48. A. L. Lehninger, *in* "The Mitochondrion," p. 199. W. A. Benjamin, New York, 1964.