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THE RELEASE OF NUCLEOTIDE FROM THE ADRENAL MEDULLA BY INDIRECTLY ACTING SYMPATHOMIMETIC AMINES*

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Abstract—Cat adrenal glands were perfused with Locke's solution, and phenylethylamine, *d*-amphetamine, or acetylcholine was added to the perfusion medium for 1 or 2 min. The catecholamine (CA) and AMP content of the perfusate was determined. Molar ratios (CA/AMP) of approximately 6 were obtained when amphetamine, phenylethylamine, or acetylcholine was employed as a secretogogue. It is concluded that the sympathomimetic amines, like acetylcholine, release medullary catecholamines from a granular fraction; however, the mechanism of release is not one of simple displacement.

PREVIOUS studies have shown that the indirectly acting sympathomimetic amines including the amphetamines, phenylethylamine, and tyramine, release catecholamines (CA) from the perfused adrenal gland of the cat in a manner which, under the experimental conditions employed, closely resembles the stimulant action of acetylcholine (ACh).[1] Like ACh, the sympathomimetic amines produced their maximal release during the first minute following their addition to the perfusion medium; required the presence of calcium for their activity; were inhibited by the presence of high concentrations of magnesium (5–10 mM) or hexamethonium (10^{-4}–10^{-3} g/ml); and were potentiated by the removal of potassium from the perfusion fluid. Since the sympathomimetic amines were equally effective as secretogogues in both chronically and acutely denervated glands, they apparently release CA by a direct action on the chromaffin cells.[1]

The close resemblance between the sympathomimetic amines and ACh as secretogogues was difficult to reconcile with studies done on preparations of isolated chromaffin granules, in which the sympathomimetic amines enhanced the release of CA whereas ACh was ineffective.[2,3] *In vitro*, the amount of CA lost from the granules was determined to be approximately equivalent to the concentration of the sympathomimetic agent which was taken up into the granules. Furthermore, there was no loss of ATP from the granules. Such findings have led many to assume that the CA-releasing activity of the sympathomimetic amines may be ascribed to their first being taken up into the chromaffin cell and then displacing the endogenous CA from its complex with ATP in the storage granules. Under the conditions of the displacement theory then, if the sympathomimetic amines release CA by simply displacing the natural amine

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from its ATP-binding site, little or no nucleotide should appear with the CA in the effluent obtained from an intact adrenal gland when CA release is evoked by the sympathomimetic amines. Conversely, if the sympathomimetic amines release medullary CA by some other mechanism which involves also the release of other components of the granules, more specifically nucleotides, then nucleotide should appear in the adrenal venous effluent in the same molar ratio observed with ACh and other types of medullary stimulating agents.[4–6]

METHODS

Adrenal gland perfusion. Cat adrenal glands were perfused *in situ* at room temperature with Locke's solution according to the method originally described by Douglas and Rubin.[7] The Locke's solution had the following composition (mM): sodium chloride 154; potassium chloride 5·6; calcium chloride 2; magnesium chloride 0·2 sodium bicarbonate 6; dextrose 10. All solutions were oxygenated with 95% oxygen and 5% carbon dioxide and had a pH close to 7·0. Rate of perfusion was maintained at 1·2–1·5 ml/min by regulation of perfusion pressure.

Analysis of catecholamines. The catecholamine content of the effluent was estimated by a modification (see Rubin and Jaanus[1]) of the fluorometric method of Anton and Sayre.[8] Values were expressed as mμmoles epinephrine plus norepinephrine per ml. Any sympathomimetic amine which was present in the perfusion medium was also added to standard epinephrine and norepinephrine solutions before analysis, in order to ascertain that the sympathomimetic agent was not affecting the assay in any way.

Analysis of AMP. AMP was measured by the enzymatic spectrophotometric method of Kalckar[9] as modified by Furchgott and DeGubareff.[10] To 1 ml perfusate were sequentially added 0·1 ml succinate buffer (pH 6·5), 10 [λ] of 1M $MgCl_2$, and 25λ of the adenylic deaminase preparation dissolved in 1 M KCl. Change in optical density was then followed at 265 mμ with the aid of a multiple-sample absorbance recorder (Gilford 2000). Completion of the reaction usually took 20–30 min. All calculations were made after substraction of enzyme blank. The presence of the sympathomimetic amines in the sample was found not to affect the AMP determination in any way.

