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The Stimulation of Cathecholamine Release from Chromaffin Granules by Valinomycin

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SUMMARY

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Valinomycin stimulates the release of catecholamine from isolated guinea pig adrenal medullary chromaffin granules incubated *in vitro*. Valinomycin-stimulated catecholamine release is dependent on the ionophore concentration and on the concentration of K^+ in the incubation medium. In. 0.15 M KCl–5 mM $Na{\cdot}PO_4$, pH 7.0, valinomycin at 1 μM increases catecholamine release about 5-fold. The finding that catecholamine release is accompained by the release of adenine nucleotides and of dopamine β-hydroxylase from the chromaffin granules indicates that valinomycin induces the lysis of the granules. The effect of valinomycin on catecholamine release is also dependent upon the anions present in the incubation medium. The anions tested support valinomycin-stimulated catecholamine release in the order SCN^-, I^-, $Br^- > Cl^-$ > acetate, F^-, isethionate. These data suggest that, in the presence of a membrane-permeable anion, valinomycin-mediated transport of K^+ into chromaffin granules leads to the osmotic lysis of these granules.

INTRODUCTION

Recent studies have shown that the ionophore lasalocid (X-537A) stimulates the release of catecholamines from isolated chromaffin granules *in vitro* (1, 2).[3] Because lasalocid is known to form lipid-soluble complexes with catecholamines (3), it has been proposed that its effect is due, at least in part, to an ion-exchange mechanism: lasalocid transports extragranular cations into the granules in exchange for catecholamines (1, 2). In the course of our studies on the effects of lasalocid on chromaffin granules, we observed that the specific K^+ ionophore, valinomycin, also stimulated the release of catecholamines from chromaffin granules. This paper reports our investigation of this phenomenon.

MATERIALS AND METHODS

Chromaffin granules were isolated from guinea pig adrenal glands. Adrenal glands were homogenized in 0.25 M sucrose–0.05 M Tris HCl, pH 7.4, in a Potter-Elvehjem homogenizer with a Teflon pestle. The ho-

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mogenate was centrifuged for 10 min at 800 × g. The supernatant fraction was diluted 5-fold with sucrose–Tris and then centrifuged for 10 min at 20,000 × g. The particulate fraction obtained after this centrifugation was washed once by centrifugation in 0.3 M sucrose and then resuspended in 0.3 M sucrose. All steps in the isolation procedure were done at 4°, and the chromaffin granules were maintained at 0–4° until use. Most experiments were performed with freshly isolated granules, and all experiments were performed within 30 hr after isolation of the granules.

Aliquots of the chromaffin granule suspensions, containing 3–5 nmoles of catecholamine (about 0.1 mg of protein), were incubated under the conditions described below. Unless otherwise indicated, incubations were carried out for 5 min at 30°, in a final volume of 0.1 ml. Incubations were terminated by the addition of 0.5 ml of ice-cold 0.27 M sucrose–5 mM $Na{\cdot}PO_4$, pH 7.0. After centrifugation of the incubation mixtures for 10 min at 20,000 × g, the supernatants were assayed for released chromaffin granule constituents. Catecholamines were assayed by the method of Anton and Sayre (4). Adenine nucleotides were measured by the luciferase method of Cole *et al.* (5), using the adaptations introduced by Imai *et al.* (6). Dopamine β-hydroxylase (EC 1.14.17.1) was assayed by a modification of the method of Nagatsu and Udenfriend (7). In this modification the tyramine concentration was increased to 40 mM, and octopamine was eluted from the cation-exchange columns with 3 ml of 4 M NH_4OH. One unit of dopamine β-hydroxylase activity catalyzes the formation of 1 nmole of octopamine per minute. Release was estimated after subtraction of the small amounts of these substances which were found in unincubated control samples. All experiments were performed at least three times.

Valinomycin (Calbiochem), a gift from Dr. T. H. Wilson, Harvard Medical School, was dissolved in ethanol at a concentration of 1 mM. The final concentration of ethanol in the experiments never exceeded 1%; control experiments showed that this concentration of ethanol had no effect on the release of catecholamines from chromaffin granules. All other chemicals were reagent grade. Glass-distilled water was used throughout.

