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CHARACTERISTICS OF STORAGE AND RELEASE OF METARAMINOL IN THE RAT HEART*

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Abstract—Field stimulation or incubation with tyramine of heart slices from rats treated with tritium-labeled metaraminol caused a release of the false adrenergic transmitter. Regardless of whether unlabeled metaraminol was given 16 or 40 hr either before or after the labeled compound, the release of labeled metaraminol by either field stimulation or tyramine reflected the specific activity of the total heart metaraminol content. Similarly, giving unlabeled metaraminol 40 hr after administration of the isotope resulted in a specific activity in the intact animal heart which did not change over a further 4-day period, even though total content decreased markedly. It is concluded that intraneuronal release of metaraminol by nerve stimulation or by tyramine occurs from either a single pool or from a functional site in rapid equilibrium with the main storage pool rather than from a site semi-sequestered from a semi-inert main storage pool.

THE MANNER in which norepinephrine (NE) is stored and released from the adrenergic neuron has been the subject of considerable investigation.[1–4] Some evidence suggests that the transmitter is bound within the neuron in at least two sites or pools, one containing only recently accumulated or synthesized transmitter which is immediately available for release, the other serving only as a semi-inert reservoir. Other findings, however, suggest that release may occur either directly from the main storage pool or from a site in rapid and complete equilibrium with the main storage pool.

In the present study, the storage and release of metaraminol, a nonmetabolizable congener of NE, which displaces NE and acts as a false adrenergic transmitter,[5,6] were investigated. The results suggest that the release of this amine by either neurogenic stimulation or by tyramine is from either a single pool or from a functional site in rapid equilibrium with the main storage pool.

MATERIALS AND METHODS

Drug solutions and dosage. [^{3}H]metaraminol injection solution ([^{3}H]MA) was prepared by placing 100 μl of a solution of *dl*-[7-^{3}H]metaraminol (8·2 c/m-mole; New England Nuclear) into 1·15 ml of a saline solution containing 50 μg/ml of unlabeled *l*-metaraminol (Sterling–Winthrop Research Institute). Each animal received 1 ml/kg i.v. of this solution as a standard dose of [^{3}H]MA. A previous report from this laboratory[5] has indicated that *d*-metaraminol is not appreciably bound to rat heart, the heart content of this isomer being insignificant 3 hr after intravenous injection. In the present experiments, [^{3}H]MA levels were always measured at least 4 hr after injection; therefore, references in the text to either total metaraminol (MA)

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or to the labeled compound ([^{3}H]MA) refer to the *l*-isomer only. The standard dose of the unlabeled *l*-metaraminol solution (MA) was 50 μg/kg administered similarly.

For use *in vitro*, tyramine (5×10^{-5} M) was dissolved in Krebs–Ringer bicarbonate solution. In one experiment, a concentration of 10^{-5} M was used.

Animals. Female Sprague–Dawley rats weighing between 200 and 225 g were used throughout this study. All injections were made via the tail vein.

Field stimulation studies. Field stimulation experiments were carried out by a modification of the method described by Baldessarini and Kopin.[7] In the present study, animals were killed by a blow on the head at various times after the injection of [^{3}H]MA, and the hearts were removed and placed into ice-cold saline. A slice of ventricular tissue (0·3 mm, 100–200 mg) was placed into a 5-ml plastic chamber containing Krebs–Ringer bicarbonate supplemented with tricarboxylic cycle intermediates[8] and bubbled with 95% O_2–5% CO_2. Temperature in the bath was maintained at 37°.

During the initial 25 min, the chamber was evacuated at various intervals and refilled with fresh medium; subsequent medium exchanges were generally made at 1-min intervals. After 30 min in the bathing medium, the tissue was stimulated by a sinusoidal 60-cycles/sec alternating electrical field provided by platinum coil electrodes about 10 mm apart. The stimulus intensity was 5 V and was continued for 1 min. Current flow through the bath was about 70 mA.

At the end of each experiment, the heart was analyzed for total metaraminol and for [^{3}H]MA. Samples of the bathing media were analyzed for [^{3}H]MA only.

Tyramine studies. Tissues were prepared and placed into the chamber as described above. In one experiment, after 30 min in the bathing media, the tissue was exposed to 10^{-5} M tyramine for 15 min with the medium exchanged once each minute. Each of the individual 1-min samples was analyzed for [^{3}H]MA content.

