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DIFFERENCES IN AMINE STORAGE IN RAT HEART AND BRAIN

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1 The characteristics of storage of amphetamine-releasable amine in rat heart and brain were studied *in vitro* with labelled and unlabelled metaraminol ([^{3}H]-MA and MA).

2 In one series of experiments, heart and brain slices were incubated with [^{3}H]-MA prior or subsequent to incubation with the same concentration of MA.

3 When brain slices, thus treated, were subjected successively to field stimulation and to amphetamine, it was found that the ratio of [^{3}H]-MA release by field stimulation to release by amphetamine was dependent upon the order in which brain tissue was exposed to [^{3}H]-MA and MA.

4 With heart slices, on the other hand, the field stimulation/amphetamine ratio of [^{3}H]-MA release remained the same whether the tissue was exposed to [^{3}H]-MA before or after MA.

5 The (+)-isomer of amphetamine released three to four times more [^{3}H]-MA from brain slices than did the (−)-isomer, while the isomers were equipotent with regard to [^{3}H]-MA release from heart slices.

6 Amphetamine-induced [^{3}H]-MA release from heart slices was unaffected by the presence of α-methyl-*p*-tyrosine, while release from brain slices was inhibited.

7 On the basis of the foregoing results it appears that amine storage differs in brain and heart, with brain exhibiting more than one functional amine pool.

Introduction

An impressive amount of evidence has accumulated indicating that the site of amphetamine-induced amine release differs in central and peripheral tissue. For example, pretreatment with reserpine abolishes the pressor response to amphetamine (Burn & Rand, 1958) but has no effect on amphetamine-induced central excitation (Rech & Stolk, 1970). That the central action of amphetamine is, however, mediated by amine release is indicated by the finding that the central stimulating action of the drug is blocked by the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine (Weissman & Koe, 1965; Weissman, Koe & Tenen, 1966).

Another difference between central and peripheral responses to amphetamine is that (+)-amphetamine is several times more potent as a central stimulant than is the (−)-isomer, whereas the two isomers are equipotent with regard to peripheral pressor activity (Stein, 1964).

In the present investigation, an attempt was made to characterize further these organ differences by comparing the release from brain and heart slices of metaraminol, a noradrenaline congener which acts as a non-metabolizable false transmitter at adrenergic nerve endings (Shore, Busfield & Alpers, 1964; Crout, Alpers, Tatum & Shore, 1964).

Some of these results were given at the Fifth International Congress on Pharmacology in San Franciso, 1972.

Methods

Tissue preparation and incubation

Female Sprague-Dawley rats (200-225 g) were decapitated, and the brain or heart removed and placed into ice cold 0.9% w/v NaCl solution (saline). In all studies, tissue slices were obtained with a Harvard tissue slicer. For brain studies, either a coronal slice taken at the level of the hypothalamus or a cortical slice (0.3 mm, 30-60 mg) was used; with heart studies, a ventricle slice (0.3 mm, 60-80 mg) was used. Tissue slices

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were placed in 20 ml beakers containing 3 ml Krebs-Ringer bicarbonate buffer (pH 7.4) and bubbled with 95% O_2 and 5% CO_2 (Dengler, Michaelson, Spiegel & Titus, 1962). Tissues were incubated at 37° C in a Dubnoff metabolic shaker. For 'hot-cold' experiments, after a 15 min pre-incubation of a slice, 10 µl of a labelled (±)-metaraminol ([^{3}H]-MA) solution was added to the beaker and incubation continued for another 15 minutes. The tissue was then transferred to another beaker containing an identical amount of buffer to which had been added 10 µl of only unlabelled (±)-metaraminol (MA) and incubation continued for a final 30 minutes. 'Cold-hot' experiments were conducted in the same manner except that the tissue was incubated with MA for 15 min prior to a 30 min incubation with [^{3}H]-MA. The initial concentration of both the [^{3}H]-MA and MA solutions in the incubation medium was 13 ng/ml.

In all other experiments, after a 15 min pre-incubation, tissue slices were incubated for 30 min with [^{3}H]-MA only.

