

## EFFECTS OF VERAPAMIL, DANTROLENE AND LANTHANUM ON CATECHOLAMINE RELEASE FROM RAT ADRENAL MEDULLA

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- 1 The release of catecholamines (CA) from rat adrenal incubated *in vitro* in Locke solution was studied.
- 2 Acetylcholine-induced release of CA and CA release by 56 mM KCl were inhibited by verapamil and lanthanum chloride which block calcium permeability.
- 3 CA secretion induced by salbutamol or by theophylline was unaffected by either verapamil or lanthanum chloride.
- 4 Dantrolene-sodium inhibited the CA secretion induced by theophylline but only partially reduced potassium-induced release of CA.
- 5 Verapamil enhanced the secretion of CA induced by salbutamol (in a calcium-free medium).
- 6 Tyramine-induced secretion of CA was unaffected by lanthanum chloride, verapamil or dantrolene-sodium.
- 7 It is suggested that cyclic adenosine 3',5'-monophosphate-mediated CA secretion (induced by theophylline or salbutamol) depends on release of calcium from intracellular stores, and that CA secretion induced by tyramine is independent of intra- or extracellular calcium.

### Introduction

Catecholamine (CA) release from the adrenal medulla can be induced by various methods. Acetylcholine (ACh) and elevated potassium concentrations have long been known to increase CA release (Smith & Winkler, 1972). The effect of these agonists is completely dependent on the presence of  $\text{Ca}^{2+}$  in the incubation medium (Douglas, 1968). On the other hand, tyramine can induce release of CA from adrenergic nerve terminals or to a lesser extent, from adrenal medulla. This release is independent of the presence of  $\text{Ca}^{2+}$  in the medium (Philippu & Schümann, 1968). Other agents acting through increase of cyclic adenosine 3',5'-monophosphate (cyclic AMP), as e.g. theophylline (Peach, 1972) or salbutamol (Boonyaviroj & Gutman, 1977) also increase CA release independently of  $\text{Ca}^{2+}$  in the medium. However, it has been suggested that cyclic AMP affects *intracellular*  $\text{Ca}^{2+}$  by acting on storage or release from intracellular organelles.

To study the possibility of the involvement of intracellular calcium in the action of cyclic AMP on CA release, it seemed to us that the interesting characteristics of two recently introduced drugs would be useful: verapamil, used as an antiarrhythmic drug, has been reported to block the permeability of cell membranes to  $\text{Ca}^{2+}$ , an effect similar to that of lanthanum

(Fleckenstein, 1971). Dantrolene-sodium, a new muscle relaxant, has been reported to increase *intracellular* calcium binding to storage sites and thus reduce the concentration of free  $\text{Ca}^{2+}$  in the cell (Ellis & Carpenter, 1972).

We, therefore, used several stimulants of CA release which act through different mechanisms: ACh and elevated  $\text{K}^{+}$  which are dependent on extracellular calcium; salbutamol, theophylline and tyramine which act in the absence of extracellular calcium. The effect of lanthanum, verapamil and dantrolene-sodium on the release caused by these agents was then studied.

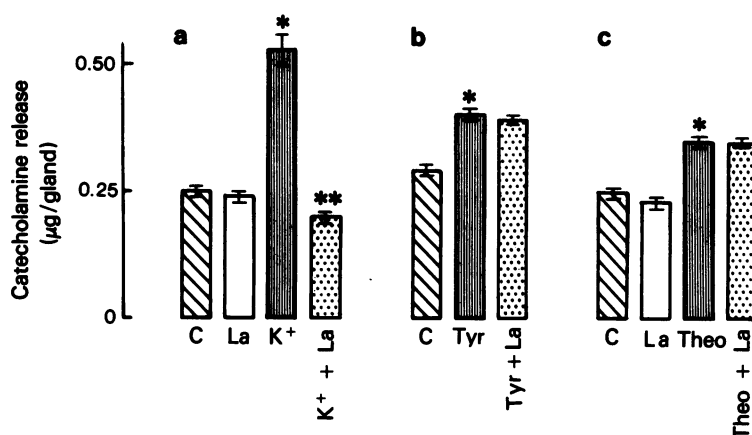
The results presented in this paper indicate that cyclic AMP-dependent release of CA involves intracellular  $\text{Ca}^{2+}$ .

A preliminary account of this work has been presented at the meeting of the Israel Physiological and Pharmacological Society, November, 1977.