Drug and reagents used. *d*-Amphetamine, tyramine, and phenylethylamine were used as their chloride salts and their concentrations expressed in grams of the free base per ml. Acetylcholine concentrations were expressed in grams of the chloride salt per ml. The adenosine-5′-monophosphate·H_2O (AMP) was obtained from Calbiochem (Los Angeles) and the 5′-adenylic acid deaminase was purchased from Sigma Chemical Co. (St. Louis).

RESULTS

In the present study, amphetamine, phenylethylamine, or tyramine was added to the perfusion medium for 1 or 2 min and the concentration of CA and AMP determined in the perfusate. The addition of either phenylethylamine or amphetamine to the perfusion medium resulted in a large increase in CA secretion, which was accompanied by the appearance of AMP in the perfusate. By contrast, control samples which were taken just prior to the addition of the stimulating agent and which contained only very small amounts of CA (< 1 mμmole/ml) had undetectable amounts of AMP as determined by this method. Although there was some variation in the

relation of CA to nucleotide in the perfusion medium, which was probably due to the fact that we were working at a lower limit of sensitivity of this method of determining nucleotide, it can be seen that not only was nucleotide detected in the perfusion medium during each stimulation period but the AMP concentration could be correlated with the quantity of CA in the effluent (Table 1).

When phenylethylamine was added in a concentration (10^{-4} g/ml), which usually exerts near maximal activity for this amine, CA output varied from 10·7 to 28·6 mμmoles/ml. Such a variation in CA output is frequently observed from one preparation to another. However, with this range of CA output, the CA/AMP molar ratio varied between 4 and 8, with a mean of 6·76 ($\pm$0·88). With varying concentrations of amphetamine, CA output ranged from 16·5 to 37·7 mμmoles/ml, yet again the mean molar ratio tended to remain constant (6·22 $\pm$ 0·67). The mean molar ratios of the CA output and AMP concentration obtained when sympathomimetic amines were used to evoke secretion were strikingly similar to the molar ratio obtained with acetylcholine (6·75 $\pm$ 0·12). It is of interest to note that the calculated mean molar ratio when we used the more potent stimulus, ACh, was obtained over a wide range of CA output (14·5–115·7 mμmoles/ml) yet it was still in close agreement with the mean molar ratios found when phenylethylamine and *d*-amphetamine were used to evoke release.

Further evidence was obtained to show that the amount of AMP appearing in the effluent depended on the amount of CA released by sympathomimetic amines. Tyramine has previously been shown to release much smaller amounts of medullary CA than the nonhydroxylated sympathomimetic amines such as phenylethylamine and amphetamine.[1] In the present series of experiments, when tyramine was used in a concentration which produces its near maximal secretory activity (2×10^{-4} g/ml), CA outputs of 6·51, 6·6, and 7·82 mμmoles/ml were obtained on three different preparations. These CA outputs were less than those found in this study with the other stimulating agents (See Table 1), and no AMP could be detected in the perfusate by the method employed. A more sensitive method for determining nucleotide is required to ascertain whether tyramine-evoked release produces a CA/AMP ratio similar to that of phenylethylamine and amphetamine.

DISCUSSION

It has previously been shown that regardless of the type of stimulus used to evoke medullary CA release, adenine nucleotide appeared in the adrenal venous effluent of the perfused cat adrenal gland in a constant molar ratio of approximately 6:1.[4–6] Most of the adenine nucleotide appeared as the monophosphate (AMP), apparently owing to the presence of endothelial phosphatases which split over 80 per cent of the tri- and diphosphates during their sojourn through the circulation.[4–6] The results of the present study have shown that stimulation of the adrenal medulla by indirectly acting sympathomimetic amines also causes a concomitant release of CA and AMP in a molar ratio of 6:1. This CA/AMP ratio is somewhat higher than the 3·7:1 ratio found in the chromaffin granules of the cat by chemical studies *in vitro*.[11] However, Douglas and Poisner[5] have shown recently that when all the nucleotides and their metabolites that appear in the adrenal venous effluent after stimulation are added—i.e. ATP, ADP, AMP, and adenosine—the molar ratio then becomes 4·0:1.