Washout of [^{3}H]-metaraminol

After incubation, the tissue was placed into a 5 ml plastic chamber filled with Krebs-Ringer bicarbonate buffer. Temperature in the bathing medium was maintained at 37° C. During the initial 25 min, the chamber was emptied at various intervals and refilled with fresh medium. Washout was continued for a further 5 min by changing the medium at 1 min intervals. Subsequent serial samples were taken at 1 min intervals for the duration of the experiment. In 'hot-cold' and 'cold-hot' studies, after the 30 min washout period, the tissue was subjected to field stimulation by a sinusoidal alternating electrical field (Baldessarini & Kopin, 1967). The stimulus intensity was 5 V and was continued for 1 minute. Current flow through the bath was about 80 mA. Following field stimulation, when the rate of [^{3}H]-MA efflux had returned to baseline, the tissue was exposed, for 1 min, to buffer containing 10^{-5} M (+)-amphetamine, followed by continued medium exchanges until isotopic efflux returned to baseline.

In studies comparing (+)-amphetamine to (−)-amphetamine, after the 30 min washout, the tissue was exposed for either 1 or 2 min to 10^{-5} M (+)-or (−)-amphetamine.

In experiments with α-methyl-*p*-tyrosine (α-MT), washout took place in buffer containing the inhibitor at a concentration of 5×10^{-6} M. These tissues were exposed to (+)-amphetamine only, at two separate times during the experiment, each exposure lasting for 1 minute.

Analytical determinations

The amount of radioactivity contained in the samples of bathing fluid removed during the washout was determined by the addition of 2 ml portions to 10 ml of scintillation solution (Aquasol, New England Nuclear) and counting in a Beckman liquid scintillation spectrometer. Each vial was counted to a statistical accuracy of ±5% or less; the counting efficiency was 39%.

Drugs used

The drugs used in this study and their sources were: (±)-[7-^{3}H]-metaraminol, 8.2 Ci/mmol, New England Nuclear (Boston, Mass.); (+)- and (−)-metaraminol, Sterling-Winthrop Research Inst. (Rensselaer, N.Y.); (+)- and (−)-amphetamine sulphate, Smith, Kline & French Labs. (Philadelphia, Pa.); (−)-α-methyl-*p*-tyrosine, Merck, Sharp & Dohme (West Point, Pa.).

Results

'Cold-hot' and 'hot-cold' studies

After heart slices were incubated with unlabelled MA for 15 min prior to a 30 min incubation with [^{3}H]-MA ('cold-hot'), both field stimulation and amphetamine evoked a substantial increase in efflux of isotope from the tissue (Figure 1). Similar results were obtained after heart slices were incubated with [^{3}H]-MA for 15 min prior to a 30 min incubation with MA ('hot-cold'). That the amount of [^{3}H]-MA released in the 'hot-cold' experiment was less than that seen with the 'cold-hot' experiment is understandable since the latter was exposed to [^{3}H]-MA for twice as long as the former and therefore contains a higher concentration of the isotope. However, it should be noted that the amount of [^{3}H]-MA released from the heart by amphetamine relative to the amount released by field stimulation did not vary with the order in which the tissue was exposed to [^{3}H]-MA and MA.

When brain cortical slices were incubated with MA for 15 min prior to a 30 min incubation with [^{3}H]-MA ('cold-hot'), about twice as much [^{3}H]-MA was released after field stimulation than after amphetamine exposure (Figure 2). Following incubation in the reverse order ('hot-cold'), the amount of ^{3}H-release by amphetamine relative to field-stimulated ^{3}H-release was much diminished (Figure 2).

To analyse the data more quantitatively, the amount of [^{3}H]-MA released by field stimulation