In most of the tyramine experiments, 30 min after the start of incubation, the tissue was exposed for 20 min to a single sample of buffer containing 5×10^{-5} M tyramine. At the end of this time, both the heart slice and the 5 ml medium were analyzed for total MA and [^{3}H]MA.

Metaraminol determinations. Metaraminol was extracted with a butanol–heptane mixture from the 20-min tyramine bathing medium and from heart tissue as described by Shore and Alpers.[9] After extraction, aliquots of the final aqueous phase were analyzed fluorometrically for total metaraminol, and by liquid scintillation spectroscopy for [^{3}H]MA. The labeled compound was determined by placing a 1-ml aliquot of the final aqueous phase into 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 per cent or less; the counting efficiency was 38 per cent.

In the case of the media taken during the field stimulation experiments and during the 15-min tyramine experiment, determination of [^{3}H]MA was carried out by placing 2-ml aliquots of media directly into 10 ml of scintillation solution and counting as above.

RESULTS

Release of [^{3}H]MA by field stimulation. Slices of heart ventricle from rats given the standard dose of [^{3}H]MA were incubated for 30 min in buffer to allow the rate of

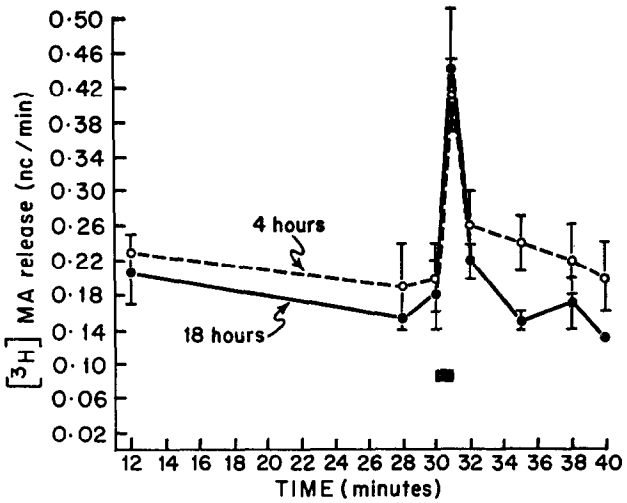

FIG. 1. Release of [^{3}H]MA from rat heart slices by a 1-min field stimulation 4 or 18 hr after i.v. administration of a standard dose of [^{3}H]MA. Each point represents the mean ± S.E. of three experiments. The bar indicates the time of stimulation.

spontaneous efflux of the labeled amine to become constant. After this time, a mild, brief electrical stimulus (5 V, 1 min) produced a striking and rapid increase in the efflux of [^{3}H]MA to several times the rate just prior to stimulation (Fig. 1). No significant difference in the stimulated release of [^{3}H]MA was noted between animals injected 4 or 18 hr prior to stimulation.

TABLE 1. CONCENTRATION OF [^{3}H]MA IN RAT HEART WHEN ADMINISTERED BEFORE OR AFTER UNLABELED MA*

Treatment sequence	Heart MA (μg/g)	Heart [^{3}H]MA (nc/g)	% of control [^{3}H]MA
[^{3}H]MA alone (18 hr)	0·22 ± 0·01	146 ± 7·3	100
MA–[^{3}H]MA (20 hr)	0·38 ± 0·01	165 ± 8·2	113
[^{3}H]MA–MA (20 hr)	0·39 ± 0·03	91 ± 11·4	62·3
[^{3}H]MA–MA (44 hr)	0·35 ± 0·02	75 ± 10·7	51·4

* Experimental animals received the standard dose of [^{3}H]MA and unlabeled MA. Control animals received [^{3}H]MA only. The numbers in parentheses represent the time between the first injection and death. In all cases, the second dose was administered 4 hr prior to killing. Each value is the mean ± S.E. of 11 experiments for the control, and six experiments each for the experimentals.

