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A CALCIUM IONOPHORE STIMULATING THE SECRETION OF CATECHOLAMINES FROM THE CAT ADRENAL

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SUMMARY

1. Experiments were performed on perfused cat adrenal glands to examine the effect of a calcium ionophore A-23187 in the secretion of catecholamines.

2. Ionophore (1–10 μM) caused a dose-dependent release of catecholamines and the output was about 100-fold greater at 10 μm than at 1 μm.

3. Release of catecholamines by the ionophore was dependent on the calcium concentration of the perfusion medium. Omission of calcium blocked the response to the ionophore while excess calcium facilitated it.

4. Magnesium antagonized the secretory response to the ionophore. Excess calcium overcame the inhibitory effect of magnesium.

5. The ionophore did not modify release of catecholamines by induced splanchnic nerve stimulation.

6. The results suggest that the ionophore, like depolarization, introduces calcium into the chromaffin cell to cause release of catecholamines.

INTRODUCTION

Secretion of catecholamines from the adrenal gland elicited by acetylcholine or high potassium concentrations is mediated by the influx of calcium ions from the extracellular medium (Douglas, 1968). It has been recently shown that ionophores which act as cation carriers selectively increase the permeability of cell membranes to several ions (Pressman, Harris, Jagger & Johnson, 1967; Pressman, 1973). The antibiotics X537A (Hoffman–La Roche) and A-23187 (Eli Lilly) increase the permeability of membranes to calcium and other divalent ions. As an example of their biologic activity, both ionophores have been shown to cause release of histamine from mast cells (Foreman, Mongar & Gomperts, 1973; Cochrane & Douglas, 1974), A-23187 to cause a massive release of potassium from

parotid glands (Sellinger, Eimerl & Schramm, 1974), and X537A to increase tension and contracture of skeletal and cardiac-muscle preparations (Levy, Cohen & Inesi, 1973). Since calcium is required for the release of catecholamines from the adrenal medulla, and since A-23187 selectively increases the permeability of cells to calcium ions, we have studied the effects of this ionophore on catecholamine secretion from the perfused cat adrenal gland in an attempt to obtain further insight into the role of calcium ions in stimulus-secretion coupling.

METHODS

Cat adrenals were perfused with Krebs-bicarbonate solution at room temperature essentially by the method of Douglas & Rubin (1961). The rate of perfusion was about 1–2 ml/min. Krebs solution has the following composition: (mmol $l.^{-1}$): NaCl, 119; KCl, 4·17; $MgSO_4.7H_2O$, 1·2; $CaCl_2$, 2·5; KH_2PO_4, 1·2; $NaHCO_3$, 25; glucose, 11; EDTA, 0·03. This solution was equilibrated with 95 % O_2–5 % CO_2, and the final pH was 7·4–7·5. Krebs solution containing greater than 7·5 mM calcium and/or high magnesium was prepared by adding the required amount of calcium and/or magnesium, and isotonicity was maintained by omitting appropriate amounts of sodium. These solutions contained Tris buffer (5 mM) and no bicarbonate or phosphate. They were equilibrated with 100 % O_2 and the pH was adjusted to 7·4 with HCl (1 N).

The ionophore A-23187, which is very poorly soluble in water, was dissolved in ethanol. Small aliquots from this alcoholic solution were then added to the perfusing solution and the final concentration of ethanol did not exceed 1 %. At the concentrations used, ethanol did not release catecholamine in control experiments. Catecholamine release was measured during perfusion with the ionophore solution for 10 min. In later experiments the ionophore solution was perfused for only the first 10 min, and the effects of perfusion with different calcium concentrations on release were studied. Venous samples were continuously collected at intervals of 2 min. Catecholamine (expressed in noradrenaline equivalents) in the venous perfusate was measured by the method described by Anton & Sayre (1962) without the intervening step of alumina adsorption. A-23187 at the concentration used in these experiments did not interfere with the catecholamine assay.

