Biochemical Pharmacology, Vol. 19, pp. 505–514. Pergamon Press. 1970. Printed in Great Britain

EFFECTS OF VARIOUS AGENTS ON THE Mg^{2+}-ATP STIMULATED INCORPORATION AND RELEASE OF CATECHOLAMINES BY ISOLATED BOVINE ADRENOMEDULLARY STORAGE VESICLES AND ON SECRETION FROM THE ADRENAL MEDULLA*

R. M. FERRIS, O. H. VIVEROS† and N. KIRSHNER

Department of Biochemistry, Duke University Medical Center, Durham, N.C. 27706, U.S.A.

(*Received* 15 *April* 1969; *accepted* 6 *June* 1969)

Abstract—The effects of several agents on the Mg^{2+}-ATP stimulated incorporation and release of catecholamines by isolated bovine adrenomedullary storage vesicles suggest that both of these processes may employ a similar or identical set of reactions for a portion of their respective pathways. *N*-ethylmaleimide and *N*-*N*-di-isopropyl-*N'*-isoamyl-*N'*-diethylaminoethylurea (P-286) inhibited equally both incorporation and release. Prenylamine inhibited incorporation to a greater extent than it inhibited release. Reserpine and propranolol, at concentrations which almost completely blocked incorporation, had little or no effect on release. *N*-ethylmaleimide, P-286 and prenylamine, when present in the perfusion medium at concentrations which blocked release from isolated vesicles, also blocked the secretory response of the isolated perfused adrenal gland to acetylcholine. In isotonic salt solutions, Mg^{2+} plus ATP stimulates to an equal extent the release of both soluble dopamine-β-hydroxylase and catecholamines. An hypothesis is proposed to explain these observations and to serve as a model for additional experiments.

THE CATECHOLAMINE storage vesicles of the adrenal medulla, when incubated with Mg^{2+} and ATP, exhibit widely different behavior which is dependent upon the composition of the medium. In isotonic sucrose the vesicles incorporate ^{14}C-adrenaline into their endogenous stores and only very slowly release their contents into the medium.[1, 2] In isotonic salt solutions the vesicles rapidly release their contents of amines, ATP and protein.[3–5] Both the Mg^{2+}-ATP stimulated incorporation and release are inhibited by *N*-ethylmaleimide, but only the incorporation is inhibited by reserpine.[1, 2, 5] For both processes Mn^{2+} can replace Mg^{2+}. These observations suggest that both processes may have some common features even though the two may be different.

* This work was supported by grants from the National Science Foundation (GB 5812), the National Institutes of Health (AM-05427), and the American Medical Association Education and Research Foundation.

The authors also wish to acknowledge the following sources of support: R. Ferris, Post-doctoral Fellow, National Institute of Mental Health (T1-MH-8394); O. H. Viveros, International Post-doctoral Fellow, National Institutes of Health; N. Kirshner, Research Career Awardee, National Institutes of Health (K3-GM-15, 184).

† Present address: Institute of Physiology, School of Medicine, University of Chile, Santiago, Chile.

In response to cholinergic stimulation, the intact adrenal medulla releases the soluble contents of the storage vesicles directly to the exterior of the cell.[6-12] This process requires a source of metabolic energy.[13, 14] The similarities between secretion and the Mg^{2+}-ATP stimulated release of amines, ATP and protein from isolated storage vesicles led to the suggestions that the latter phenomenon may be a step in the secretory response of the adrenal medulla[5] as well as a generalized reaction for the secretion of other hormones which are stored in vesicles.[15]

The availability of compounds which block incorporation of catecholamines into the storage complex or which block secretion from the adrenal gland provides an experimental approach which may resolve the fundamental similarities and differences between the release and incorporation of catecholamines by the storage vesicles, and which may also provide information to evaluate the role of the Mg^{2+}-ATP stimulated release in the secretory process. The effects of several drugs on the release and incorporation of catecholamines by isolated storage vesicles and on secretion from isolated perfused adrenal glands are reported here.

METHODS

Preparation of adrenal medullary catecholamine storage vesicles. The catecholamine storage vesicles were prepared from bovine adrenal medulla as previously described.[1] The washed vesicles were finally suspended in a volume of 0·3 M sucrose equivalent to the original weight of the fresh tissue.