### Methods

#### Animals

Male rats of the Hebrew University strain were used throughout (weight 200 to 250 g). Adrenal glands were taken out immediately after dislocation of the



**Figure 1** Effects of lanthanum on catecholamine secretion from rat adrenal gland. (a) Release of catecholamines induced by 56 mM KCl ( $K^+$ ); (b) release of catecholamines induced by  $10^{-3}$  M tyramine HCl (Tyr); (c) release of catecholamines induced by  $10^{-2}$  M theophylline (Theo); control = C; La indicates that lanthanum choride 1 mM was added to the incubation medium. Vertical bars show s.e. mean;  $n = 10$  for each column. \* $P < 0.001$  compared to control; \*\* $P < 0.001$  compared to  $K^+$ .

neck. Each gland was cut into two halves and incubated in a conical flask (50 ml) containing 10 ml of Locke solution, kept in a thermostatic bath ( $37^\circ\text{C}$ ) with constant shaking. Incubation was carried out for 10 min. In experiments where tyramine, salbutamol or theophylline were added, the incubation medium was Ca-free Locke solution to which 1,2-bis,2-aminoethoxyethane-NNN'-tetra-acetic acid (EGTA, final concentration 2 mM) was added. At the end of the incubation, the medium was separated from the gland and CA content was assayed by the trihydroxy-indole method, as previously described (Feuerstein, Boonyaviroj & Gutman, 1977).

### Drugs

Acetylcholine iodide, and theophylline were purchased from Sigma. Salbutamol was a gift from Allen & Hanbury Research Ltd. Verapamil was kindly given by Ikapharm, Israel. Dantrolene-sodium was generously sent by Dr Keith O. Ellis of Norwich Pharmacal. Co., Norwich, New York. Tyramine hydrochloride was purchased from BDH.

Results are expressed as mean  $\pm$  s.e.mean. Student's  $t$  test was used to evaluate differences between control and experimental groups.

### Results

#### *Effect of lanthanum on catecholamine secretion by adrenal medulla*

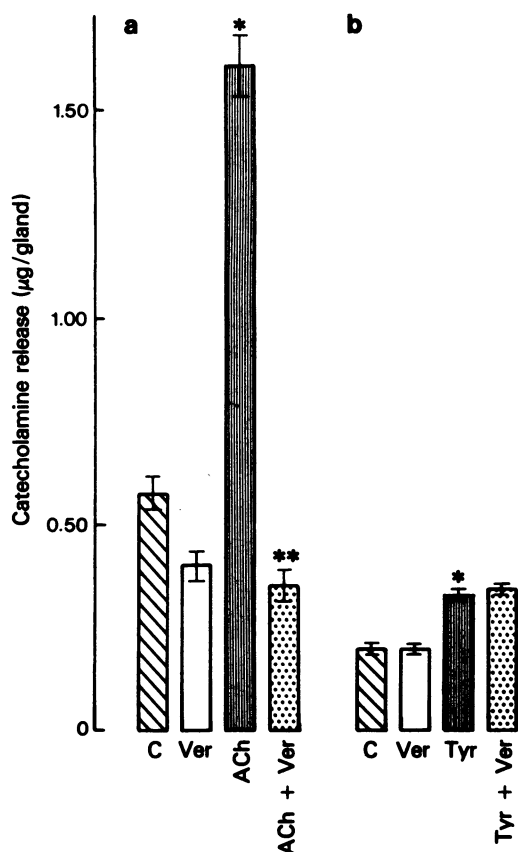
Rat adrenal medulla, incubated *in vitro*, secretes CA into the incubation medium. This secretion can be

stimulated by several methods. Figure 1 shows that increased  $K^+$  concentration in the medium (56 mM), tyramine ( $10^{-3}$  M) and theophylline ( $10^{-2}$  M) stimulated CA secretion. Lanthanum antagonizes the action of Ca in the medium by reducing Ca permeability. Figure 1 shows that lanthanum (1 mM) did not affect spontaneous CA secretion, but completely blocked the secretion of CA caused by 56 mM  $K^+$ . On the other hand, tyramine, which causes CA release by displacing the CA from storage vesicles, was as effective in releasing CA in the presence of lanthanum as in the absence of this ion. Finally, the increase of CA release by theophylline was unaffected by the addition of lanthanum to the medium.

#### *Effect of verapamil on catecholamine secretion in adrenal medulla*

Figure 2 shows that ACh can increase CA secretion from rat adrenal incubated *in vitro* (similar increases in CA secretion were observed when ACh ( $10^{-4}$  M) was added to slices of human adrenal medulla and bovine adrenal *in vitro*). When verapamil (1 mM) was added to the incubation medium, the increase of CA secretion by ACh was abolished completely. The release of CA caused by tyramine was unchanged in the presence of verapamil.

