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RELEASE OF CATECHOLAMINE FROM THE CAT HEART BY SOME DIRECTLY AND INDIRECTLY ACTING SYMPATHOMIMETIC AMINES*

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Abstract—The effect of various directly and indirectly acting sympathomimetic amines on the inotropic, chronotropic and release of noradrenaline was investigated on the isolated cat heart. Tyramine, ephedrine and metaraminol produced positive inotropic and chronotropic effects with simultaneous release of noradrenaline while amphetamine and dopamine were unable to release any significant amount of noradrenaline although they both produced marked cardiac effects. We have further shown that amphetamine produces positive inotropic and chronotropic effects on cat heart depleted of catecholamines by low doses of reserpine, guanethidine and α-methyl tyrosine. It is therefore concluded that amphetamine has a direct effect on the heart and produces its cardiac effect by a different mechanism than that of tyramine.

BURN and Rand[1] proposed that part of the pressor activity of tyramine and some other indirectly acting sympathomimetic amines is brought about by the release of noradrenaline from its storage sites. Although the validity of this hypothesis has been confirmed by a number of other workers,[2–4] there are a number of reports which indicate that tyramine has some sympathomimetic action of its own which is not related to the release of catecholamines.[5,6]

The effect of various directly and indirectly acting amines on the inotropic, chronotropic and release of noradrenaline from the isolated heart has been studied by Lindmar and Muscholl.[7] They have shown that tyramine, β-phenylethylamine and 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) increased the rate and the force of contraction of the isolated rabbit heart and caused the release of noradrenaline. They have further suggested that tyramine, besides releasing noradrenaline from the tissue store, also enhances the effect of released noradrenaline at the receptor sites.[8] A very good correlation between the increase in heart rate and increase in the amount of noradrenaline released was not seen in their experiments with DMPP and tyramine on the isolated heart. Swaine,[9] studying the inhibition of release of noradrenaline by various α-adrenergic blockers has shown that tyramine induced release of noradrenaline was inhibited by phenoxybenzamine.

Out of all the indirectly acting sympathomimetic amines tyramine seems to be most widely studied as far as release of noradrenaline is concerned. We were interested to investigate the release of noradrenaline by other directly acting amines, namely, dopamine, metaraminol and some indirectly acting amines namely, amphetamine and tyramine.[10] The last two indirectly acting amines were specially chosen because it is reported that the mechanism by which amphetamine and tyramine release noradrenaline is different and possibly both these substances are not acting on the same pool of noradrenaline.[11]

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METHODS

Hearts were removed from cats anaesthetised with pentobarbitone sodium and were perfused with Ringer solution at 37° by the method of Langendorff. The coronary flow was measured by timing the flow in a graduated cylinder using a stopwatch. Heart rate was recorded by an electronic counter. In some experiments the rate and the force of contraction was recorded on a 7 channel Grass polygraph by means of a force displacement transducer FT10C. The perfusates samples were collected in measuring cylinders containing 1 ml of N HCl and 0·75 mg of EDTA. The perfusates were concentrated, adsorbed on alumina, eluted and catecholamines concentration determined as noradrenaline as described by Crout *et al.*[12] Many of the perfusates were also assayed biologically using pithed rat blood pressure. The identification of noradrenaline was checked both biologically (on the blood pressure of a pithed rat) and chemically by comparing the fluorescence spectra of pure samples of noradrenaline added to the perfusates. Out of the two methods used for identification of noradrenaline, it was felt necessary to give more weight to the biological assay than the chemical one (details in result section). Recovery of the pure noradrenaline added to the perfusates was of the order of 80–90 per cent.