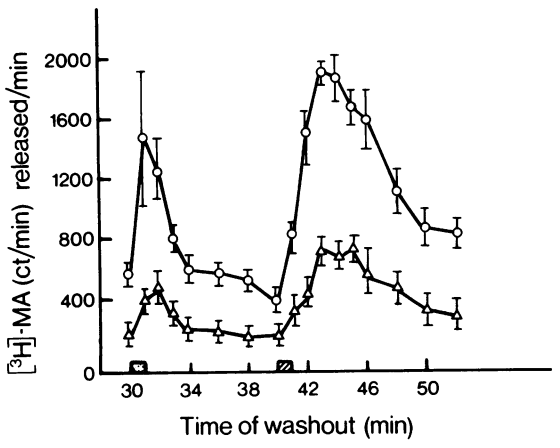

Fig. 1 Release of labelled metaraminol ([^{3}H]-MA) from rat heart ventricular slices after a 1 min field stimulation and a 1 min exposure to (+)-amphetamine. Cold-hot (○) represents tissues allowed to accumulate unlabelled metaraminol prior to the uptake of [^{3}H]-MA. Hot-cold (△) tissues were treated in the reverse fashion. The stippled bar represents the duration of field stimulation (5 V, 80 mA), the hatched bar represents the duration of amphetamine (10^{-5} M) exposure. Each point is the mean ± s.e. of three experiments.

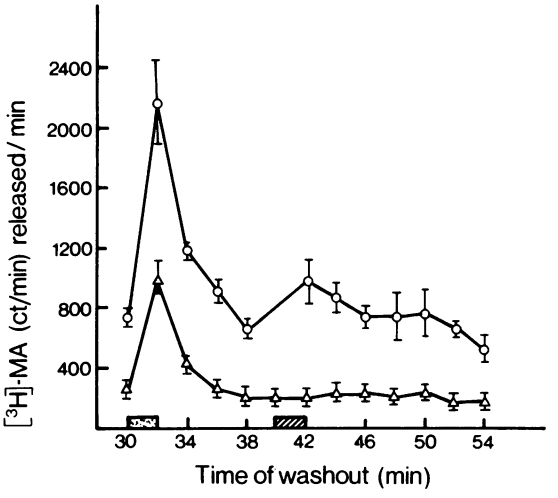

Fig. 2 Release of labelled metaraminol ([^{3}H]-MA) from rat brain cortical slices after a 1 min field stimulation and a 1 min exposure to (+)-amphetamine. Cold-hot (○) represents tissues allowed to accumulate unlabelled metaraminol prior to the uptake of [^{3}H]-MA. Hot-cold (△) tissues were treated in the reverse fashion. The stippled bar represents the duration of field stimulation (5 V, 80 mA), the hatched bar represents the duration of amphetamine (10^{-5} M) exposure. Each point is the mean ± s.e. of three experiments.

and by amphetamine was determined by calculation of the net release of [^{3}H]-MA for the 1 min during and 3 min following exposure to field stimulation or amphetamine. Table 1 shows that, from brain tissue exposed to [^{3}H]-MA after MA ('cold-hot'), 1.74 times more [^{3}H]-MA was released in response to field stimulation than in response to amphetamine. However, from brain tissue exposed to MA after [^{3}H]-MA ('hot-cold'), 3.59 times more [^{3}H]-MA was released in response to field stimulation than in response to amphetamine. This ^{3}H-release dependence on the order of incubation in brain tissue is in contrast to heart tissue, where the ratio of field stimulation to amphetamine release was the same regardless of whether the tissue stored [^{3}H]-MA before or after MA. Similar results were obtained when the experiments were performed with amphetamine exposure preceding field stimulation.

Table 1 Comparison of [^{3}H]-metaraminol release by field stimulation and by amphetamine from rat heart and brain slices incubated with [^{3}H]-metaraminol ([^{3}H]-MA) before or after unlabelled metaraminol.

Tissue	*Treatment*	*[^{3}H]-MA released* (ct/min)*		*Ratio (Field stim/amphet)*
		Field stim	*Amphet*	
Cerebral cortex	'cold-hot'	1977 ± 448	1139 ± 338	1.74
	'hot-cold'	844 ± 217	235 ± 17	3.59
Heart	'cold-hot'	1924 ± 491	4523 ± 302	0.43
	'hot-cold'	695 ± 141	1573 ± 104	0.43

*Values represent net release of [^{3}H]-MA for 1 min during and 3 min following field stimulation or amphetamine. Each value is the mean ± s.e. of three experiments.