Release of catecholamines and incorporation of ^{14}C-adrenaline in salt solutions. Incubations were carried out in polyethylene centrifuge tubes at 30° without shaking. The standard incubation medium contained NaCl, 5 mM; KCl, 160 mM; TES* buffer (pH 7·0), 10 mM; EDTA, 0·05 mM; ATP, 5 mM; Mg^{2+}, 5 mM and 0·2 ml of the vesicle preparation in a final volume of 1·0 ml. When incorporation studies were carried out, the reaction medium contained 0·1 μc of *dl*-adrenaline-2-^{14}C. The reaction was initiated by the addition of ATP plus Mg^{2+} immediately before placing the tubes in the 30° water bath. The incubation time varied from 0 to 30 min, and the reaction was terminated by the addition of 2·0 ml of ice-cold 0·3 M sucrose containing 2 mM EDTA. When dopamine-β-hydroxylase was to be assayed, EDTA was omitted from both the incubation medium and the cold 0·3 M sucrose used to stop the reaction. The tubes were chilled in ice and then centrifuged at 26,000 *g* for 20 min. The supernatants were decanted and saved for measurement of catecholamines and for the measurement of radioactivity. The 26,000 *g* pellets were lysed in 3·0 ml of distilled water and centrifuged at 26,000 *g*. The supernatant was saved for assay. Catecholamines were measured by the methods of von Euler and Hamberg[16] and radioactivity assayed in a liquid scintillation spectrometer.

Incubations in all studies were carried out at 0° and at 30° with and without ATP plus Mg^{2+} to enable calculation of the temperature dependent and the Mg^{2+}-ATP dependent release at 30°. At 0° there was little or no difference in extravesicular catecholamines in the presence or absence of Mg^{2+}-ATP. At 30° the extravesicular catecholamines generally increased approximately 2-fold in the absence of Mg^{2+}-ATP but increased 3- to 8-fold in the presence of Mg^{2+}-ATP depending upon the preparation and length of incubations.

When the effects of various drugs were studied, the adrenal vesicles were pre-

* TES is *N*-Tris (hydroxymethyl)-2-aminoethane sulfonic acid.

incubated for 10 min at 30° in the absence of Mg^{2+}-ATP with and without the drugs at the concentration shown in Table 1. The incubation vessels were then chilled in ice and the reaction subsequently initiated by the addition of Mg^{2+}-ATP. Concentrations of the drugs higher than those shown in Table 1 frequently caused a spontaneous release of catecholamines at 0° or at 30° which may have been due to lysis of the storage vesicles.

Release and incorporation in 0·3 *M sucrose.* When incubations were carried out in a sucrose medium, the standard incubation mixtures contained: sucrose, 300 mM; TES buffer, pH 7·0, 10 mM; EDTA, 0·05 mM; ATP, 5·0 mM; Mg^{2+}, 5·0 mM; and 0·2 ml of the vesicle preparation in a final volume of 1·0 ml. All other procedures were the same as those used for studies in salt solutions.

Measurement of dopamine-β-hydroxylase. Dopamine-β-hydroxylase released into the incubation medium and the amounts present in the lysates of the storage vesicles was measured as previously described[17] using *p*-hydroxymercuribenzoate (10^{-5} M) to inactivate the endogenous inhibitor.

ATPase activity. The effects of the various drugs on the Mg^{2+}-stimulated ATPase activity of the storage vesicles were carried out as previously described.[18]

Calculations. The amounts of adrenaline incorporated into the storage complex were calculated from the formula:

$$\text{Incorporation } (\mu g) = \frac{\text{C.P.M. Lysed Vesicle Supernatant}}{\text{Av. Sp. Act. Adr. in Medium} \times (1\text{-Fraction of Catecholamine Released})}.$$

In these calculations it is assumed that the amine taken up is homogenously diluted in the vesicular pool of releasable amines.