Effect of unlabeled MA given before or after [^{3}H]MA on the heart concentration, specific activity and stimulation-induced release of [^{3}H]MA. When the standard dose of [^{3}H]MA was administered either 16 hr before or after the standard dose of unlabeled MA, the concentration of total MA in the heart 20 hr after the first dose was about 70 per cent greater than in control animals, which had received only [^{3}H]MA (Table 1); however, the concentration of [^{3}H]MA was dependent upon the order in which the

TABLE 2. RELATIONSHIP BETWEEN [^{3}H]MA RELEASED BY FIELD STIMULATION AND SPECIFIC ACTIVITY IN THE HEART*

Treatment sequence	Heart MA (μg/g)	Heart [^{3}H]MA specific activity (nc/μg)	Stimulated release of [^{3}H]MA (nc/min)	% of control	
				Heart specific activity	Stimulated release of [^{3}H]MA
[^{3}H]MA alone (18 hr)	0·22 ± 0·01	661 ± 18·6	0·21 ± 0·03	100	100
MA–[^{3}H]MA (20 hr)	0·38 ± 0·01	433 ± 16·6	0·14 ± 0·01	65·5	64·3
[^{3}H]MA–MA (20 hr)	0·39 ± 0·03	233 ± 13·5	0·08 ± 0·01	35·3	39·7
[^{3}H]MA–MA (44 hr)	0·35 ± 0·02	207 ± 19·5	0·07 ± 0·01	31·3	34·9

* Experimental animals received the standard dose of [^{3}H]MA and MA. Control animals received [^{3}H]MA only. Numbers in parentheses represent the time between the first injection and death. In all cases, the second treatment was administered 4 hr prior to killing. Tissues were stimulated for 1 min at 5 V. Each value shown for stimulated release of [^{3}H]MA is the mean ± S.E. of three experiments, and represents the electrically stimulated rate of release above spontaneous efflux. Each of the other values is the mean ± S.E. of 11 experiments for the control, and six experiments for the experimentals.

labeled and unlabeled compounds were given. Thus, hearts of animals which received [^{3}H]MA 16 hr after the same dose of unlabeled MA had about the same concentration of labeled compound 4 hr later as did controls; whereas, animals which received the two injections in reverse order over the same time period had only 60 per cent of the control [^{3}H]MA level. Further, the concentration of [^{3}H]MA in hearts of animals which received unlabeled MA 40 hr after [^{3}H]MA was much lower than control, but was not significantly different from the similarly treated 20-hr animals.

As shown in Table 2, the specific activity of heart MA was less than that of controls in all cases of dual treatment, the magnitude of the decrease being dependent upon the order in which the [^{3}H]MA and unlabeled MA were administered. Of special note is the finding that the decrease in specific activity in the heart was accompanied by a proportional decrease in the amount of [^{3}H]MA released by field stimulation. Furthermore, when the time period between injections was increased to 40 hr, the specific

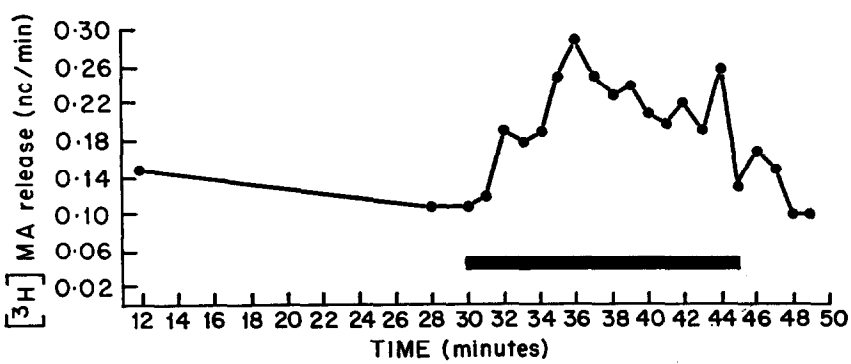

FIG. 2. Tyramine-induced release of [^{3}H]MA from rat heart slice. Animals were treated with the standard dose of [^{3}H]MA 18 hr prior to death. The bar represents the period the tissue was exposed to 10^{-5} M tyramine.

TABLE 3. RELATIONSHIP BETWEEN THE SPECIFIC ACTIVITY OF [^{3}H]MA RELEASED BY TYRAMINE AND THE SPECIFIC ACTIVITY IN THE HEART*

Treatment sequence	Specific activity (nc/μg)		% of control	
	Heart	Medium	Heart	Medium
[^{3}H]MA alone (18 hr)	641 ± 22·9	604 ± 11·6	100	100
MA–[^{3}H]MA (20 hr)	438 ± 23·3	411 ± 6·5	68·33	68·05
[^{3}H]MA–MA (44 hr)	242 ± 18·3	223 ± 14·6	37·75	36·92

* Experimental animals received the standard dose of [^{3}H]MA and MA. Control animals received [^{3}H]MA only. Numbers in parentheses represent the time between the first injection and death. In all cases, the second treatment was administered 4 hr prior to killing. Medium values represent the specific activity of the fluid bathing the tissue after a 20-min exposure to 5×10^{-5} M tyramine. Each value is the mean ± S.E. of three experiments.

activity and amount of [^{3}H]MA released did not change significantly from that of animals treated over a 16-hr period.