RESULTS

The effect of the ionophore (A-23187) on release of catecholamine

Perfusion with the ionophore caused a concentration-dependent release of catecholamine from the cat adrenal glands. Fig. 1 shows that the catecholamine release was minimal at an ionophore concentration of 1 μm in normal Krebs solution (3 ng/min). As the concentration was increased from 1 to 10 μM, proportionally greater release of catecholamine occurred (335 ng/min). The effect of the ionophore concentration on release was graded and the catecholamine release was about 100-fold greater at 10 μm than at 1 μM. Similar results were obtained in five more experiments.

In order to compare the rate of release of catecholamine by the ionophore with other stimuli, catecholamine release was evoked either by stimulation of the splanchnic nerve (30 Hz) or by infusion of acetylcholine (10^{-5} M); this released 572 ± 96 and 691 ± 177 ng catecholamine/min respectively. The rate of release of catecholamine by the ionophore (10 μM) was usually less than either splanchnic nerve stimulation or acetylcholine infusion.

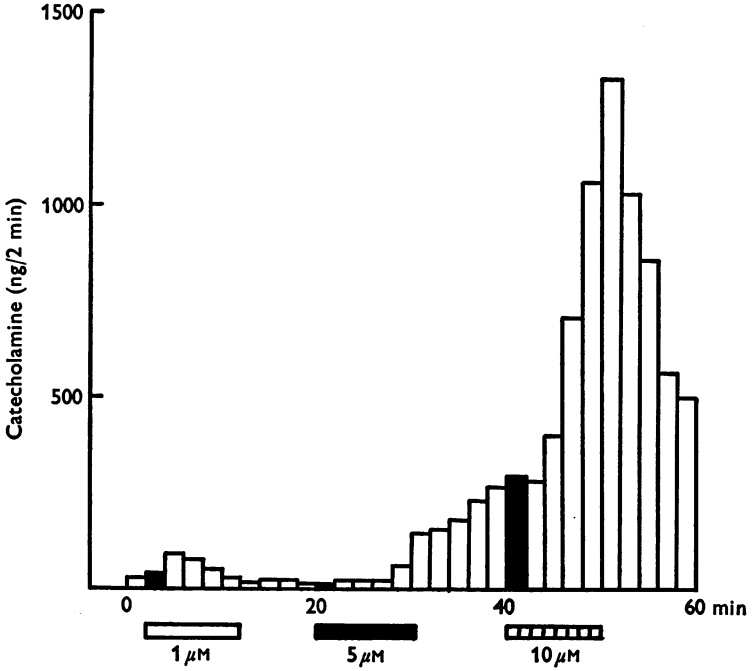

Fig. 1. Effect of different concentrations of A-23187 on catecholamine release from the adrenal medulla. In this and subsequent Figures, each bar represents the catecholamines found in a 2 min collection period. Filled columns represent the first sample collected after introduction of each new concentration of the drug.

The time course of release of catecholamines induced by the ionophore did not show a consistent pattern. In some experiments a high rate of release was maintained throughout the perfusion (10–20 min), while in others, after an initial rise, it fell off somewhat and then increased again. In most experiments the release of catecholamine after the initial 10 min perfusion with the ionophore was sustained for more than 1 h after washout of the drug.

The relationship between calcium and the release of catecholamines induced by the ionophore

Fig. 2 shows that the ionophore failed to release catecholamine when the calcium concentration of the perfusion medium was reduced to zero. During a 10 min perfusion with calcium-free solution the release of catecholamine was almost completely blocked. On reperfusion with normal Krebs solution, release was not only restored but appeared to be slightly potentiated.