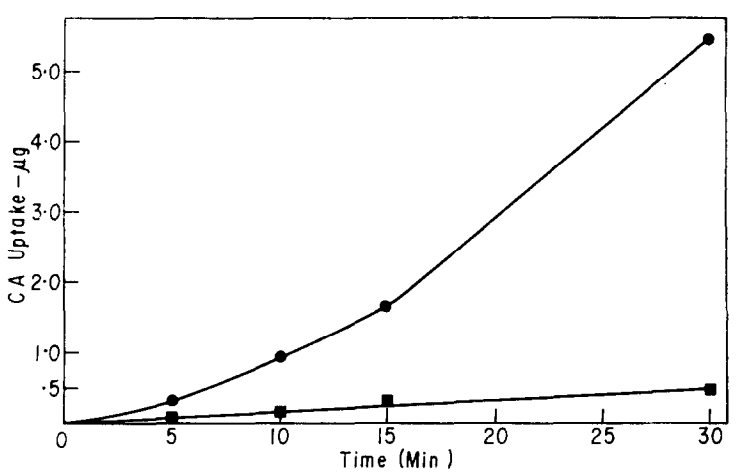

FIG. 1. Incorporations (uptake) of ^{14}C-adrenaline by isolated adrenomedullary vesicles in isotonic salt solution in the absence, ■, and in the presence, ●, of added ATP and Mg^{2+}. Incubations were carried out at 30°.

Release of catecholamines from isolated perfused adrenal glands. Isolated bovine adrenal glands were perfused with Locke's solution and stimulated with acetylcholine as previously described.[10] Two-min samples were collected and assayed for catecholamines. The 2-min secretion rate immediately before perfusing with acetylcholine (1×10^{-4} M) was recorded as the resting level. Acetylcholine in Locke's solution was then perfused for 2 min and the perfusion medium then changed to Locke's solution. The secretion rate during the first 6 min starting with the perfusion of acetylcholine was recorded as the response.

After the initial response to acetylcholine, drugs were added to the perfusion medium at the different concentrations shown (Fig. 3) and perfused for 15 min before stimulation with acetylcholine. Both the Locke's solution containing acetylcholine and the Locke's solution perfused through the gland immediately after stimulation contained one of the drugs tested. At the end of the first response period, the gland was then perfused for 15 min with the next higher concentration of the same drug and the response to acetylcholine was again measured. Only one drug was tested on each gland. In separate experiments, it was found that perfusion of the drug at the lower concentrations had little or no effect on the response to the higher concentrations. The same degree of inhibition was observed at the higher concentration whether or not the drugs were first perfused at the lower concentrations.

MATERIALS

^{14}C-adrenaline and uniformly labeled ^{3}H-tyramine were obtained from New England Nuclear Corp. The following drugs were obtained as gifts: P-286 (*N*, *N*-diisopropyl-*N'*-isoamyl-*N'*-diethylaminoethylurea), Pittman-Moore; guanethidine and reserpine, Ciba; Tranylcypromine, Smith, Kline & French; Chlorthiazide, Merck, Sharpe & Dohme; Neohydrin, Lakeside Laboratories; prenylamine, Hoechst.

RESULTS

Release and incorporation by isolated vesicles. The release of catecholamines was approximately linear for 25–30 min (Fig. 2) when the incubations were carried out as described in Methods. In sucrose solution the incorporation was linear for 15–20 min[1, 2] but in salt solutions (Fig. 1) the rate of incorporation increased with time due to the increased concentration of amines in the medium.[19]

The effects of the various drugs on inhibition of release and incorporation of catecholamines by isolated vesicles are shown in Table 1. *N*-Ethylmaleimide had been previously reported[1, 2] to inhibit uptake in sucrose solutions at concentrations (1 or 2×10^{-4} M) which also inhibited the Mg^{2+}-dependent ATPase.[20] Poisner and Trifaro[5] have also reported that *N*-ethylmaleimide inhibited the Mg^{2+}-ATP dependent release from isolated vesicles. P-286 inhibited equally both the release of endogenous catecholamines and the incorporation of ^{14}C-adrenaline. Prenylamine[2] and reserpine[1, 2] have been reported to inhibit incorporation of ^{14}C-adrenaline from sucrose media. Prenylamine inhibited both release and incorporation in salt solutions but incorporation was inhibited to a much greater extent than was release at equal doses of the drug. Poisner and Trifaro[5] reported that reserpine (10^{-5} M) had no effect on the release from the isolated vesicles. In our experiments 10^{-5} M reserpine had no effect, but lower concentrations of reserpine caused a small but consistent inhibition of release and higher concentrations stimulated the release (Table 1).