Release in vitro *of [^{3}H]MA by tyramine.* A slice of heart ventricle from a rat treated 18 hr previously with the standard dose of [^{3}H]MA was incubated for 15 min in the presence of 10^{-5} M tyramine. The incubation chamber was evacuated and refilled every minute during this time period, and the [^{3}H]MA content of the medium was measured. As shown in a typical experiment (Fig. 2), there was a significant and sustained increase in the rate of [^{3}H]MA efflux in the presence of tyramine.

Effect of unlabeled MA given before or after [^{3}H]MA on the specific activity of MA released by tyramine. When heart tissue from [^{3}H]MA-treated rats was exposed to tyramine (5×10^{-5} M) for 20 min, enough MA was released to measure fluorometrically. Thus, the specific activity in the medium as well as in the tissue could be determined by measuring total MA fluorometrically and the [^{3}H]MA by scintillation spectroscopy. As shown in Table 3, once again the specific activity in the heart decreased when animals received both MA and [^{3}H]MA, the amount of decrease being dependent upon the order in which the two injections were given. Of special

TABLE 4. MA CONCENTRATION AND SPECIFIC ACTIVITY IN RAT HEART 4 AND 50 hr AFTER ADMINISTRATION OF [^{3}H]MA TO ANIMALS PRETREATED WITH UNLABELED MA*

Time after [^{3}H]MA (hr)	Time after unlabeled MA (hr)	Heart MA (μg/g)	Specific activity (nc/μg)
4	44	0·45 ± 0·03	426 ± 36·1
50	90	0·24 ± 0·01	460 ± 6·5

* Animals received the standard dose of MA 40 hr prior to that of [^{3}H]MA, and were killed either 4 or 50 hr after the second treatment. Each value is the mean ± S.E. of three to six experiments.

note again is the finding that the specific activity of metaraminol released into the medium by tyramine was, regardless of the sequence of treatment, the same as that in the heart.

Effect of release of metaraminol in vivo *on specific activity when MA is administered prior to [^{3}H]MA.* Animals were given the standard dose of unlabeled MA 40 hr prior to the standard dose of [^{3}H]MA. At 4 or 50 hr after administration of the isotope, the rats were killed and the hearts analyzed for total MA and [^{3}H]MA. As shown in Table 4, although the heart concentration of total metaraminol decreased about 50 per cent during the 46-hr period, the specific activity of heart MA at the two times was similar.

DISCUSSION

Several reports have suggested that norepinephrine (NE) resides in the adrenergic neuron in two or more kinetically distinct pools.[3,4,10] For example, it has been found that intracisternally administered [^{3}H]NE disappears from the brain in a multiphasic fashion, suggesting the existence of more than one pool for uptake and storage of the transmitter.[10] Also, the specific activity of newly synthesized NE released from the spleen by nerve stimulation was found to be greater shortly after administration of [^{14}C]tyrosine than at later times, suggesting that newly synthesized transmitter is selectively released from a site different from the major portion of the amine stored in the nerve ending.[4] Further evidence is provided by the finding that tyramine releases only a small portion of the NE found in nervous tissue, and it has been suggested that this portion represents a readily releasable store, whereas the tyramine-resistant pool is a main storage site.[3] Thus the functional model of the adrenergic neuron based on these findings has as a basic tenet the concept of at least two pools which are semi-sequestered from one another.[11]

Other work on the nature of storage and release in the adrenergic neuron has found no such evidence for multiple pools. Neff *et al.*[2] have reported that tracer amounts of [^{3}H]NE decline as a single exponential and they suggest that multiphasic decay curves for exogenous NE do not reflect the behavior of the endogenous stores, but rather represent the disappearance of NE from sites where it is not normally present. This interpretation is reinforced by indications that early after L-dopa administration catecholamines are formed and temporarily stored in a spurious fashion in serotonergic neurones.[12,13] Moreover, it has been demonstrated that the specific activity of NE in the rat heart after administration of the labeled substance was not altered by partial release by tyramine.[2] In an additional study, it was reported that tyramine can deplete NE levels by 90 per cent or more if a sufficient blood concentration of the drug is maintained, thus casting doubt on the existence of separate tyramine-releasable and tyramine-resistant pools.[1]