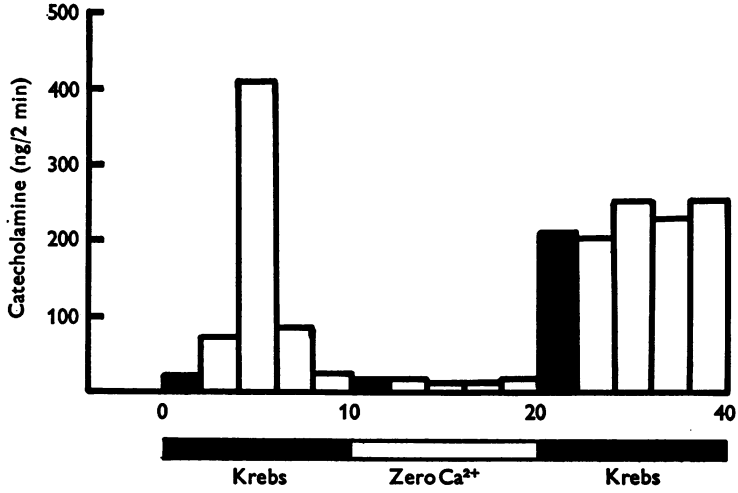

Fig. 2. An experiment showing that the ionophore A-23187 does not release catecholamine in the absence of calcium. An adrenal gland was perfused alternately with Krebs solution and calcium-free Krebs solution containing the ionophore (10 μm) for 10 min. Filled columns represent the first sample collected after introduction of a new solution.

In six experiments we perfused the adrenal gland with 10 μM of the ionophore in zero calcium–Krebs solution for the initial 10 min period. Following washout of the ionophore solution the gland was then perfused successively with Krebs solution containing various concentrations of calcium for 20 min. Because of its essentially irreversible action the ionophore was only initially perfused for 10 min. A typical experiment of the series is shown in Fig. 3. It can be seen that the ionophore did not release catecholamine in the absence of calcium. However, after washout of the ionophore, perfusion with 0·8 mM calcium–Krebs solution markedly enhanced the spontaneous release (87 ng/min). Increasing the calcium concentration of the Krebs solution to 2·5 and 7·5 mM enhanced catecholamine release even further, to 145 ng and 283 ng/min, respectively. Upon finally perfusing the gland once more with calcium-free Krebs solution, the

output was depressed almost completely after about 10 min. The initial release during the first 10 min of this final perfusion of calcium-free Krebs solution probably can be attributed to the delay in washout of high calcium from the extracellular spaces of this organ.

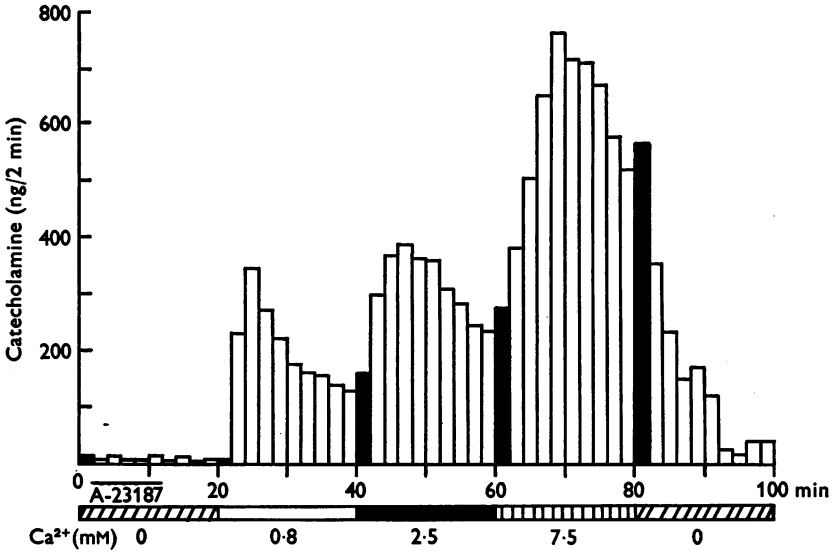

Fig. 3. The relationship between the calcium concentration of the perfusion solution and catecholamine secretion after the initial exposure to A-23187 (10 μm for 10 min). Filled columns represent the first sample collected after introduction of each calcium concentration, as indicated by the horizontal bars.