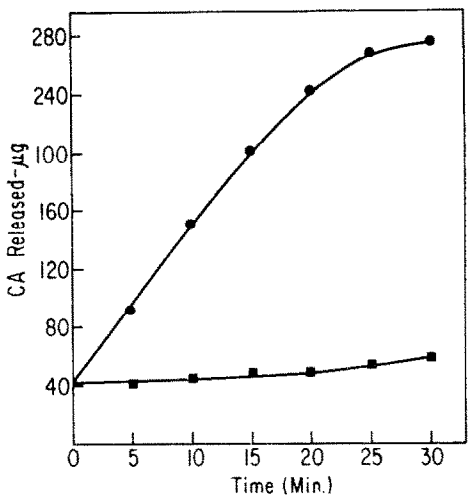

FIG. 2. Release of catecholamines by isolated adrenomedullary storage vesicles in isotonic salt solution in the absence, ■, and in the presence, ●, of added ATP and Mg^{2+}. At 25 min, 75 per cent of the total amine content of the vesicles had been released. The results of a single experiment are shown and are typical of several different preparations of vesicles. Incubations were carried out at 30°.

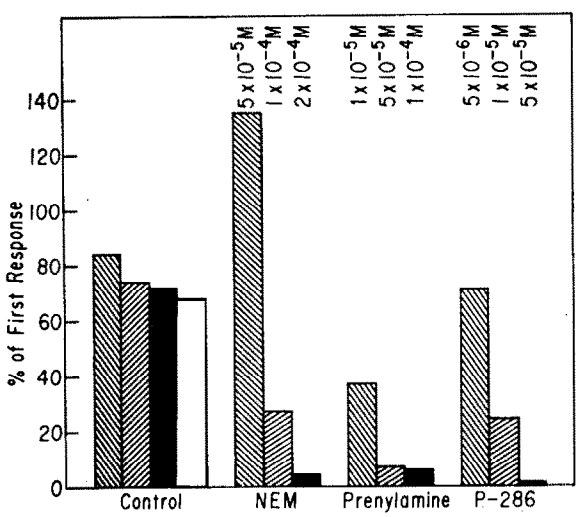

FIG. 3. Effect of *N*-ethylmaleimide (NEM), prenylamine and P-286 on the secretory response of the adrenal medulla to perfused acetylcholine (10^{-4} M). In each set of data, the first response to acetylcholine was recorded prior to the administration of the drug. The corresponding shaded bars refer sequentially from left to right to the second, third, fourth, and fifth response of each gland to acetylcholine.

The enhancement of release by reserpine was consistent, but the extent of enhancement varied considerably as indicated by the large standard error of the mean. Propranolol had no effect on release but did inhibit incorporation of ^{14}C-adrenaline from sucrose media. Studies of the effects of propranolol on incorporation in salt solutions were also carried out. Propranolol decreased the amount of radioactivity found in the vesicles compared to controls incubated without propranolol, but

because of the decrease in specific activity of the ^{14}C-adrenaline in the medium due to release of endogenous catecholamines there appeared to be little or no inhibition of incorporation.

Effect of other drugs. In addition to the compounds listed in Table 1, a wide variety of other agents were tested for their ability to inhibit release. Guanethidine (10^{-5} to 10^{-3} M) tranylcypromine (10^{-5} to 10^{-3} M), chlorthiazide (5×10^{-6} to 10^{-4} M), neohydrin (5×10^{-6} to 5×10^{-5} M) and dithiothreitol (10^{-5} to 10^{-3}M) had no

TABLE 1. EFFECT OF VARIOUS AGENTS ON RELEASE AND INCORPORATION OF CATECHOLAMINES BY ISOLATED STORAGE VESICLES*

	Release KCl	Incorporation Sucrose	Incorporation KCl
	% of control value		
NEM			
1×10^{-5}	100 ± 18		
5×10^{-5}	49 ± 16		
1×10^{-4}	37 ± 6		
2×10^{-4}	10 ± 5		
P-286			
1×10^{-5} M	70 ± 4	48 ± 3	58 ± 3
5×10^{-5} M	28 ± 2	14 ± 5	22 ± 0·2
1×10^{-4} M	13 ± 5	1 ± 1	11 ± 2
2×10^{-4} M	4 ± 2	2 ± 2	1 ± 1·0
Prenylamine			
1×10^{-5} M	63 ± 8		8 ± 1
2×10^{-5} M	43 ± 3		5 ± 5
5×10^{-5} M	24 ± 7		5 ± 5
Propranolol			
1×10^{-5} M	100 ± 1	77 ± 2	
1×10^{-4} M	100 ± 1	29 ± 10	
2×10^{-4} M	100 ± 1	22 ± 5	
Reserpine			
5×10^{-7}	72 ± 3		
5×10^{-6}	80 ± 4		5 ± 5
1×10^{-5}	92 ± 4		5 ± 5
5×10^{-5}	171 ± 29		
1×10^{-4}	186 ± 49		