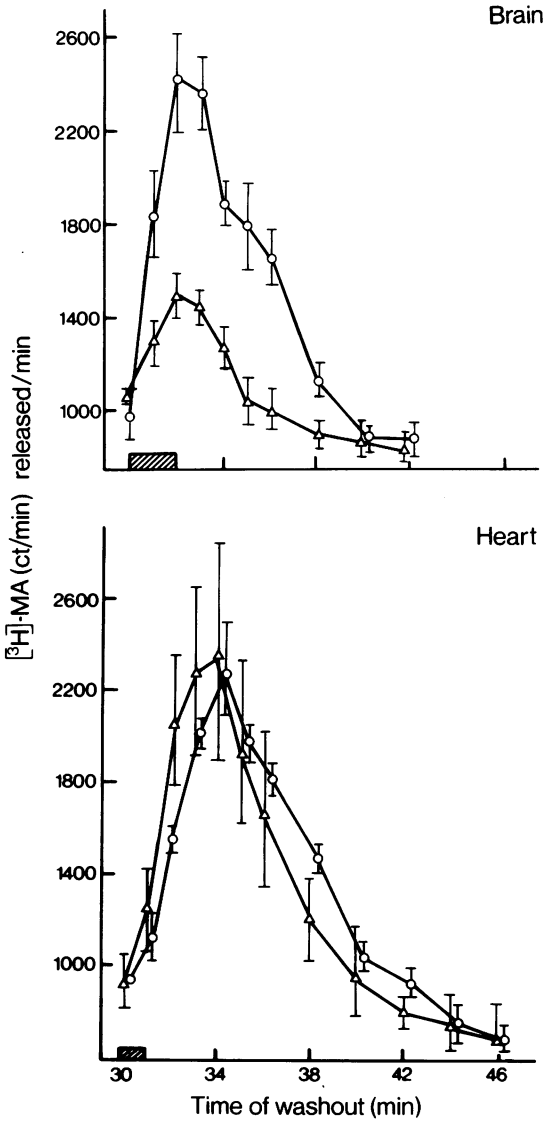

Fig. 3. Release of labelled metaraminol ([^{3}H]-MA) by (+)- and (−)-amphetamine from rat brain and heart slices. Each value is the mean ± s.e. for three experiments. Bars represent the presence of amphetamine, (○) (+)-amphetamine treated, (△) (−)-amphetamine treated.

Release of [^{3}H]-metaraminol by (+)- or (−)-amphetamine

After incubation of brain coronal slices or heart ventricular slices in [^{3}H]-MA for 30 min, the tissue was washed as described above for 30 min and was then exposed to 10^{-5} M (+)- or (−)-amphetamine. As shown in Fig. 3, a 2 min exposure to (+)-amphetamine evoked a greater release of [^{3}H]-MA from brain slices than did a 2 min exposure to (−)-amphetamine, whereas in heart slices, the two isomers evoked an equal ^{3}H-release.

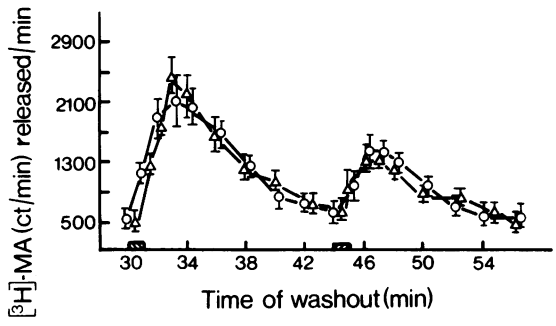

Fig. 4 Effect of α-methyl-*p*-tyrosine (α-MT) on the release of labelled metaraminol ([^{3}H]-MA) from rat heart slices by (+)-amphetamine. Each value is the mean ± s.e. for 3-6 experiments. Bars represent the presence of (+)-amphetamine and (○) control and (△) α-MT-treated heart slices.

Effect of α-methyl-p-tyrosine on release of [^{3}H]-metaraminol from heart

Heart slices, preloaded *in vitro* with [^{3}H]-MA, were washed for 30 min with buffer containing 5×10^{-6} M α-MT. The tissue was then exposed for 1 min to 10^{-5} M (+)-amphetamine followed 13 min later by a second 1 min exposure to the drug. As shown in Fig. 4, α-MT had no effect on the amphetamine-induced release of [^{3}H]-MA during either exposure to the stimulant.

