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DIRECT STIMULANT EFFECT OF AMINOPHYLLINE ON CATECHOLAMINE RELEASE FROM THE ADRENAL MEDULLA

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Abstract—Bovine adrenal glands, perfused *in vitro*, responded to single injections or continuous infusions of aminophylline by increased release of catecholamines. This effect was not mediated by the release of acetylcholine in the gland, since it was not blocked by cholinergic blocking agents. Stimulation for short periods of time (4 min or less) showed that the catecholamine release was depressed when extracellular calcium was reduced; however, even in the absence of calcium, aminophylline still evoked catecholamine release. The stimulant effect of aminophylline was present during perfusion with high potassium media and was inhibited by the local anesthetic, dibucaine. Papaverine potentiated the response to aminophylline. The results are discussed in terms of two different mechanisms for aminophylline-induced secretion, one dependent on influx of extracellular calcium, and one dependent on release of intracellular calcium.

THE METHYL xanthines, theophylline and caffeine, have diverse pharmacological effects, including actions on the nervous system, on metabolism, and on smooth and cardiac muscle. Recently a large body of evidence has accumulated on the effect of theophylline and caffeine on secretory processes. Depending on the concentration used, theophylline[1–7] or aminophylline[8–11] (theophylline ethylenediamine) and caffeine[6,7,12] have been reported to initiate secretion or to potentiate the actions of other secretogogues. The conclusions from these studies have concerned the role of cyclic AMP in secretion, since the methyl xanthines have been demonstrated to inhibit phosphodiesterase, the enzyme responsible for cyclic AMP breakdown. Another feature which these secretory processes have in common is the requirement for calcium ions.[13] The purpose of the present study was to examine the effect of a methyl xanthine on catecholamine release from the adrenal medulla with special attention to the role of calcium.

A preliminary account of some of this work has been reported.[14]

METHODS

Perfusion of glands. Bovine adrenal glands obtained from a local slaughterhouse were perfused at 10 ml/min as described previously.[15] Glands were always perfused for 40 min before beginning an experiment in order to obtain low spontaneous secretion rates. In some cases the effluent from the gland flowed directly into the sample tubing of an Autoanalyzer manifold for catecholamines.[16] The standard perfusion medium was a modified Locke's solution of the following composition (mM): NaCl, 154; KCl, 5·6; $CaCl_2$, 2·2; $MgCl_2$, 1·0; $NaHCO_3$, 6·0 and dextrose, 10·0. The solution was equilibrated with 5% CO_2 in O_2. In some experiments 5 mM Na_2HPO_4 replaced the $NaHCO_3$ and the solution was bubbled with 100% O_2.

Assay of catecholamines. The automated trihydroxyindole fluorimetric method was used for catecholamine determinations.[16]

Drugs and chemicals. Aminophylline was obtained from Searle as the intravenous preparation (500 mg/20 ml). Nicotine was used as the bitartrate salt. Hexamethonium and atropine were employed as the chloride and sulfate salts respectively. Dibucaine was obtained through the courtesy of CIBA and paperverine was supplied by Eli Lilly & Company.

RESULTS

Effect of single injections of aminophylline on catecholamine release. When perfused adrenal glands, connected directly to the Autoanalyzer manifold, were injected with aminophylline, there was an increased release of catecholamines. Figure 1 shows an