* Incubations were carried out as described in Methods. The data are expressed as the per cent of control values, the controls being the observed release or incorporation in the presence of Mg^{2+} and ATP. Each value represents the mean ± the standard error of the mean for three to five determinations.

effect on release. Higher concentrations of neohydrin (5×10^{-5} to 5×10^{-4} M) lysed the storage vesicles. Metabolic inhibitors (oligomycin, 5 μg/ml; antimycin, 10 μg/ml; iodoacetate, 10^{-4} M; cyanide, 10^{-4} M), by themselves, or in combinations which inhibit secretion from the adrenal medulla[13] also had no effect on release or incorporation. Tri-*n*-butyl tin chloride, a potent inhibitor of photophosphorylation and chloroplast ATPase, lysed the vesicles at concentrations of 10^{-6} M and also inhibited the vesicle ATPase by 74 per cent.

Effects on secretion from perfused adrenal glands. The effects of *N*-ethylmaleimide, prenylamine and P-286 on the acetylcholine induced secretion from the isolated perfused adrenal gland are shown in Fig. 3. Sequential administration of acetylcholine to control glands at 25-min intervals produced responses that moderately and progressively declined. When the various drugs were perfused through the glands at concentrations which inhibited release from the isolated vesicles, the response to acetylcholine was also inhibited. The lowest concentration of *N*-ethylmaleimide used consistently increased the response to acetylcholine. Prenylamine had a dual effect on the adrenal gland. At the concentration shown (Fig. 3) it inhibited the response to acetylcholine but at higher concentrations it, by itself, markedly stimulated release. Similar observations had been reported for dog adrenal glands.[21] Reserpine, when perfused at concentrations ranging from 1×10^{-4} M to 5×10^{-5} M had no effect on secretion by itself nor did it alter the response to acetylcholine. Propanolol at concentrations of 10^{-5} M to 10^{-4} M has been shown to inhibit the response to acetylcholine.[22]

Release of catecholamines and dopamine-β-hydroxylase. Poisner and Trifaro[5] have reported that ATP plus Mg^{2+} caused the release of proportionate amounts of soluble

TABLE 2. Mg^{2+}-ATP STIMULATED RELEASE OF DOPAMINE-β-HYDROXYLASE AND CATECHOLAMINES FROM ISOLATED STORAGE VESICLES*

	DBO		CA		DBO/CA	
	Med.	Lysate	Med.	Lysate	Med.	Lysate
No Mg^{2+}-ATP	4·22	15·14	43	159	0·098	0·095
Mg^{2+}-ATP	9·21	10·81	84	118	0·109	0·091
Δ	+ 4·99	− 4·33	+ 41	− 41	0·121	0·105
No Mg^{2+}-ATP	51·36	257·5	56	187	1·04	1·38
Mg^{2+}-ATP	119·20	195·3	106	141	1·14	1·38
Δ	+ 67·84	− 62·2	+ 50	− 46	1·36	1·35
No Mg^{2+}-ATP	168·8	458·2	52	133	3·25	3·44
Mg^{2+}-ATP	244·4	378·0	82	102	2·98	3·71
Δ	+ 75·6	− 80·2	+ 30	− 31	2·521	2·59
No Mg^{2+}-ATP	125·4	560·0	58	239	2·16	2·34
Mg^{2+}-ATP	232·2	384·0	105	180	2·21	2·14
Δ	+106·8	−176·0	+ 47	− 59	2·27	2·98

* The release was measured for a 15-min incubation period in salt media as described in Methods. Catecholamines are expressed in μg. Dopamine-β-hydroxylase activity (DBO) is expressed as mμmoles of octopamine formed per hr in each of the fractions. Dopamine-β-hydroxylase was assayed in the presence of 5, 50, 250 and 500 μM ^{3}H-tyramine, respectively, for experiments 1, 2, 3 and 4. Different vesicle preparations were used in each of the experiments.

protein, ATP and catecholamines from isolated vesicles. Since dopamine-β-hydroxylase and catecholamines are released upon stimulation of perfused adrenal glands with acetylcholine in the same ratios as they are found in the soluble fraction of lysed storage vesicles,[23] it was of interest to determine whether the enzyme was also released by the action of Mg^{2+} plus ATP on the isolated storage vesicles. The data of Table 2 show that Mg^{2+} plus ATP causes a release of dopamine-β-hydroxylase and catecholamines in the same proportion as that found in the soluble fraction of

the vesicles. When P-286 or prenylamine was added to the medium, there was a proportionate decrease in the release of dopamine-β-hydroxylase and catecholamines.

