CATECHOLAMINE EQUILIBRATION GRADIENTS OF ISOLATED CHROMAFFIN VESICLES INDUCED BY THE IONOPHORE X-537A

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1. Introduction

The ionophore X-537A, a monocarboxylic acid antibiotic, is able to form lipophilic complexes with monovalent and divalent ions [1,2]. A number of studies have reported the ability of X-537A to increase calcium fluxes through certain biological membranes such as mitochondria [3] and sarcoplasmic reticulum [4,5].

More recently, X-537A has been used to investigate the role of Ca^{2+} as secretagogue of neurotransmitters and hormones from endogenous stores of perfused organs. Accumulating evidence has shown that X-537A prompts a release of histamine from mast cells [6,7], neurotransmitters from chick retina [8], vasopressin from neurohypophysis [9], norepinephrine from peripheral adrenergic neurones [10], and from endogenous stores of myocardia [11]. These studies have interpreted the secretagogue effect of X-537A as being a consequence of Ca^{2+} mobilization across the storage vesicles. However, X-537A was also shown to form lipophilic complexes with biological amines and to transport them across apolar moieties of model systems [11,12]. Direct transport of norepinephrine from endogenous stores of perfused hearts has been recently postulated [13,14].

This study was undertaken to examine the effect of X-537A on catecholamine release from chromaffin vesicles isolated from beef adrenal medulla. The results suggest that X-537A induces catecholamine release directly by facilitating norepinephrine transport across the lipid phase of the membrane, rather than indirectly by increasing the permeability of the vesicles to Ca^{2+}. A preliminary report has already been published [15].

2. Materials and methods

Bovine adrenal medulla chromaffin granules were isolated on a D_2O-Ficoll–Sucrose density gradient by the method of Trifaro and Dworkind [16]. Although expensive, this procedure allows the granules to be isolated in an isotonic medium unlike previous preparatory procedures [17,18]. Once isolated, the granules were suspended in 0.27 M sucrose–10 mM Tris (hydroxymethyl) aminomethane, pH 7.0. The granules were stable for several days if stored at 4°C. After addition of the ionophore, 1 ml samples were taken from the granule suspension and spun down rapidly in an Eppendorf Model 3200 Centrifuge. The catecholamines were assayed fluorimetrically in the supernatant on a Fluorispec Model SF-100 Fluorescence Spectrophotometer by a modified method of von Euler and Lishajko [19] with excitation at 410 nm and emission at 520 nm. All experiments were carried out at 4°C. Protein concentration was measured by the biuret method.

Catecholamine transport across a chloroform phase was measured using the method of Pinkerton et al. [20] in a vessel modified as described previously [11]. Aliquots of the buffers were withdrawn at intervals and counted in a Tricarb scintillation counter for both ^{14}C and ^{3}H.

Norepinephrine HCl and epinephrine bitartrate were purchased from Sigma, St. Louis, Mo. X-537A and its derivatives were generous gifts of Dr J. Berger of Hoffman-La Roche. A23187 and monensin were supplied by Dr R. Hamill of Eli Lilly & Co. d,l-Norepinephrine-7-^{3}H (specific activity 3.7 Ci/mM) and d,l-epinephrine-7-^{14}C (specific activity 55.3 m Ci/mM) were purchased from New England Nuclear.

3. Results

Fig. 1 illustrates the dose-dependent release of catecholamines from isolated chromaffin vesicles induced by X-537A. The addition of various concentrations of X-537A to vesicles incubated at 4°C in the absence of added Ca^{2+} caused, after 30 min, a release of catecholamines comparable to that obtained after extensive ultrasonic disruption of the vesicles. At shorter times, the relase of catecholamines was dependent upon the concentrations of X-537A and was kinetically resolvable. Faster rates of release were observed at higher temperatures. In all the X-537A concentrations, a biphasic release was obtained, which suggested that one catecholamine may be preferentially transported. Bovine chromaffin granules contain 3 times more epinephrine than norepinephrine. On the other hand, it has been reported that, in toluene-butanol, the affinity of X-537A for norepinephrine is 28 times greater than that for epinephrine [12]. Reliable measurements of norepinephrine and epinephrine in the same sample are difficult to obtain and accurate experiments using both radiochemical and fluorescence techniques are now under way to determine whether norepinephrine is preferentially released during the first initial phase after addition of X-537A.