TABLE 1. EFFECT OF VARIOUS DRUGS ON THE NORADRENALINE RELEASE* FROM THE ISOLATED CAT HEART

Series	Treatment	Dose (μg/ml)	Noradrenaline released (ng/min) I Dose		Noradrenaline released (ng/min) II Dose		Concentration of noradrenaline (μg/g) (whole heart)†	
1	Tyramine	15	25·5 ± 2	(19)	20·13 ± 4		0·80	(2)
2	Amphetamine	50	0	(10)	0	(10)	1·23 ± 0·06	(5)
3	Metaraminol	20	26·4 ± 7	(4)	16·1 ± 3	(4)	0·66 ± 0·1	(6)
4	Ephedrine	50	17·5 ± 3	(8)	8·3 ± 3	(6)	1·06 ± 0·1	(6)
5	Dopamine	2	0	(9)	0	(9)	1·67 ± 0·13	(8)

All values are the mean ± S.E.M. The figures in the parenthesis indicate the number of observations.
* Noradrenaline estimated spectrophotofluorimetrically.
† Control values for the cat heart were 1·3 ± 0·08 (range 1·00–1·99, 21 cats).

The general procedure used for catecholamine release was as follows: The hearts were allowed to perfuse for about half an hour. When the heart rate and amplitude were fairly constant, the first sample of the perfusate was collected. In these control samples there was a small reading which was used as a blank figure for the calculation of noradrenaline released when the drugs were incorporated (blank). The drugs under study were then incorporated in the perfusing fluid and were perfused for 2 min. The perfusion fluid was then changed back to Ringer solution. The collection of the perfusate was started as soon as the cardiac effects were seen and was continued for 10 min. In many cases the same dose of the drug was again repeated a second time after about 30–45 min. In some cats reserpine, guanethidine and α-methyl tyrosine pretreatment was given for 2–3 days (doses given in Table 5).

Extraction of noradrenaline from the cat heart was carried out by perchloric acid. The catecholamines were adsorbed on acid washed alumina at pH 8·4 and eluted with 0·2 N acetic acid as described above.[12]

To test that none of the drugs used in the present investigation interfered with the assay of noradrenaline, a series of experiments were carried out in which different concentrations of drugs used were incorporated with pure solution of noradrenaline and assayed as described above. Under our experimental conditions we could pick up as little as 0·3 ng/ml of noradrenaline in the perfusates (about 40–50 ng total in about 150 ml).

Drugs used were l-noradrenaline-*d* hydrogen tartrate, *d*-amphetamine sulphate, tyramine hydrochloride, guanethidine sulphate, ephedrine hydrochloride, metaraminol, 3-hydroxytyramine hydrochloride and *dl*-α-methyltyrosine. All the doses refer to their salts, except for noradrenaline which is expressed as a base.

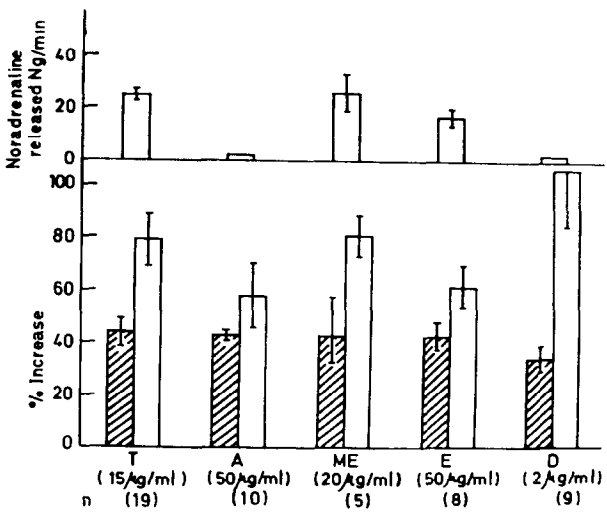

FIG. 1. Effect of tyramine (T), amphetamine (A), metaraminol (ME), ephedrine (E), and dopamine (D) on the inotropic (open columns), chronotropic (crossed columns) and noradrenaline release in the isolated cat heart. Note that amphetamine and dopamine did not release any measurable amount of noradrenaline.