Effect of drugs on ATPase. None of the drugs at the concentrations shown in Table 1, except *N*-ethylmaleimide, had any effect on the ATPase activity of the storage vesicles.

DISCUSSION

The effects of the various drugs suggest that the mechanisms of the Mg^{2+}-ATP stimulated incorporation and release of catecholamines may not be as dissimilar as they appear, and also suggest that each of these processes may employ a similar or identical set of reactions for a portion of their respective pathways. *N*-Ethylmaleimide and P-286 each caused a parallel inhibition of release and incorporation by the isolated vesicles. However, the mechanism by which these two drugs act would seem to be different, indicating at least two reactions for incorporation and for release. *N*-Ethylmaleimide, at concentrations which block release and incorporation, inhibits the Mg^{2+}-ATPase activity of the storage vesicles, but P-286, as well as prenylamine, propranolol and reserpine, had no effect on the ATPase activity. The inhibition of incorporation by reserpine, prenylamine and propranolol at concentrations which had little or no effect on release further suggests that a third reaction is required for incorporation.

The inhibition of both release and secretion by *N*-ethylmaleimide, P-286 and prenylamine is consistent with the hypothesis[5, 15] that the Mg^{2+}-ATP stimulated release observed in isolated vesicles is an event of the secretory response. However, since *N*-ethylmaleimide reacts with a wide variety of sulfhydryl or imidazole groups within the cell and upon the cell surface, and since propranolol as well as several of the other agents tested had no effect on release but did block secretion, it is not unlikely that *N*-ethylmaleimide, P-286 and prenylamine may also block secretion at some site remote from the storage vesicle.

Since the vesicle membrane is permeable to catecholamines and to ATP at or near 0° in sucrose media,[24–26] it is presumed that the vesicle membrane is also permeable to these materials in both sucrose and salt media at higher temperatures. If this is true, then the stimulation of both incorporation and release by Mg^{2+} plus ATP would not involve enhanced transport across the vesicle membrane. Stjärne[27] has proposed that the catecholamines, ATP and protein may be stored in membrane-bound subgranules within the vesicles and that Mg^{2+}-ATP stimulates transport into the subgranules. Since a membrane-bound ATPase activity appears to be involved in both release and incorporation, Stjärne's proposal offers a localization for the enzyme.[27] However, the presence of subgranules is not necessary for this requirement; the enzyme may be bound to an intravesicular reticulum forming a three-dimensional web containing the amine storage complex. Neither subvesicular structures nor a reticulum has been observed within the storage vesicles.

The effects of the different compounds suggest the following hypothesis as a tentative model for the release and incorporation of catecholamines:

(1) ATP, in a reaction that requires Mg^{2+}, either produces an intermediate or induces a conformational change in a protein. In this process ATP is hydrolyzed to ADP.

(2) The intermediate or the modified protein is in a high-energy state. In a subsequent

reaction this energy is utilized to activate the catecholamine-ATP-protein storage complex.

(3) In the "activated state" the complex is labile and can exchange with or incorporate exogenous amines from media of low ionic strength.

(4) In media of isoionic strength, univalent cations (K^+, Na^+, perhaps others) compete with the amines for exchange with the "activated complex," or the presence of a high concentration of univalent cations can, by itself, disrupt the "activated" complex. The increased osmolarity due to release of catecholamines and ATP within the vesicles, and the subsequent influx of water may result in further disruption of the complex leading to lysis of the vesicles or distension of the vesicle surface to the extent that they become permeable to protein. This sequence of events may explain the observed release of catecholamines, ATP and protein by the isolated vesicles. In the intact gland, the disruption of the complex and the influx of water after the vesicles have become attached to the plasma membrane may be an important physiological mechanism for the extrusion of the vesicle contents to the exterior of the cell.