RESULTS

When chromaffin granules are incubated at 30° in 0.15 M KCl–5 mM $Na{\cdot}PO_4$, pH 7.0, they release a small percentage of their stored catecholamines into the incubation medium. The addition of valinomycin greatly increases the rate of catecholamine release. Catecholamine release is dependent both on the time of incubation (Fig. 1) and on the valinomycin concentration (Fig. 2). At a concentration of 1 μM, valinomycin stimulates catecholamine release about 5-fold. Effects of valinomycin can be detected at ionophore concentrations as low as 50 nM. At high concentrations of valinomycin, all the catecholamines in the granules can be released within 5 min. In the experiment illustrated in Fig. 2, valinomycin had little effect on catecholamine release in 5 min at 0°. However, valinomycin does cause a significant increase in catecholamine release after prolonged incubation (30–45 min) at 0°. The stimulation of catecholamine release by valinomycin is not inhibited by 0.2 mM N-ethylmaleimide, by 0.01 mM reserpine, or by 1 mM EDTA (not shown).

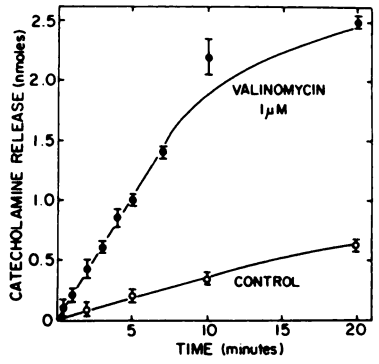

FIG. 1. *Time course of valinomycin-induced catecholamine release*

Chromaffin granules containing 3 nmoles of catecholamine were incubated at 30° in 0.15 M KCl–5 mM $Na{\cdot}PO_4$, pH 7.0, in the presence (●) and absence (○) of valinomycin (1 μM). Catecholamine release is expressed as the means ± standard errors of four samples.

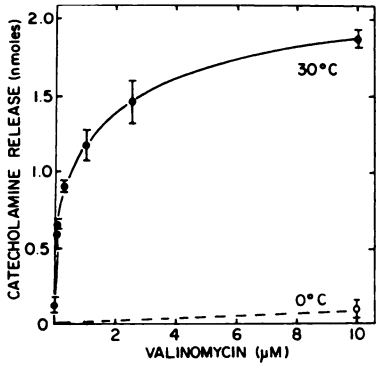

FIG. 2. *Dependence of catecholamine release on valinomycin concentration*

Chromaffin granules containing 3 nmoles of catecholamine were incubated for 5 min at 30° (●) or at 0° (○) in 0.15 M KCl–5 mM $Na{\cdot}PO_4$, pH 7.0, containing valinomycin at the concentrations indicated. Catecholamine release is expressed as the means ± standard errors of five samples.

If valinomycin-stimulated catecholamine release is due to the function of valinomycin as a K^+ ionophore, the effect of valinomycin should be a function of the K^+ concentration in the incubation medium. The data in Fig. 3 indicate that this is the case: valinomycin-induced catecholamine release is dependent upon the concentration of K^+ in the incubation medium.

It was of interest to determine whether valinomycin would cause the release of other chromaffin granule constituents, in addition to catecholamines. Attempts to measure ATP release were frustrated by the rapid hydrolysis of ATP in our incubation mixtures. We therefore chose to measure total adenine nucleotide release from the granules. Valinomycin stimulates the release of adenine nucleotides from chromaffin granules (Table 1). In the presence of valinomycin, catecholamines and adenine nucleotides are released in a molar ratio of 4.6, which is similar to the ratio in which they are stored in chromaffin granules (8). Valinomycin causes the release of 0.86 unit of dopamine β-hydroxylase per nanomole of catecholamine. Again, this ratio is similar to the ratio of dopamine β-hydroxylase activity to catecholamine content in the soluble fraction of the granules. Lysis of the granules in hypotonic media results in the release of 0.95 unit of dopamine β-hydroxylase per nanomole of catecholamine (about 85% of the total dopamine β-hydroxylase activity in the granules is released by hypotonic lysis).

We then investigated the effects of anions on valinomycin-induced catecholamine release. In these experiments chromaffin granules were incubated with valinomycin in 0.15 M solutions of a variety of K^+ salts. All samples were buffered with 5 mM $Na{\cdot}PO_4$, pH 7.0, and had a final pH between 7.0 and 7.2. Changes in pH over this range had no significant effect on the stability of the granules. Valinomycin-induced catecholamine release is pronounced in KSCN, KI, and KBr, but is small in potassium isethionate (Table 2). The order of effectiveness of various anions in supporting this action of valinomycin is, approximately, SCN^-, I^-, $Br^- > Cl^- >$ acetate, F^-, isethionate. Valinomycin also has little effect on catecholamine release in 0.137 M $K{\cdot}PO_4$, pH 7 (not shown). KSCN also promotes catecholamine release in the absence of valinomycin, but the effect of KSCN alone is small compared to the effect of valinomycin. The mechanism of this effect of KSCN is not known.