Fig. 2 shows the results of an experiment in which release of endogenous catecholamines was measured

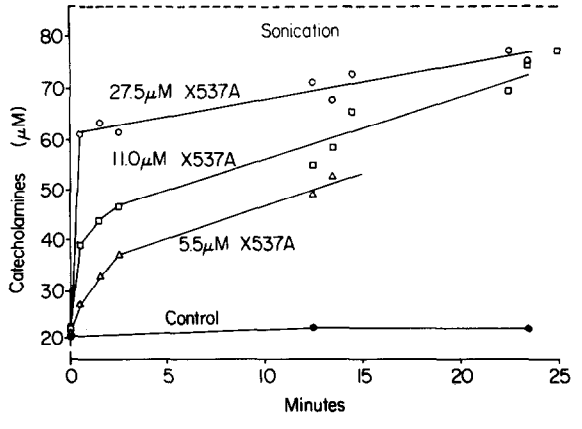

Fig. 1. The effect of various concentrations of X-537A on catecholamine release by chromaffin granules. The reaction mixture contained 270 mM sucrose–10 mM Tris (hydroxymethyl) aminomethane, pH 7.2 and 0.55 mg chromaffin vesicles/ml. Vol. 10 ml. Temperature, 4°C.

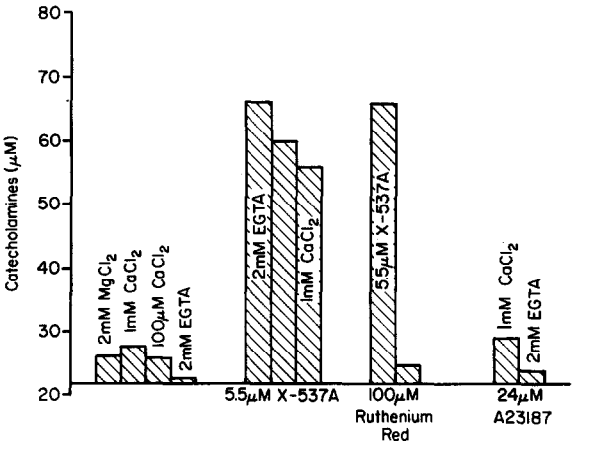

Fig. 2. The release of catecholamines induced by ionophores X-537A and A23187 under the conditions shown. Experimental conditions as in fig. 1. Catecholamine release was measured 25 minutes after the additions reported in the figure.

under various conditions. After 25 minutes of incubation of the granules in an isotonic buffered reaction mixture, only a small fraction of the endogenous catecholamines was found in the supernatant. The amount released was slightly increased in the presence of Ca^{2+} or Mg^{2+}. The presence of X-537A caused a release of catecholamines of about 80% of the total content of isolated chromaffin vesicles. The release was greater and faster in the presence of ethylene glycol-bis(β-aminoethyl ether)*N,N'*-tetracetic acid (EGTA). The presence of Ca^{2+} diminishes both the amount and rate of catecholamine release. The effect of X-537A was insensitive to ruthenium red, a compound known to compete with Ca^{2+} for binding sites and block Ca^{2+} transport in a variety of biological membranes [21,22]. A23187, another ionophore known to equilibrate Ca^{2+} across several biological membranes more effectively than X-537A [23,24], produced only a small release of catecholamines. Even at a concentration of 80 μM (not shown), A23187 was unable to produce a significant release of catecholamines. Contrary to X-537A, the effect of A23187 was greater in the presence of added Ca^{2+}. Other ionophores known through different mechanisms to transport monovalent cations across articial and biological membranes (valinomycin, monencin, nigericin) were without effect, even at high concentrations (5–10 μM). These data suggest that the release of catecholamines induced by X-537A may not