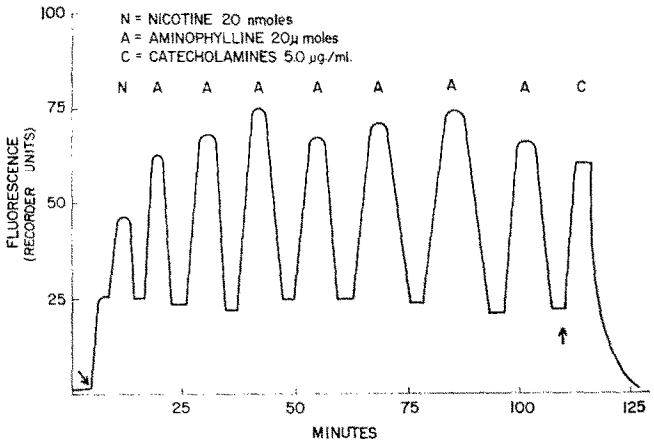

FIG. 1. Effect of single injections of aminophylline on catecholamine release from a perfused adrenal gland continuously monitored by means of the Autoanalyzer. At the first arrow, the effluent from the gland was connected to the manifold. N represents injection of nicotine (20 nmoles); A represents injection of aminophylline (20 μmoles). At the second arrow, the sample line of the manifold was connected to a standard catecholamine solution (5 μg/ml) for 2 min and then placed in water.

experiment in which a gland responded to seven successive injections of aminophylline without any evidence of tachyphylaxis. Also shown is the response to an injection of nicotine (20 nmoles) which produced a peak secretory rate of about one-half of that evoked by aminophylline (20 μmoles). This same type of response was observed in three other glands injected with aminophylline.

Effect of increasing concentrations of aminophylline infused into the adrenal gland. In another series of experiments, different concentrations of aminophylline were infused into adrenal glands for 4-min periods and the release of catecholamines was determined by assaying aliquots of the perfusates.

The results from one experiment are shown in Fig. 2. Aminophylline at concentrations from 1·6 to 5·5 mM produced a graded increase in catecholamine secretion. This type of response was seen in each of five other glands.

Effect of extracellular calcium concentration on aminophylline-induced catecholamine release. A series of glands were infused with increasing concentrations of aminophylline for 4-min periods during perfusion with Locke's solution at three different calcium

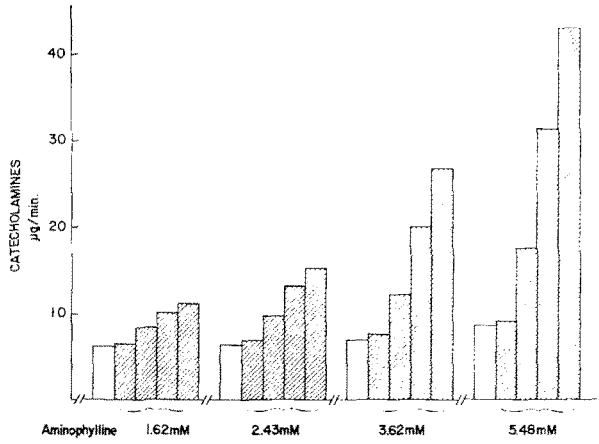

FIG. 2. Effect of increasing concentrations of aminophylline on catecholamine release from a perfused adrenal gland. After a control collection, aminophylline was infused for 4 min followed by 10 min of perfusion with Locke's solution. The vertical bars show the rate of catecholamine release in 1-min collection periods.

concentrations. Figure 3 shows dose–response curves of the peak secretory rate in response to aminophylline. The response to aminophylline was reduced at calcium concentrations below that of the regular Locke's solution (2·2 mM).

Effect of cholinergic blocking agents on aminophylline-induced catecholamine release. In order to rule out the possibility that the effect of aminophylline was due to release of acetylcholine in the gland, several experiments were performed in which high concentrations of hexamethonium (5×10^{-4} g/ml) plus atropine (10^{-5} g/ml) were present in the perfusion media to block cholinergic receptors.[17] In four glands stimulated