The anion dependence of valinomycin-induced catecholamine release was studied

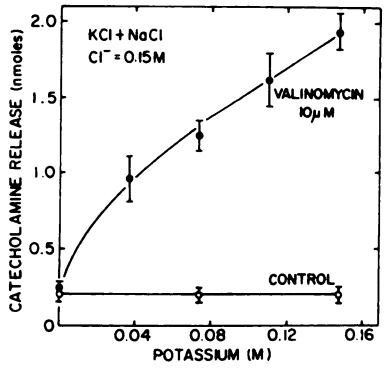

FIG. 3. *Potassium dependence of valinomycin-induced catecholamine release*

Chromaffin granules containing 3 nmoles of catecholamine were incubated for 5 min at 30° in mixtures of NaCl and KCl, in the presence (●) and absence (○) of valinomycin (10 μM). The chloride concentration was maintained constant at 0.15 M. All samples contained, in addition, 5 mM $Na{\cdot}PO_4$, pH 7.0. Catecholamine release is expressed as the means ± standard errors of six samples.

TABLE 1

Valinomycin-induced release of catecholamines, adenine nucleotides, and dopamine β-hydroxylase

Chromaffin granules were incubated for 5 min at 30° in 0.15 M KCl–5 mM $Na{\cdot}PO_4$, pH 7.0, with and without valinomycin (10 μM). In experiment 1 the release of catecholamines and of adenine nucleotides was measured. The granules used in this experiment contained 5 nmoles of catecholamine per sample, and were incubated in a volume of 0.5 ml. In experiment 2 the release of catecholamines and of dopamine β-hydroxylase was measured. The granules used in this experiment contained 4 nmoles of catecholamine per sample, and were incubated in a volume of 0.1 ml. The data are expressed as the means ± standard errors of four samples.

Conditions	Catecholamine release	Adenine nucleotide release	Catecholamine/adenine nucleotide
	nmoles	*nmoles*	*moles/mole*
Experiment 1			
Control	0.39 ± 0.06	0.25 ± 0.09	1.6
Valinomycin	4.60 ± 0.34	1.01 ± 0.11	4.6

Conditions	Catecholamine release	Dopamine β-hydroxylase release	Dopamine β-hydroxylase/catecholamine
	nmoles	*units*	*unit/nmole*
Experiment 2			
Control	0.36 ± 0.10	0.07 ± 0.02	0.19
Valinomycin	1.90 ± 0.16	1.64 ± 0.11	0.86

TABLE 2

Effect of anions on valinomycin-induced catecholamine release

Chromaffin granules containing 4 nmoles of catecholamine were incubated for 5 min at 30°, with and without valinomycin (10 μM), in 0.15 M solutions of the potassium salts listed. All samples contained 5 mM $Na{\cdot}PO_4$, pH 7.0, and had a final pH between 7.0 and 7.2. Catecholamine release from the granules is expressed as the means ± standard errors of the number of samples in parentheses.

Salt	Catecholamine release	
	No valinomycin	10 μM valinomycin
	nmoles	
KSCN	0.68 ± 0.05	3.31 ± 0.09 (8)
KI	0.23 ± 0.04	3.27 ± 0.13 (15)
KBr	0.36 ± 0.09	3.07 ± 0.11 (17)
KCl	0.20 ± 0.02	1.90 ± 0.07 (15)
K acetate	0.20 ± 0.03	0.49 ± 0.08 (7)
KF	0.17 ± 0.03	0.32 ± 0.04 (7)
K isethionate	0.10 ± 0.04	0.21 ± 0.06 (8)

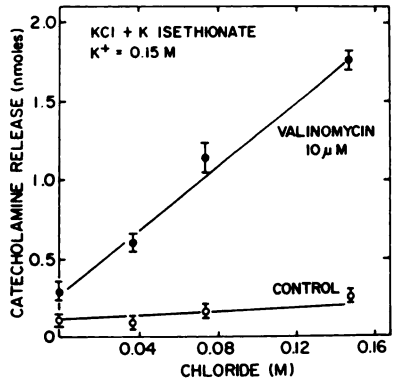

FIG. 4. *Chloride dependence of valinomycin-induced catecholamine release*

Chromaffin granules containing 3 nmoles of catecholamine were incubated for 5 min at 30° in mixtures of KCl and potassium isethionate, in the presence (●) or absence (○) of valinomycin (10 μM). The potassium concentration was maintained constant at 0.15 M. Each sample contained, in addition, 5 mM $Na{\cdot}PO_4$, pH 7.0, and had a final pH between 7.0 and 7.2. Catecholamine release is expressed as the means ± standard errors of four samples.