RESULTS

In the absence of any drug no measurable amount of noradrenaline was detected in the perfusates. The effects of various drugs on the inotropic, chronotropic and noradrenaline release are shown in Figs. 1 and 2 and Tables 1 and 2. In the last column of Table 1, the noradrenaline concentration of the whole heart of the cat is shown. These hearts were taken after experiments with the corresponding drug were over. This measurement was thought necessary to see if there is any significant change in the noradrenaline content of the heart after perfusion with these drugs. Ephedrine, tyramine and metaraminol produced a marked change in the noradrenaline concentration (Table 1, Series 1, 3 and 4). All the substances showed a positive inotropic and chronotropic response with simultaneous release of noradrenaline (Fig. 1) except for dopamine (2 μg/ml) and amphetamine (50 μg/ml) (Table 1 and 2). Despite the fact that amphetamine produced positive inotropic and chronotropic effects, it did not release any significant amount of noradrenaline which could be measured either chemically or biologically (Fig. 1). In the case of dopamine, however, the perfusates

TABLE 2. EFFECT OF VARIOUS DRUGS ON THE NORADRENALINE RELEASE* FROM THE ISOLATED CAT HEART

Series	Treatment	Dose (μg/ml)	Noradrenaline released (ng/total)	Noradrenaline released (ng/min)
1	Tyramine	15	247 ± 14 (5)	24·7 ± 1·4 (5)
2	Amphetamine	50	0 (3)	0 (3)
3	Metaraminol	20	131 ± 31 (3)	13·1 ± 3 (3)
4	Ephedrine	50	126 ± 34 (3)	12·6 ± 3 (3)
5	Dopamine	2	0 (3)	0 (3)

* Noradrenaline estimated biologically on the blood pressure of a pithed rat.

when assayed chemically seemed to show a release of noradrenaline from the cat heart. The fluorescence spectra were positive, but when the same perfusates were assayed biologically on the blood pressure of a pithed rat, they gave no pressor responses indicating no release at all. Dopamine in low concentration (200 ng) did not interfere in our assay of noradrenaline but higher concentration of dopamine (80–100 times more) as were present in our perfusates did interfere in the assay of noradrenaline, thus giving a positive fluorescence spectrum indicative of release of noradrenaline.

The effect of 15 and 50 μg/ml of amphetamine on the heart rate, amplitude and noradrenaline release is shown in Table 3. It can be seen that this substance produces positive inotropic and chronotropic effect without any measurable release of noradrenaline in the perfusates. A total of 16 experiments were carried out with both the

TABLE 3. EFFECT OF AMPHETAMINE (15 and 50 μg/ml) ON THE INOTROPIC, CHRONOTROPIC AND NORADRENALINE RELEASE IN THE ISOLATED CAT HEART

Series	Dose	% Increase Chronotropic effect	% Increase Inotropic effect	Noradrenaline release (ng/ml)
1	Amphetamine (15 μg/ml)	41·4 ± 6 (6)	33·0 ± 8 (6)	Nil
2	Amphetamine (50 μg/ml)	42·7 ± 2 (10)	57·9 ± 12 (10)	Nil

All values are the means ± S.E.M.

doses. Three experiments did not show any difference in the fluorescence readings from the controls. In the rest of the thirteen experiments, a slight difference in the fluorescence readings between the blank and the sample were noted. However, none of these samples gave a positive fluorescent spectra for noradrenaline nor gave any pressor response in the pithed rat. These results therefore indicate that amphetamine at these dose levels, although producing the normal cardiac effects similar to many other drugs studied in the present study, was unable to release any measurable amount of noradrenaline or if it did release it was so small that one could not detect it (less than 40–50 ng per sample). This study was further checked by doing a few more experiments in three different ways:

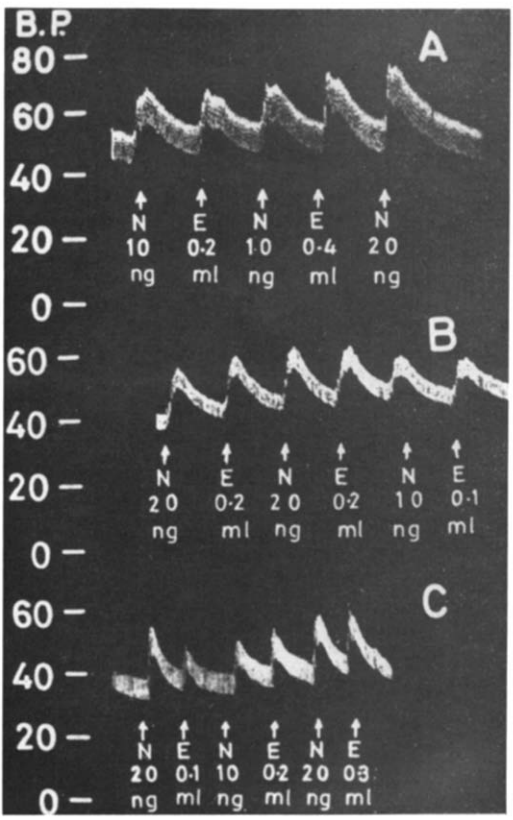

FIG. 2. Pithed rat blood pressure. Responses to standard noradrenaline and noradrenaline isolated from the perfusates of tyramine (A), metaraminol (B) and ephedrine (C) treated cats. For biological assays two doses of standard noradrenaline (N) and two doses of the extract (E) were compared with each other.

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(a) In the first series, amphetamine (50 μg/ml) was perfused as described before and this was followed by tyramine (15 μg/ml). Both the perfusates were collected and assayed.
(b) In the second series, tyramine (15 μg/ml) was given first followed by amphetamine (50 μg/ml).
(c) In the last series a dose of tyramine was followed by two different doses of amphetamine and at the end a dose of tyramine was repeated again. The time intervals between the two doses were at least half an hour.

The results of these three sets of experiments are shown in Table 4 and Fig. 3. As can be seen from Table 4, there was no measurable release of noradrenaline by amphetamine while tyramine produced a considerable release of noradrenaline (Table 4, Series A). The fluorescence readings obtained after amphetamine (15 μg/ml)

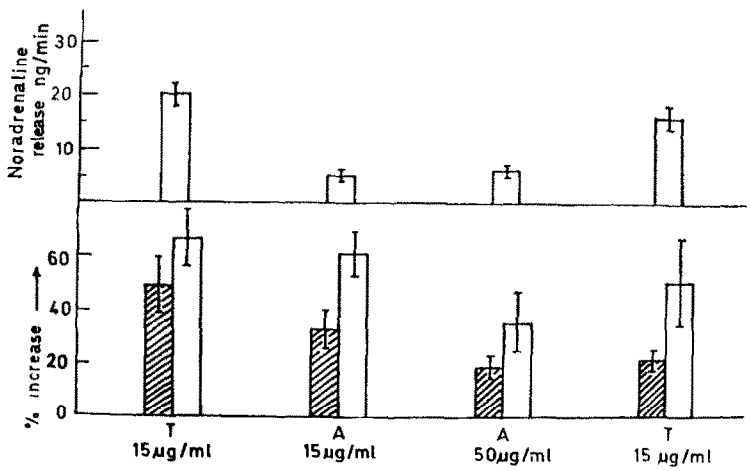

FIG. 3. Effect of tyramine (T) and amphetamine (A) on the inotropic (open columns) and chronotropic (crossed columns) and noradrenaline release from the isolated cat heart. Note that even the last dose of tyramine released as much noradrenaline as the first dose but amphetamine did not release any measurable amount of noradrenaline.