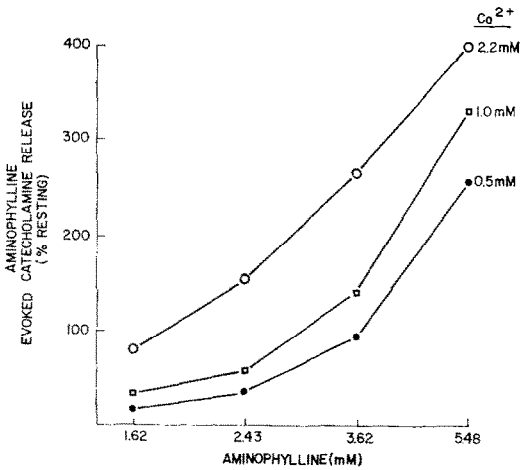

FIG. 3. Dose–response curves for aminophylline at different calcium concentrations. Perfused adrenal glands were exposed to four concentrations of aminophylline in 4-min periods and the peak secretory response is plotted as the increment in release as a percentage of the spontaneous rate. Each point represents the mean value from two experiments on different glands. In each gland, a 10-min period of perfusion in the absence of drug separated the stimulation periods.

with aminophylline (5·0 mM) in the presence of hexamethonium plus atropine, the release of catecholamines was 92·6 ± 2·2 per cent of the first stimulation in the absence of blocking drugs.

Effect of calcium depletion on aminophylline-induced catecholamine release. Although the response to aminophylline was reduced when the calcium concentration was lowered, it was observed that adrenal glands still responded to aminophylline in the absence of extracellular calcium, even in the presence of 2 mM EDTA to chelate traces of calcium. This effect of aminophylline in the absence of calcium was particularly evident when stimulation was prolonged. Figure 4 contrasts the stimulant effects of aminophylline with that of nicotine in the presence or absence of calcium.

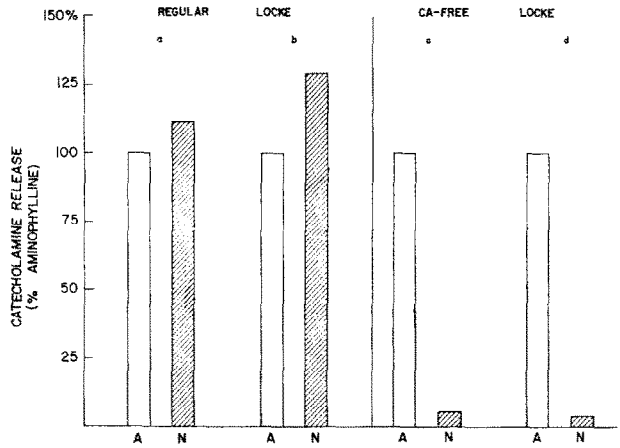

FIG. 4. Comparison of the stimulant effects of aminophylline and nicotine in the presence or absence of calcium. Four different adrenal glands were stimulated for 8 min with (A) aminophilline (5×10^{-3} M) and with (N) nicotine ($1{\cdot}6 \times 10^{-5}$ M). In glands a and b, the calcium concentration of the perfusion medium was 2·2 mM; in c and d, no calcium was added to the medium. The results in each gland are expressed relative to the response to aminophylline (pictured as 100 per cent). The actual values of aminophylline-induced release were (in micrograms/8 min): a, 66·1; b, 26·7; c, 198 and d, 168. These absolute secretory rates are not to be compared one with another, since the four glands were of different sizes and condition.

The release of catecholamines during 8-min of perfusion with aminophylline or nicotine in four different glands showed that aminophylline (5×10^{-3} M) was less potent than nicotine ($1{\cdot}6 \times 10^{-5}$ M) in evoking catecholamine release in the presence of calcium, but was about 25 times more potent in the absence of calcium (Fig. 4).

In another series of experiments, adrenal glands were first stimulated with aminophylline (8·8 mM) for 4 min to obtain a reference response. A second stimulation was then obtained after 16 min of perfusion with the regular medium or with calcium-free medium containing 1·0 mM EGTA [ethylene glycol *bis*-(β-aminoethyl ether)-*N,N'*-tetraacetic acid]. The response in calcium-free medium in comparison to the reference response was not significantly different from that of the control glands (Table 1).

Effect of papaverine on aminophylline-induced catecholamine release. To obtain evidence linking the action of aminophylline to cyclic AMP, papaverine, a more potent inhibitor of phosphodiesterase, was employed. Papaverine (10^{-4} M) potentiated the action of aminophylline in inducing catecholamine release (Table 1).