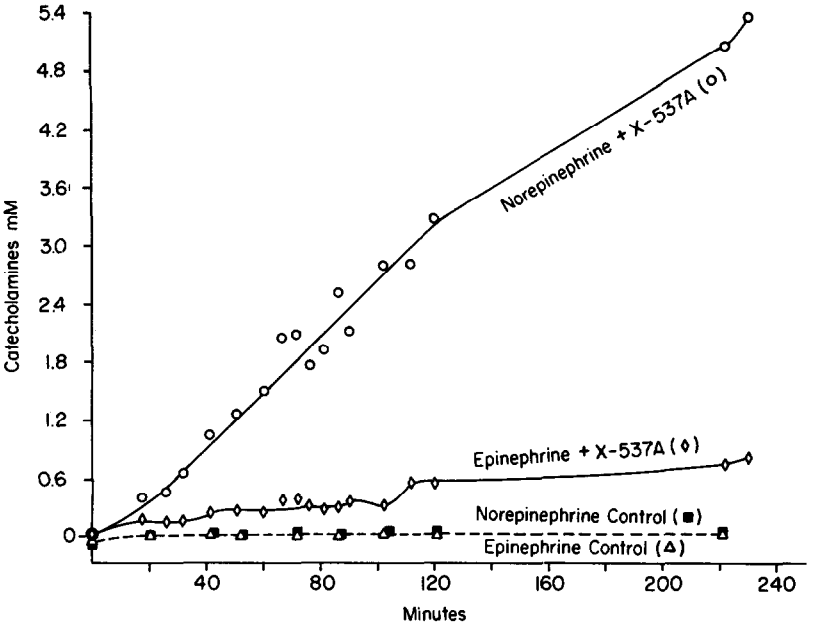

Fig. 3. X-537A-mediated transport of catecholamines across a chloroform phase as described in Materials and methods. 120 mM [^{3}H]norepinephrine and 120 mM [^{14}C]epinephrine plus 5 mM ascorbate and 20 mM Na morpholinopropane sulfonate (MOPS), pH 7.2 were placed in one aqueous phase. Transport was monitored by the appearence of [^{3}H]norepinephrine and [^{14}C]epinephrine in the opposite aqueous compartment. X-537A was added to the chloroform phase such that the final concentration was 330 μM.

be secondary to Ca^{2+} mobilization induced by the ionophore.

The ability of X-537A to directly transport catecholamines across the vesicle membrane and its specificity toward epinephrine–norepinephrine transport were tested in the experiment of fig. 3. A layer of chloroform was used to separate two sides of a U-shaped chamber containing two buffered aqueous phases. [^{3}H]norepinephrine and [^{14}C]epinephrine were added to one of the aqueous phases on one side of the chamber, and aliquots of the other aqueous layer on the other side of the chamber were removed for counting at intervals. X-537A was added to the chloroform layer and the transport of catecholamines across the chloroform phase was measured as the appearance of labelled material in the order aqueous compartment. As shown in the figure, X-537A was able to transfer both epinephrine and norepinephrine through the organic solvent. At equimolar concentrations, the transport of norepinephrine was 3–6 times greater than that of epinephrine. A23187 and other monovalent cation ionophores were without effect. These data show that X-537A is able to transport catecholamines across an apolar moiety and provide further evidence that the release of catecholamine by the granules may result from primary transport through the granules rather than equilibration of Ca^{2+} gradients.

4. Discussion

Calcium's role as releasing element in the adrenal medulla has been well documented [25]. Douglas and Rubin have proposed that calcium acts as such by serving as the stimulus–secretion coupling agent on the medullary chromaffin cell [26]. Release then would be expected by X-537A in the presence of calcium in light of its ability to act as a mobile carrier for calcium across biological membranes [3–5,12]. X-537A was recently shown to release neurotransmitters and hormones from endogenous stores of various tissues and this effect has been attributed to the disturbance of Ca^{2+} gradients induced by the ionophore [6–10].

Our results show that X-537A is able to release

catecholamines from chromaffin vesicles isolated from beef adrenal medulla. The evidence presented also indicates that catecholamine release from the vesicles results primarily from a direct transport of catecholamines across the membranes via X-537A rather than secondarily to an increase of membrane Ca^{2+} permeability induced by the ionophore. This conclusion is based on the following evidence:

(a) The release of catecholamines induced by X-537A was independent of calcium ion concentrations surrounding the vesicles. If there was any effect, Ca^{2+} presence decreased both extent and rate of catecholamine release. This may be attributable to the formation of a complex between calcium and X-537A, with consequent decrease of the amount of ionophore available for the transport of catecholamines.

(b) The effect of X-537A was specific and occurred in a variety of reaction mixtures of different composition. A23187, another ionophore which equilibrates Ca^{2+} gradients across several biological membranes [23,24] was ineffective in releasing catecholamines. Also without effect were several other ionophores which affect protons and monovalent cation distribution across membranes.

(c) X-537A (and no other ionophores) effectively transports both norepinephrine and epinephrine through apolar moieties of model systems (ref. [11,12] and fig. 3).

In view of these results and the ability of X-537A to form lipophilic complexes with several biological amines [12], recent studies which have shown a release of various transmitters induced by X-537A and attributed the release as being secondary to Ca^{2+} mobilization [6,7,10] may require reconsideration. In addition, our results offer a reasonable explanation for the powerful inotropic action of X-537A when injected in perfused heart preparations. Since the effect of X-537A on increasing heart work, flow rate and heart rate was absent in hearts from reserpinized animals and in the presence of β-blocking agents, it was suggested that the inotropic effect of X-537A was due to a mobilization of norepinephrine from myocardial endogenous stores [11,13,14]. The results presented indicate that this mechanism is feasible and that disturbances of Ca^{2+} distribution may not be required.

Compounds that affect specific permeabilities of biological membranes offer tools for perturbing cellular functions and provide useful methods for investigating transport mechanisms. Along this line, X-537A offers a powerful tool for the study of the nature of the secretory processes and storage complexes in chromaffin granules and in other systems.

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