In four experiments, perfusion of the adrenal with graded calcium concentrations ranging from 0 to 22·5 mM for periods of 10 min after an initial 10 min perfusion with the ionophore (10 μm) in calcium-free medium caused a very marked and graded increase in catecholamine secretion. The outputs in different calcium concentrations were expressed as a percentage of the initial output of catecholamine in zero calcium solution. Thus, the outputs in 0·8, 2·5, 7·5 and 22·5 mM calcium-Krebs solutions were approximately 7-, 11-, 22- and 100-fold greater than the output obtained during perfusion with zero mM calcium-Krebs solution. Reperfusion with calcium-free Krebs solution for 10–15 min after perfusion with 22·5 mM calcium solution abolished release.

It should be pointed out that perfusion of the adrenal gland with the same graded calcium concentrations for 10 min periods, without the prior perfusion of ionophore solution, did not induce secretion of catecholamine at all under our experimental conditions.

The effect of magnesium on the release of catecholamine induced by the ionophore

A competition between calcium and magnesium has been described for the release of acetylcholine from motor nerves (del Castillo & Engbaek, 1954), catecholamine from adrenal medulla (Douglas & Rubin, 1963) and noradrenaline from post-ganglionic sympathetic nerves (Kirpekar & Misu, 1967; Boullin, 1967). It was therefore of some interest to determine whether excess magnesium would suppress the release catecholamine induced by the ionophore.

In the first group of experiments, the glands were perfused with the ionophore (10 μM) for only 10 min and secretion of catecholamine was obtained during and 10 min after its perfusion. Glands were then perfused with 20 mM magnesium-Krebs solution (containing 2·5 mM calcium) for 20 min and then with normal Krebs solution for a similar period. In three experiments the control output was 81 ± 14 ng/min, which was reduced to 22 ± 12 % during perfusion with 20 mM magnesium. On reperfusion with normal Krebs solution, the outputs were essentially restored.

In the second group of experiments interaction of magnesium and calcium was investigated. The ionophore (10 μM) was perfused for only 10 min and secretion of catecholamines was obtained during these 10 min and during a similar period after its perfusion. Fig. 4 shows that the initial control output was 61 ng/min. During perfusion with 20 mM magnesium-Krebs solution (containing 2·5 mM calcium), the release was depressed (26 ng/min). However, during a simultaneous perfusion with 20 mM magnesium and 25 mM calcium-Krebs solution, not only was the release restored but it was markedly potentiated (127 ng/min). Similar results were obtained in three additional experiments. This experiment shows that the depressant effect of magnesium on release of catecholamine by the ionophore was overcome by increasing the calcium concentration of the magnesium-Krebs solution.

The effect of lanthanum on the release of catecholamines induced by the ionophore

Since lanthanum is known to inhibit transmitter release presumably by competing with calcium (Miledi, 1971; Kirpekar, Prat, Puig & Wakade, 1972) experiments were also performed to study the effect of lanthanum on catecholamine release by the ionophore. These experiments were rather inconclusive since lanthanum (1 mM) itself caused a very marked release of catecholamines. This probably masked any inhibitory effect of this ion on the secretory response to the ionophore. Lanthanum is known to enhance the spontaneous release of acetylcholine at the neuromuscular

junction of the frog (Heuser & Miledi, 1971; Kajimoto & Kirpekar, 1972) and a similar mechanism may probably account for the spontaneous release of catecholamines from the adrenal gland.

The effect of splanchnic nerve stimulation on catecholamine release in the presence of the ionophore

Since the release of catecholamines by the ionophore and splanchnic nerve stimulation have many features in common, it was of some interest to determine whether the release induced by splanchnic nerve stimulation was modified in any way by the ionophore.