Discussion

A recent report from this laboratory demonstrated that metaraminol released from rat heart following either field stimulation or exposure to tyramine is derived from a single storage pool or from a functional site in rapid equilibrium with the main storage pool (Enna & Shore, 1972). That peripheral amine is stored in a single pool in the adrenergic neurone is further suggested by the finding that treatment with reserpine, which depletes the main storage pool of transmitter, abolishes the pressor response to amphetamine (Burn & Rand, 1958).

In contrast, it appears that in brain tissue transmitter is stored in at least two separate pools since amphetamine-induced central effects are not

blocked by reserpine (Rech & Stolk, 1970), but are blocked by the tyrosine hydroxylase inhibitor, α-MT, at a time when the cerebral concentration of noradrenaline is still over 50% of normal (Weissman & Koe, 1965). More recently it has been shown that this action of α-MT in brain may not be due entirely to its ability to block amine synthesis, since α-MT also caused a direct inhibition of amphetamine-induced amine release from brain slices (Enna, Dorris & Shore, 1973). Another indication that transmitter storage and release may differ in heart and brain is apparent in that the two isomers of amphetamine differ in potency with respect to central stimulation, but are equipotent with respect to pressor activity (Stein, 1964).

In the present experiments, when brain slices were incubated in [^{3}H]-MA prior or subsequent to an incubation with MA, it was found that the order in which incubation took place determined the amount of [^{3}H]-MA released by field stimulation relative to that released by amphetamine. Similar results were obtained when the tissue was exposed to amphetamine prior to field stimulation, indicating that the order in which the tissue was exposed to the releasing agents was not important in determining the relative difference in response to the two stimulants. These findings thus suggest that in brain tissue, amphetamine, as compared with field stimulation, releases transmitter preferentially from a site containing most recently acquired amine. With heart tissue, on the other hand, both amphetamine and field stimulation apparently release amine from a single site, since the order of amine loading did not alter ^{3}H-release. This finding with heart tissue is in agreement with the previous conclusion reached with hearts loaded *in vivo* with labelled and unlabelled MA (Enna & Shore, 1972).

Further evidence that the amphetamine-sensitive site differs in heart and brain is provided by the finding that (+)-amphetamine was more potent as a releaser of [^{3}H]-MA from brain than was (−)-amphetamine, whereas in heart, the amount of isotope liberated by the two isomers was the same. These results with brain are in agreement with a recent report by Ziance, Azzaro & Rutledge (1972) who demonstrated *in vitro* that (+)-amphetamine released more [^{3}H]-norepinephrine from rat brain cortical minces than did (−)-amphetamine.

It has been reported that (+)-amphetamine is 10 times more potent than (−)-amphetamine in inhibiting uptake of transmitter into adrenergic neurones in rat brain (Taylor & Snyder, 1970) and that the (+)-isomer is 20 times more potent than the (−)-isomer in inhibiting uptake into rat heart (Iversen, 1967). In the present study, (+)-amphetamine was three to four times as potent as (−)-amphetamine in the brain and the two isomers were equipotent in the heart, suggesting that the appearance of [^{3}H]-MA in the washout medium was due primarily to release of the isotope by amphetamine rather than to blockade of reuptake.

Another indication of the difference between heart and brain in the storage site of amphetamine-releasable transmitter is suggested by the results obtained with α-MT. Previous work has demonstrated that amphetamine-induced release, but not field stimulation-induced release, of [^{3}H]-MA or [^{3}H]-noradrenaline from brain slices is inhibited by the presence of α-MT in the washout medium (Enna *et al.*, 1973). The present study shows that α-MT does not have a similar inhibitory action in heart tissue.

In summary, the present study presents three lines of evidence suggesting that in brain, but not in heart, there exists an amphetamine-sensitive amine storage site which is separate from the general amine pool. These are: (1) the special susceptibility of brain as opposed to heart to release of newly acquired amine by amphetamine relative to field stimulation; (2) the stereospecific nature of release of brain amine but not heart amine by (+)-amphetamine as compared with (−)-amphetamine; (3) the inhibitory action of α-MT on amphetamine-induced release of brain amine but not heart amine.

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