Figure 3 shows that both salbutamol ( $6 \times 10^{-6}$  M) and theophylline ( $10^{-2}$  M) caused increased CA secretion. Addition of verapamil to the incubation medium did not suppress the effect of salbutamol or theophylline on CA secretion; CA release was even further increased after addition of verapamil to these

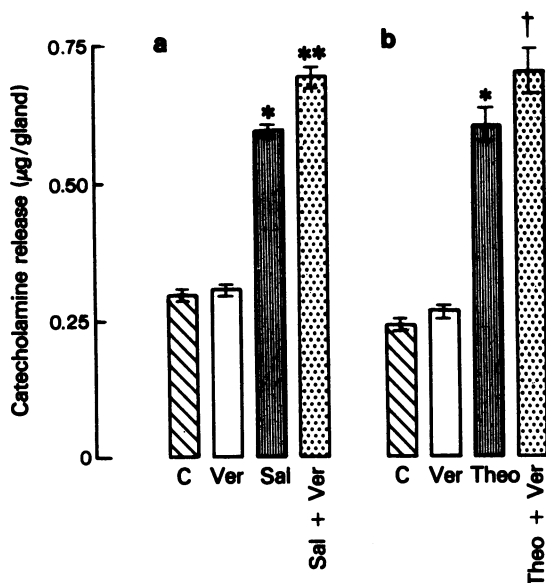


**Figure 2** Effects of verapamil on catecholamine secretion from rat adrenal gland. (a) Release of catecholamines induced by  $10^{-4}$  M acetylcholine (ACh); (b) release of catecholamines induced by  $10^{-3}$  M tyramine HCl (Tyr); control = C; Ver indicates that verapamil 1 mM was added to incubation medium. Vertical bars show s.e.mean;  $n = 10$  for each column. \*  $P < 0.001$  compared to control; \*\*  $P < 0.001$  compared to ACh.

secretagogues, while verapamil alone had no significant effect on CA secretion from adrenal medulla.

#### *Effect of dantrolene-sodium on catecholamine secretion in adrenal medulla*

Figure 4 shows the effect of dantrolene-sodium on CA release induced by different secretagogues. Dantrolene-sodium (1 mM) had no significant effect on CA secretion caused by tyramine. The increased CA secretion caused by 56 mM  $K^+$  was only partially reduced by the addition of dantrolene-sodium but the secretion rate was still significantly higher than in control adrenal glands or in glands exposed only to dantrolene. On the other hand, addition of dantro-



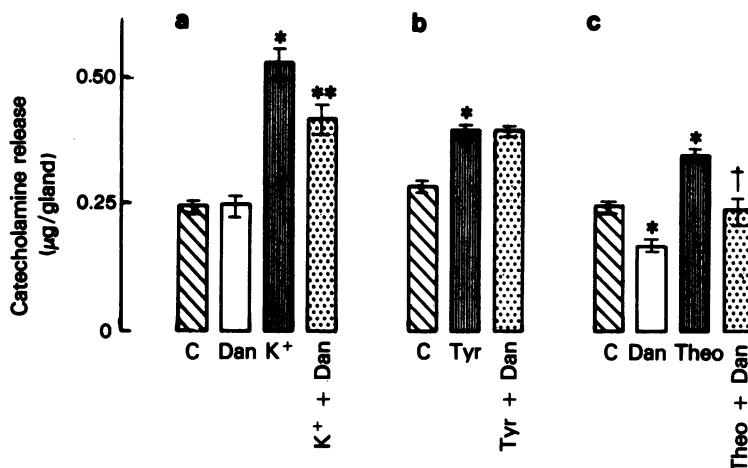
**Figure 3** Effect of verapamil on catecholamine secretion induced by salbutamol and theophylline. (a) Release of catecholamines induced by  $6 \times 10^{-6}$  M salbutamol (Sal); (b) release of catecholamines induced by  $10^{-2}$  M theophylline (Theo); control = C; Ver indicates that verapamil 1 mM was added to incubation medium. Vertical bars show s.e.mean;  $n = 10$  for each column in experiment (a) and  $n = 15$  for each column in experiment (b). \*  $P < 0.001$  compared to control; \*\*  $P < 0.001$  compared to Sal; †  $P < 0.05$  compared to Theo.

lene-sodium to the medium completely abolished the increased secretion of CA caused by theophylline.

#### **Discussion**

The experiments described in the present paper show that stimulation of CA secretion which depends on the presence of extracellular calcium (i.e. by ACh or by 56 mM  $K^+$ ) can be abolished by addition of either lanthanum or verapamil to the incubation medium. Since lanthanum can compete with calcium for transport, it is to be expected that inhibition of Ca-dependent secretion will result. Verapamil has been shown to decrease calcium conductance of the membrane (Kohlhardt, Bauer, Krause & Fleckenstein, 1972). This effect would also result in reduction of Ca-dependent secretion of CA. Thus, the inhibition of CA secretion by these two agents (lanthanum and verapamil) was to be expected and no differentiation of their mechanism of action is evident from this type of experiment.