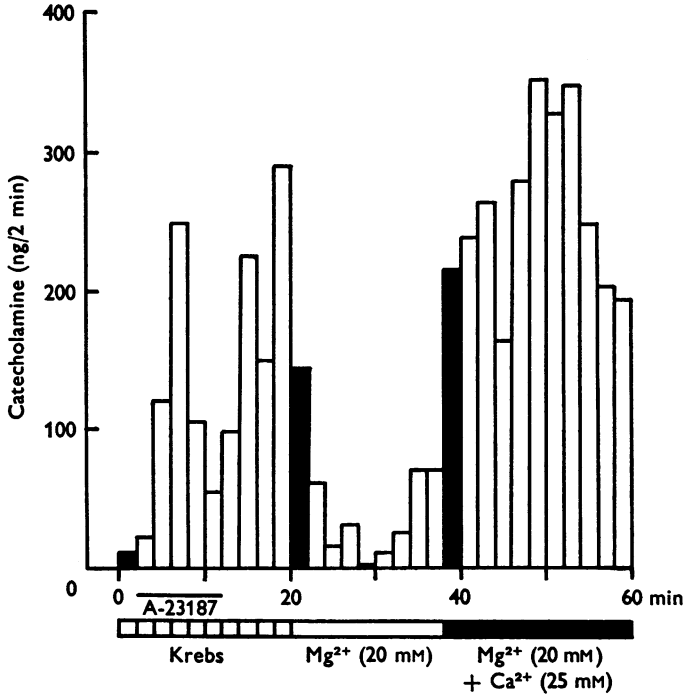

Fig. 4. The interaction between calcium and magnesium on the secretion of catecholamines evoked by A-23187. Filled vertical bars represent the first sample collected after introduction of each new solution.

In four experiments, the output of catecholamines in response to splanchnic nerve stimulation (5 Hz for 1 min) without the ionophore was $2 \pm 0{\cdot}46$ μg/min, which was not appreciably altered after treatment of the gland with the ionophore ($1{\cdot}86 \pm 0{\cdot}5$ μg/min). In one experiment when the nerve was stimulated at 30 Hz for 1 min the control output of 2·78 μg was slightly increased to 3·66 μg after treatment with the ionophore.

DISCUSSION

Even though the importance of calcium in the release of neurotransmitters and hormones has been well established, very little is known about the specific entry of calcium ions during a release process. It is generally believed, from experiments on giant axons and ganglia of the squid, that transmitter release evoked by depolarization is related to the late tetrodotoxin-insensitive phase of calcium entry (Katz & Miledi, 1969; Baker, Meves & Ridgway, 1973). It therefore appears that depolarization is the stimulus for calcium entry, which in turn initiates release of neurotransmitters. The channels through which calcium enters during depolarization appear to be available only from the outside (Miledi & Slater, 1966; Garcia & Kirpekar, 1973), and the intracellular calcium probably is not involved in the secretory process.

The ionophore A-23187 mimicks the effect of depolarization in causing secretion of catecholamines from the adrenal gland. There is thus a striking similarity between secretion of catecholamine induced by splanchnic nerve stimulation or acetylcholine and the ionophore. Release occurs only in the presence of calcium ions, and the release is graded with the calcium concentration of the extracellular medium. Just as acetylcholine-induced secretion of catecholamine is suppressed by magnesium and restored by high calcium (Douglas & Rubin, 1963), the ionophore-induced secretion is blocked by high magnesium and restored by high calcium. Effects of the ionophore on secretion appear to be very specific, since secretion could not be evoked even with high concentrations of this agent in the complete absence of calcium. The ionophore did not appreciably interfere in the release of catecholamine by splanchnic nerve stimulation. A result of this kind would suggest that depolarization would further enhance the calcium permeability over and above the increase due to the action of the ionophore alone, and that the ionophore probably did not cause release by depolarization of the chromaffin cell membrane.

Our interpretation of these results is based on the knowledge that A-23187 can facilitate transport of calcium and magnesium ions (Reed & Lardy, 1972), and because of the well known role of calcium in secretory systems, we favour calcium as the most likely ion involved. Since secretion of catecholamine does not occur by merely increasing the calcium concentration of the perfusion medium, and since it does occur in the presence of the ionophore and is related to the extracellular calcium concentration, it would seem that the ionophore probably transports calcium across biological membranes. Because of the ability of A-23187 to bind calcium and solubilize the ion in an organic phase, the possibility exists that it is a carrier of calcium ions.

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