Finding the CA and nucleotide in the effluent in proportions similar to those in the granules has been advanced to support the idea that the chromaffin granule is the locus of the "readily releasable" CA and that the so-called extragranular or unbound CA plays no direct role in the physiological release process.[4-6] In relation to the present study, the finding that the nucleotide and CA appear concomitantly

TABLE 1. EFFLUX OF CATECHOLAMINES (CA) AND ADENOSINE MONOPHOSPHATE (AMP)

Conc. (g/ml)	(mμmoles/ml) CA output	AMP	Molar ratio* (CA/AMP)
	d-Amphetamine		
2×10^{-5}	16·5	4·1	4·02
5×10^{-5}	22·0	3·9	5·64
$7{\cdot}5 \times 10^{-5}$	17·9	2·3	7·70
$7{\cdot}5 \times 10^{-5}$	31·4	4·2	7·48
$7{\cdot}5 \times 10^{-5}$	37·7	6·5	6·28
			Mean and S.E. 6·22 ± 0·67
	Phenylethylamine		
10^{-4}	10·7	1·78	6·0
10^{-4}	20·1	1·97	10·2
10^{-4}	24·4	4·35	5·6
10^{-4}	27·1	4·1	6·6
10^{-4}	28·6	5·33	5·4
			Mean and S.E. 6·76 ± 0·88
	Acetylcholine		
10^{-6}	14·5	2·19	6·62
5×10^{-5}	42·7	6·35	6·72
5×10^{-5}	69·25	9·6	7·20
5×10^{-5}	73·7	11·20	6·58
5×10^{-5}	115·7	17·43	6·64
			Mean and S.E. 6·75 ± 0·12

* Each value was obtained from a different preparation.

after addition of one or another sympathomimetic amine to the perfusion medium indicates that the sympathomimetic amines also release medullary CA which is contained within the granule stores. In addition, the results suggest that the sympathomimetic amines do not release medullary CA simply by displacing the natural amine from its ATP binding site within the granule. If the displacement theory were valid, nucleotide should not have appeared in the perfusate with the CA after release evoked by amphetamine and phenylethylamine, as Schümann and Philippu,[12] using various sympathomimetic amines, observed during release of CA from isolated chromaffin granules. It should not be inferred that these indirectly acting sympathomimetic amines cannot *replace* the endogenous CA in its granular binding site, for indeed there is strong evidence to support the idea that these amines can be taken up by chromaffin granules.[12, 13]

One can explain the findings of a stoichiometric replacement of the natural amine by the sympathomimetic agent in an alternative manner. It appears that the CA bound within the chromaffin granule may be in dynamic equilibrium with CA in the surrounding medium, and exchange between these two fractions continually occurs.[18] Thus, if a sympathomimetic amine is present in the extragranular fraction, and it too can be taken up by granules,[12, 13] it is not surprising that after a prolonged period of incubation a certain amount of sympathomimetic amine is found in the granules and a similar amount of CA is lost. In the intact adrenal gland, when the chromaffin granules are depleted of CA after stimulation, granular uptake of the sympathomimetic amine may occur to return the granule amine content toward normal. Consistent with this idea are studies indicating that chromaffin granules possess a limited number of storage sites which may be occupied by a variety of amines.[13–17] Thus, from the results of the present study, it is suggested that the hypothesized displacement theory of amine-induced CA release is actually only a replacement phenomenon; i.e. the uptake of the sympathomimetic amine is only a consequence of the release of endogenous amine and is not the immediate cause of the release itself.

If the displacement does not trigger the release of CA from its granular binding site, then how is CA release effected by the sympathomimetic amines? Stjärne[18, 19] has suggested that CA release induced by the sympathomimetic amines from nerve granules might be explained by the competition between the sympathomimetic agent and the natural amine for uptake into the granule. The free CA, unable to re-enter the granule, would be extruded into the circulation. However, the presence of nucleotide in the perfusate along with the CA released by the sympathomimetic amines makes such a theory untenable, at least in regard to the events occurring at the adrenal medulla. Calcium must play some key role in the secretory process, since the sympathomimetic amines, like ACh, require the presence of calcium for their releasing activity.[1] However, the interaction of the sympathomimetic amines with calcium in bringing about secretion requires elucidation (for further discussion of this point see Rubin and Jaanus[1]).

The data presented, although not consistent with the displacement theory of CA release, does point to the granular ATP-bound CA as the store that is depleted by the sympathomimetic amines. Whether the sympathomimetic amines also release extramedullary CA from a similar granular store still remains to be answered. There are those who believe that one or another of these agents does release CA from peripheral adrenergic neurons and from brain by an action on granular CA,[20–23] whereas others have suggested that these agents release from a more labile (perhaps extragranular) store.[24–26] However, this question will remain unresolved in the lack of any evidence showing the presence or absence of a constant CA:nucleotide ratio in the perfusate of an adrenergically innervated organ which has been exposed to sympathomimetic amines.

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