The Ca-dependent CA secretion was only partially inhibited by dantrolene-sodium. This agent, related



**Figure 4** Effect of dantrolene-sodium on catecholamine secretion from rat adrenal gland. (a) Release of catecholamines induced by 56 mM KCl ( $K^+$ ); (b) release of catecholamines induced by  $10^{-3}$  M tyramine HCl (Tyr); (c) release of catecholamines induced by  $10^{-2}$  M theophylline (Theo); control = C; Dan indicates that dantrolene sodium 1 mM was added to incubation medium. Vertical bars show s.e. mean;  $n = 10$  for each column. \*  $P < 0.001$  compared to control; \*\*  $P < 0.02$  compared to  $K^+$  (experiment a); †  $P < 0.001$  compared to Theo (experiment c).

to hydantoin, can suppress intracellular events which depend on Ca, e.g. muscle contraction (Ellis & Carpenter, 1972). It is assumed that dantrolene does not affect calcium entry into cells (Desmet & Hainaut, 1977). Therefore, the partial inhibition of potassium-induced CA secretion requires explanation, since it is a secretagogue dependent on Ca entry into the cells. During incubation of adrenals *in vitro*, the catecholamines released into the medium can activate adenylyl cyclase in adrenal medullary cells (Boonyaviroj & Gutman 1977) which would cause an increase of cyclic AMP and this may finally lead to further secretion of CA. Cyclic AMP-induced release of CA is independent of external Ca (Peach, 1972) but may depend on intracellular calcium, as will be discussed later. This cyclic AMP-dependent release of CA may be suppressed by dantrolene, thus causing a partial inhibition of CA release.

Cyclic AMP can be increased in the adrenal medulla either by inhibition of phosphodiesterase (e.g. by theophylline) or by stimulation of  $\beta$ -adrenoceptors, specifically by  $\beta_2$ -agonists, e.g. salbutamol (Boonyaviroj & Gutman, 1977). CA secretion from adrenal medulla can be increased by theophylline or by dibutyryl cyclic AMP (Peach, 1972). This CA secretion is independent of extracellular calcium (Peach, 1972). This is also corroborated in our studies, where both theophylline and salbutamol caused an increase of CA secretion in calcium-free Locke solution supplemented with 2 mM EGTA. As would be expected, addition of lanthanum had no effect on CA secretion stimulated by these agents, since no entry of CA into

the chromaffin cells can take place under these conditions. However, verapamil not only failed to reduce CA secretion but even enhanced the effect of cyclic AMP-mediated stimulation of CA secretion. Thus, verapamil showed some difference in its effect as compared to lanthanum. Cyclic AMP is assumed to cause release of intracellular Ca from storage sites and thus cause contraction of muscle (e.g. heart muscle). If a similar event takes place in adrenal medulla cells, i.e. cyclic AMP releases intracellular calcium from its storage sites, then the level of free cytoplasmic calcium would be affected by the rate of calcium efflux into the medium, if the latter is calcium-free and contains EGTA (as was the case in the experiment shown in Figure 3). Therefore, the presence of verapamil, a drug that lowers membrane permeability to calcium, would, under these particular circumstances, prevent loss of calcium from the cells into the medium and thus enhance the release of CA, as indeed it did, as seen in Figure 3.

Lanthanum did not show this effect, suggesting a different mode of action on calcium movement. It has been suggested that lanthanum affects mainly Na-Ca exchange and, therefore, no direct effect on calcium permeability is observed.

On the other hand, dantrolene-sodium has an opposite effect on free cytoplasmic calcium concentration. Since dantrolene prevents release of calcium from intracellular storage sites (Desmet & Hainaut, 1977), the presence of this drug would act in an opposite way to that of cyclic AMP on cytoplasmic calcium levels. This was verified by the experiment

shown in Figure 4. Thus, dantrolene completely blocked the secretion of CA induced by a rise of cyclic AMP. More recently we have found corroborating evidence for the role of intracellular calcium stores in CA release caused by cyclic AMP, i.e. introduction of EGTA into adrenal medullary cells by using EGTA loaded liposomes prevented the secretion of CA caused by theophylline (unpublished observations).

Finally, tyramine causes CA secretion by a mechanism completely independent of calcium, either extra-

cellular or intracellular. This is evident from the lack of any effect on tyramine-induced CA secretion by any of the drugs studied: lanthanum, verapamil, or dantrolene (Figures 1, 2, 4). This is to be expected if tyramine causes release by displacement of CA from the storage sites.

Y.G. is an Established Investigator of the Chief Scientist's Bureau, Israeli Ministry of Health.

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