TABLE 1. EFFECT OF CALCIUM-FREE MEDIUM AND PAPAVERINE ON AMINOPHYLLINE-INDUCED CATECHOLAMINE RELEASE

	Test stimulation/reference stimulation* (%)		
	Control	Ca-free	Papaverine
	29·6	32·5	81·9
	39·4	34·0	70·7
	46·0	53·0	83·9
	38·7	13·5	66·3
	42·2	21·7	88·9
	46·5	14·9	65·4
	45·7	55·5	77·3
	40·6	44·0	63·6
	43·4	38·7	70·5
	33·9		67·1
	52·8		71·3
	34·7		72·8
	29·8		
	34·7		
Mean	39·9	34·2	73·3
S.E.	0·9	5·1	2·3
P†		>0·25	<0·001

* Adrenal glands were stimulated twice with aminophylline (8·8 mM) at 16-min intervals. Each value represents a separate gland with the second stimulation compared to the first (reference) stimulation conducted with the regular Locke's solution. Calcium-free medium contained 1·0 mM EGTA. Papaverine concentration was 10^{-4} M.

† Significance of difference from control (*t*-test).

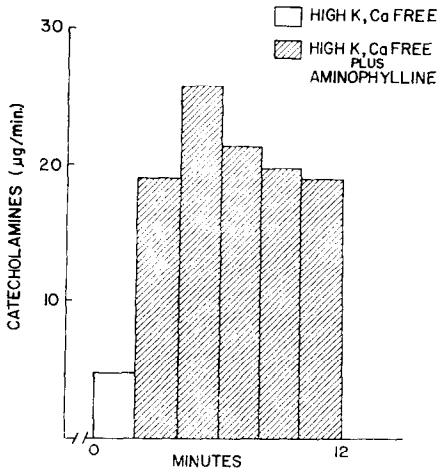

FIG. 5. Effect of aminophylline on catecholamine release during perfusion with high K^+, Ca-free medium. The vertical bars represent 2-min collection periods. Prior to the zero time on the abscissa, the gland had been perfused for 60 min. The medium had the following composition (mM): KCl, 100; NaCl, 50; $MgCl_2$, 1·0; Na_2HPO_4, 5·0; EGTA, 0·2; dextrose, 10·0. The solution was adjusted to pH 7·4 with HCl and bubbled with 100% O_2. During the period indicated by the hatched bars, aminophylline, 5·0 mM, was present in the perfusion medium.

Effect of elevated extracellular potassium on aminophylline-induced catecholamine release. When the potassium concentration was raised to 100 mM (in the absence of calcium), aminophylline was still able to evoke catecholamine release (Fig. 5). This result shows that depolarization *per se* is not required for aminophylline-induced secretion.

Effect of ethylenediamine on catecholamine release. Since aminophylline contains ethylenediamine conjugated to theophylline, four glands were perfused for 8 min with 5·5 mM ethylenediamine. The change in secretory rate during perfusion with ethylenediamine was $+1{\cdot}0 \pm 1{\cdot}2$ μg/min. Thus the effect of aminophylline is related to the theophylline moiety.

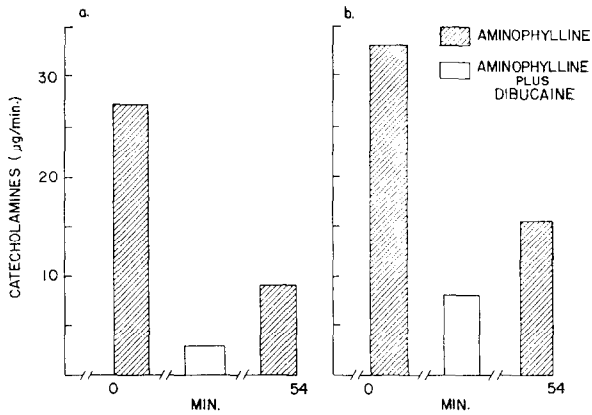

FIG. 6. Effect of dibucaine on aminophylline-evoked catecholamine release. Experiments on two different adrenal glands are illustrated. After 40 min of perfusion with Ca-free Locke's solution, each gland was stimulated three times for 8 min with aminophylline, 5·0 mM. Between stimulation periods there was a 15-min interval with no aminophylline present. Ten min prior to the second stimulation period dibucaine, $1{\cdot}5 \times 10^{-5}$ M, was introduced and maintained until the end of the 8-min stimulation.