treatment were slightly higher than those seen in experiments shown in Fig. 1 where 50 μg/ml of amphetamine was used. However, no further increase in the fluorescence reading was obtained with a higher dose of amphetamine (50 μg/ml). The only difference in these two sets of experiments was that in the first set of experiments, hearts were directly exposed to amphetamine (50 μg/ml, Fig. 1) and in the second set of experiments (Fig. 3) hearts were exposed to amphetamine (15 and 50 μg/ml) after a dose of tyramine. This pretreatment may be responsible for the slight increase seen in the fluorescence readings. However, all the fluorescence spectra for amphetamine were negative and for tyramine positive. From Fig. 3, it can be seen that tyramine given at the end of experiments (4th dose) was still able to release a significant amount of noradrenaline whereas both the doses of amphetamine given in the middle of experiments released no measurable amount of noradrenaline although the inotropic and chronotropic effects were marked. In some experiments with tyramine it was noted that the second dose of tyramine produced tachyphylaxis on the inotropic and

TABLE 4. EFFECT OF AMPHETAMINE (50 μg/ml) AND TYRAMINE (15 μg/ml) ON THE INOTROPIC, CHRONOTROPIC AND NORADRENALINE RELEASE FROM THE ISOLATED CAT HEART

Series	Test substance and dose	% Increase in chronotropic effect	% Increase in inotropic effect	Noradrenaline released (ng/ml)	Noradrenaline released (ng/min)
A	Amphetamine (50 μg/ml)	40·3 ± 4·3 (9)	48·0 ± 13 (9)	Nil (9)	Nil (9)
	Tyramine (15 μg/ml)	8·6 ± 3·1 (9)	57·9 ± 13 (9)	1·83 ± 0·21 (9)	47·8 ± 5 (9)
B	Tyramine (15 μg/ml)	46·6 ± 12 (5)	69·30 ± 14 (5)	1·03 ± 0·1 (5)	27·10 ± 5 (5)
	Amphetamine (50 μg/ml)	18·3 ± 3·6 (5)	No increase Decrease 4/5	Nil (5)	Nil (5)

All values are the means ± S.E.M. The figures in parenthesis indicate the number of observations.

In Series A the sequence of perfusing the drugs was amphetamine, tyramine and in Series B, tyramine was given first followed by amphetamine.

chronotropic effect of the heart but not on the amount of noradrenaline release. These results would indicate a lack of correlation between tachyphylaxic response for tyramine and release of noradrenaline in the cat heart.

Since we were unable to detect any noradrenaline in the perfusates of the amphetamine-treated hearts, and the fact that the cardiac effects were seen in these experiments, it seemed worthwhile to investigate as to how important noradrenaline stores are for the amphetamine response. Pretreatment of cats with different drugs were carried out and the effect of amphetamine investigated on these hearts. The results are shown in Table 5. From the results it can be seen that amphetamine can produce a significant effect in the heart virtually depleted of noradrenaline (Table 5, Series 2, 3 and 6). Besides producing depletion, high doses of reserpine seem to depress the myocardium which might in itself be responsible for the absence of effect seen in the reserpinized animals (Table 5, Series 2 and 4).

TABLE 5. EFFECT OF AMPHETAMINE (15 μg/ml) ON THE INOTROPIC, CHRONOTROPIC AND NORADRENALINE CONTENT OF THE ISOLATED CAT HEART*

Series	Treatment	No. of observations	% Increase		Noradrenaline content (μg/g)	% Depletion
			Inotropic effect	Chronotropic effect		
1	Amphetamine	6	33	41	1·38	—
2	Amphetamine	2	9	14	0	100
3	Amphetamine	2	22	33·4	0	100
4	Amphetamine	1	17	6	0·09	93
5	Amphetamine	4	20	30	0·02	98·5
6	Amphetamine	3	20	35	0·14	90·0

* In Series 2, 2 × 50 μg/kg reserpine (i.p.) was given with an interval of 4 hr. The animals were killed 16 hr after the last dose.

In Series 3, 1 × 25 μg/kg α-methyl tyrosine (i.p.) followed 4 and 20 hr later by 2 × 15 μg/kg i.p. of reserpine. Animals were killed 3–6 hr after the last dose of reserpine.