In this model *N*-ethylmaleimide would inhibit both incorporation and release by blocking the utilization of ATP in the first step in the sequence. P-286 and prenylamine would inhibit the second step in the sequence, the reaction in which the storage complex is activated. Propranolol and reserpine would block the third stage of the sequence, the reaction in which the exogenous amines exchange with the endogenous amines, but would not affect the exchange with, or disruption of, the complex by univalent metal ions. Prenylamine inhibited incorporation more effectively than release and may inhibit both the second and third stages. This hypothesis predicts that all drugs which block the Mg^{2+}-ATP stimulated release must also block the Mg^{2+}-ATP stimulated incorporation but other models may equally well explain the observed phenomena.

It is generally accepted that the Mg^{2+}-ATP stimulated incorporation is physiologically important for maintaining the catecholamine content of the storage vesicles and for taking up dopamine from the cytoplasm for synthesis of noradrenaline. Whether the Mg^{2+}-ATP stimulated release plays a role in the secretory response or whether it is an artifact remains to be established.

REFERENCES

1. N. Kirshner, *J. biol. Chem.* **237**, 2311 (1962).
2. A. Carlsson, N.-Å. Hillarp and B. Waldeck, *Acta physiol. scand.* **59**, suppl. 215, 5 (1963).
3. M. Oka, O. Ohuchi, H. Yoshida and R. Imaizumi, *Life Sci.* **5**, 433 (1966).
4. M. Oka, O. Takeshi, H. Yoshida and R. Imaizumi, *Jap. J. Pharmac.* **17**, 199 (1967).
5. A. M. Poisner and J. M. Trifaro, *Molec. Pharmac.* **3**, 561 (1967).
6. W. W. Douglas, A. M. Poisner and R. P. Rubin, *J. Physiol.* **179**, 130 (1965).
7. W. W. Douglas and A. M. Poisner, *J. Physiol.* **183**, 236 (1966).
8. P. Banks and K. Helle, *Biochem. J.* **97**, 40C (1965).
9. N. Kirshner, H. J. Sage, W. J. Smith and A. G. Kirshner, *Science, N.Y.* **154**, 129 (1966).
10. N. Kirshner, H. J. Sage and W. J. Smith, *Molec. Pharmac.* **3**, 254 (1967).
11. H. Blaschko, R. S. Comline, F. H. Schneider, M. Silver and A. D. Smith, *Nature, Lond.* **215**, 58 (1967).
12. F. H. Schneider, A. D. Smith and H. Winkler, *Br. J. Pharmac.* **31**, 94 (1967).
13. N. Kirshner and W. J. Smith, *Science, N.Y.* **154**, 422 (1966).
14. R. P. Rubin, *Fedn Proc.* **28**, 288 (1969).
15. A. M. Poisner and W. W. Douglas, *Molec. Pharmac.* **4**, 531 (1968).
16. U. S. von Euler and U. Hamberg, *Science, N.Y.* **110**, 561 (1949).

17. D. S. DUCH, O. H. VIVEROS and N. KIRSHNER, *Biochem. Pharmac.* **17**, 255 (1968).
18. N. KIRSHNER, A. G. KIRSHNER and D. L. KAMIN, *Biochim. biophys. Acta* **113**, 332 (1966).
19. O. H. VIVEROS, L. ARQUEROS, R. J. CONNETT and N. KIRSHNER, *Molec. Pharmac.* **5**, 69 (1969).
20. N. KIRSHNER, C. HOLLOWAY, W. J. SMITH and A. G. KIRSHNER, in *Mechanism of Release of Biogenic Amines* (Eds. U. S. VON EULER, S. ROSELL and B. UVNÄS) p. 109. Pergamon Press, Oxford (1966).
21. A. V. JUORIO and M. VOGT, *Br. J. Pharmac.* **24**, 566 (1965).
22. S. D. JAANUS, E. MIELE and R. P. RUBIN, *Br. J. Pharmac.* **31**, 319 (1967).
23. O. H. VIVEROS, L. ARQUEROS and N. KIRSHNER, *Life Sci.* **7**, 609 (1968).
24. A. CARLSON and N.-Å. HILLARP, *Acta Physiol. scand.* **44**, 163 (1958).
25. N.-Å. HILLARP, *Acta physiol. scand.* **47**, 271 (1959).
26. N. KIRSHNER, C. HOLLOWAY and D. L. KAMIN, *Biochim. biophys. Acta* **112**, 532 (1966).
27. L. STJÄRNE, *Acta physiol. scand.* **62**, suppl. 228 (1964).