Effect of dibucaine on aminophylline-induced catecholamine release. Local anesthetics have been shown to block caffeine-induced muscle contraction.[18] One such agent, dibucaine, was tested on aminophylline-induced catecholamine release. In two experiments, aminophylline was used to stimulate catecholamine release in the absence of calcium and this stimulation was shown to be inhibited by dibucaine (Fig. 6).

DISCUSSION

The present experiments demonstrate that aminophylline can initiate the release of catecholamines from the adrenal medulla. This finding is paralleled by the effects of theophylline on other secretory systems (see introductory section). Furthermore, the effect of aminophylline seems to be a direct effect on the chromaffin cell and not due to release of acetylcholine within the gland, since the stimulant action was not affected by hexamethonium plus atropine. There are reports that aminophylline and caffeine cause the release of catecholamines *in vivo* in animals and man.[19–21] The present study shows that aminophylline can release catecholamines from adrenal glands separated from the influence of the nervous system.

A further distinction between the cholinomimetic secretogogues and aminophylline is observed when examining the effects of calcium ions. Although the effects of extracellular calcium on aminophylline-induced catecholamine release seem to parallel those on acetylcholine-induced release (lowering the calcium concentration reduces the stimulant effect), aminophylline still evoked release in the absence of calcium; and with a high dose (8·8 mM) the slight depression of secretion was not significantly different from the control.

The studies on the effect of calcium on aminophylline-induced catecholamine release suggest that the drug is activating two mechanisms which initiate secretion: one depends on extracellular calcium, and one does not. A likely possibility is that these concentrations of aminophylline depolarize the chromaffin cell with increased influx of calcium ions triggering secretion. This might be related to the stimulant effects of the xanthines on nerve and heart muscle.[22] This would account for the calcium-dependent portion of aminophylline-induced secretion. The second mechanism might be related to the release of intracellular calcium from membrane stores. This is the explanation of the stimulant effects of caffeine on muscle in the absence of extracellular calcium.[23] Blockade of the effect of aminophylline by a local anesthetic is also consistent with this possibility.[18] One source of this bound calcium in the adrenal medulla is the membrane fraction which actively binds calcium.[24]

How could aminophylline produce such a release of intracellular calcium? One current suggestion is that methylxanthines, by inhibiting phosphodiesterase, promote the accumulation of cyclic AMP and that cyclic AMP formation or action somehow releases bound calcium.[25] Before confidence in such a role in adrenal medullary secretion can be held, it would be desirable if the four criteria of Sutherland *et al.*[26] relating to mediation by cyclic AMP of hormone action could be obtained. These criteria, as applied to the release of secretory products, are: (1) adenyl cyclase in broken cell preparations should respond to secretogogues; (2) the level of cyclic AMP in intact tissue should change in response to secretory stimuli; (3) agents which inhibit phosphodiesterase should potentiate secretory stimuli or initiate secretion directly; (4) cyclic AMP or a derivative should mimic the action of the normal secretory stimulus. After this paper was submitted, evidence has been presented that cyclic AMP can initiate catecholamine release[27] and that aminophylline and nervous activity increase cyclic AMP in the adrenal medulla.[28] Thus three of the above criteria have now been met.

There is already some indirect evidence from studies with aminophylline that cyclic AMP is involved in the release of norepinephrine from sympathetic nerves,[20,29] and it might be expected that the many parallels between release of amines from the adrenal medulla and from adrenergic neurons[13] will be extended.

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