In Series 4, 3 × 25 μg/kg reserpine (i.p.) on 3 different days; animals were killed 6 hr after the last dose.

In Series 5, 2 × 25 μg/kg reserpine (i.p.) on 2 different days; animals were killed 24 hr after the last dose.

In Series 6, 2 × 5 mg/kg guanethidine (s.c.) with an interval of 7 hr; 16 hr later 1 × 5 mg/kg guanethidine (s.c.) was given. Animals killed 24 hr after the last dose.

DISCUSSION

The use of perfusion method in the present study to measure the release of noradrenaline in the perfusion fluid leaving the organ was preferred to some other methods because, under the influence of drugs, a small proportion of transmitter is released which produces substantial effects. The measurement of this small amount of transmitter is relatively easy, in the perfusion fluid rather than if one measures the levels of the stored transmitter in the tissues.

The results presented in this paper show that most of the substances used in the present study release noradrenaline in the perfusates. The results of tyramine reported here are very similar to those reported by earlier workers.[7,9] The amount of transmitter released by these substances cannot be estimated in absolute figures as part of

it is taken up in the amine stores and part of it may even be metabolised. Out of all the substances tested, tyramine was the most potent in releasing noradrenaline. The inability of dopamine to release any noradrenaline was not surprising since this substance is known to act directly. However, the disparity observed in biological and chemical estimation in these perfusates was because of the high concentration of dopamine in our perfusates. These results therefore show that estimation of noradrenaline in the presence of high concentration of dopamine can give misleading results, and care should be taken in the interpretation of such results. The identification of noradrenaline under such conditions is very difficult since both dopamine and noradrenaline exhibit identical fluorescence spectras.

The results reported here for tyramine and amphetamine seem to throw some light on the mechanism by which these two substances produce their cardiovascular effects. Our results do not seem to support the view that both amphetamine and tyramine produce their effects by the same mechanism, e.g. release of noradrenaline. (For detailed discussion on the evidence in favour and against the noradrenaline displacement theory, see Iversen.[13]) As far as we know there is no direct evidence that amphetamine releases endogenous noradrenaline from the peripheral tissues except that of Chidsey *et al.*[14] where an increase in the noradrenaline output has been seen in the coronary sinus blood of a dog after the administration of amphetamine. Our results cannot be directly compared with their results because of the different conditions of experiments. It is of interest, however, to see that these workers used a dose of amphetamine which is five times the dose of tyramine and still released only one-fourth the amount of noradrenaline in the coronary sinus. They also concluded that tyramine was very potent in releasing noradrenaline and amphetamine was very weak. Carlsson and Waldeck[15] have also shown the inability of amphetamine to release extragranular [^{3}H]noradrenaline in normal mice brain, but they were able to show a release in reserpinized mice, treated with monoamine oxidase inhibitors.

The evidence that the cardiovascular effects of indirectly acting amines (namely, amphetamine and tyramine) are mediated by the release of noradrenaline from the storage sites is mainly based on the experiments done in reserpinized animals where fairly high doses of reserpine have been given (0·5–5 mg/kg) (Burn and Rand).[1,16] Although this hypothesis has been supported and confirmed by a number of workers,[2–4] it still does not answer all the questions concerning the cardiovascular effects of these indirectly acting amines[17–19] (for dissenting view points see Ref. 19. The ability of tyramine and inability of amphetamine to release noradrenaline seems to indicate that both these substances do not act the same way. Our results further show that amphetamine has some direct action on the myocardium since we were able to see significant cardiac responses in those hearts where no noradrenaline stores were present (Table 5). Increasing the doses of reserpine seem to depress the myocardium. Large doses of reserpine are known to produce failure of the heart.[20] It is therefore possible that such doses of the sympathomimetic amines which would have normally stimulated the normal heart would fail to do so in reserpinized heart.

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