in greater detail in the experiment illustrated in Fig. 4. In this experiment chromaffin granules were incubated with valinomycin in buffers containing mixtures of KCl and potassium isethionate, such that the final K^+ concentration was maintained at 0.15 M. As before, all samples were buffered with 5 mM $Na{\cdot}PO_4$, pH 7.0, and had a final pH between 7.0 and 7.2. Catecholamine release is small in the absence

of Cl^-, and increases with increasing concentrations of Cl^-

DISCUSSION

Valinomycin is known to form lipid-soluble complexes with K^+ and to facilitate the transport of K^+ across both artificial and biological membranes (9). Because the stimulation of catecholamine release by valinomycin is dependent upon the concentration of K^+ in the incubation medium, it seems very likely that this effect of valinomycin is due to its function as a K^+ ionophore, and not to some unrelated biological activity. There are several plausible mechanisms by which the entry of K^+ into chromaffin granules might lead to the release of catecholamines from these granules. The entry of K^+ might lead to the release of catecholamines by an ion-exchange mechanism, such as that proposed to explain the action of lasalocid (1, 2). Alternatively, the entry of K^+ might alter the electrical potential across the chromaffin granule membranes in such a way as to promote the release of catecholamines. Finally, the entry of K^+ into chromaffin granules could lead to the osmotic lysis of these granules. Our experiments are most consistent with the osmotic lysis hypothesis. If valinomycin caused catecholamine release by an ion-exchange mechanism or by changing the chromaffin granule membrane potential, its action would be independent of the anions in the incubation medium. In contrast, osmotic lysis would require the entry of anions as well as of K^+ into the chromaffin granules, and so should occur only in the presence of membrane-permeable anions. SCN^- and I^-, which support valinomycin-induced catecholamine release, are lipid-soluble anions, which are relatively permeant to many membranes (10); isethionate, which does not support this effect of valinomycin, is a large, polar anion which would not be expected to cross membranes readily. Although we have no independent estimate of the permeability of the chromaffin granule membrane to anions, our data suggest that, in the presence of membrane-permeable anion, valinomycin-induced entry of K^+ into chromaffin granules leads to the osmotic lysis of these granules. The concurrent release of catecholamines, adenine nucleotides, and dopamine β-hydroxylase supports the conclusion that valinomycin actually produces lysis of the granules. The finding that the effect of lasalocid on chromaffin granules occurs equally well in the presence of NaCl and of sodium isethionate[4] is consistent with the idea that valinomycin and lasalocid cause the release of catecholamines by different mechanisms.

The fact that valinomycin causes the lysis of guinea pig chromaffin granules implies that these granules are normally impermeable to K^+. Carlsson and Hillarp reported that bovine chromaffin granules are permeable to KCl, NaCl, and sucrose (11). There is no obvious explanation for their results. However, the known stability of freshly prepared chromaffin granules in isotonic solutions of these compounds (12) is incompatible with the idea that the granules are permeable to these substances. The stability of chromaffin granules *in vivo* must depend upon their being impermeable to the K^+ salts present within the chromaffin cells. It is possible that an alteration in the permeability of chromaffin granules may be involved in the process of catecholamine secretion. If ions enter the chromaffin granules after they have fused with the plasma membrane, secretion could occur by the osmotic lysis of these granules. Experiments to test this possibility are currently in progress in our laboratory.

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REFERENCES

1. Johnson, R. G. & Scarpa, A. (1974) *FEBS Lett.*, 47, 117–121.
2. Holz, R. W. (1975) *Biochim. Biophys. Acta*, 375, 138–152.
3. Pressman, B. C. (1973) *Fed. Proc.*, 32, 1698–1703.
4. Anton, A. H. & Sayre, D. F. (1962) *J. Pharmacol. Exp. Ther.*, 138, 360–375.

[4] R. L. Perlman, unpublished observations.

5. Cole, H. A., Wimpenny, J. W. T. & Hughes, D. W. (1967) *Biochim. Biophys. Acta*, **143**, 445–453.
6. Imai, S., Riley, A. L. & Berne, R. M. (1964) *Circ. Res.*, **15**, 443–450.
7. Nagatsu, T. & Udenfriend, S. (1972) *Clin. Chem.*, **18**, 980–983.
8. Hillarp, N.-Å. (1958) *Acta Physiol. Scand.*, **42** 321–332.
9. Pressman, B. C. (1968) *Fed. Proc.*, **27**, 1283–1288.
10. Davson, H. & Danielli, J. F. (1952) *The Permeability of Natural Membranes*, Ed. 2, Cambridge University Press, Cambridge.
11. Carlsson, A. & Hillarp, N.-Å. (1958) *Acta Physiol. Scand.*, **44**, 163–169.
12. Hillarp, N.-Å. (1958) *Acta Physiol. Scand.*, **43**, 292–302.