In the present study, the storage and release of metaraminol, a nonmetabolizable NE congener which displaces NE and acts as a false adrenergic transmitter,[5,6] were investigated in order to assess the intraneuronal source of this amine when released by neurogenic stimulation or by tyramine. Thus, after rats were treated with [^{3}H]MA 4 and 18 hr prior to measurement of field-stimulated release from slices of heart ventricle, the amount of [^{3}H]MA released should decrease at the later time, if, with time, the amine assumes a position in a more stable, less readily available amine pool. However, the amount of [^{3}H]MA released was the same at both times.

To investigate the degree of mixing which takes place in the neuron and the relationship between the degree of mixing and release, rats were given [^{3}H]MA either prior or subsequent to an identical dose of unlabeled MA. If there were incomplete mixing of labeled and unlabeled amine in the heart, then the metaraminol released by field stimulation from an animal given unlabeled MA 40 hr after [^{3}H]MA might be expected to contain little radioactivity, since the unlabeled material was the more recently acquired transmitter. However, it was found that 16 or 40 hr after the initial injection, a succeeding dose of MA readily mixed with the metaraminol already present in the heart, and the amount of [^{3}H]MA released by field stimulation reflected the specific activity of the total heart MA store.

The possibility that tyramine-induced release might show a different picture from field-stimulated release was also investigated. Animals were given [^{3}H]MA and unlabeled MA as above, and the specific activity of the tyramine-released material was compared to the specific activity of the heart content. It was found that regardless of whether unlabeled MA or [^{3}H]MA was given last, the specific activity of the tyramine-released MA was the same as that in the heart.

As evidence that these results reflect the state *in vivo*, animals were given unlabeled MA 40 hr prior to the injection of an indentical dose of [^{3}H]MA. At 4 and 50 hr after the second injection, the hearts were analyzed for total MA and [^{3}H]MA. Again, if incomplete mixing occurred, the decline in total heart metaraminol during the two periods studied should be accompanied by a changed specific activity. As shown in Table 4, although the heart content of total MA declined by about 50 per cent during this time, the specific activity remained unchanged, once again suggesting that the newer amine mixed completely with the older stored amine, and that amine released by normal nerve activity was drawn from this mixture.

These findings suggest that MA is not present in multiple semi-sequestered pools such as a readily releasable and a semi-inert storage pool, but rather that the amine is either stored in a single amine pool from which release occurs or that the amine is released from a separate but rapidly equilibrating site whose distinctive existence might be difficult to demonstrate due to rapid amine interchange.

This picture of MA storage and release in rat heart is similar to that reached in the case of NE by Neff *et al.*,[2] who showed that tracer doses of labeled NE mixed completely with endogenous NE and that tyramine released a fraction of stored NE of the same specific activity as that in the entire organ. These findings thus suggest that MA in the adrenergic neuron is stored and released in a manner similar to that of NE. The present findings with MA also support the concept of Neff *et al.*[2] that any multiple amine pools in the adrenergic neuron must be in rapid equilibrium rather than semi-sequestered from one another.

REFERENCES

1. N. H. Neff, T. N. Tozer, W. Hammer and B. B. Brodie, *Life Sci.* **4**, 1869 (1965).
2. N. H. Neff, T. N. Tozer, W. Hammer, E. Costa and B. B. Brodie, *J. Pharmac. exp. Ther.* **160**, 48 (1968).
3. L. T. Potter, J. Axelrod and I. J. Kopin, *Biochem. Pharmac.* **11**, 254 (1962).
4. I. J. Kopin, G. R. Breese, K. R. Krauss and V. K. Weise, *J. Pharmac. exp. Ther.* **161**, 271 (1968).
5. P. A. Shore, D. Busfield and H. S. Alpers, *J. Pharmac. exp. Ther.* **146**, 194 (1964).
6. J. R. Crout, H. S. Alpers, E. L. Tatum and P. A. Shore, *Science, N.Y.* **145**, 828 (1964).
7. R. J. Baldessarini and I. J. Kopin, *J. Pharmac. exp. Ther.* **156**, 31 (1966).
8. H. J. Dengler, I. A. Michaelson, H. E. Spiegel and E. Titus, *Int. J. Neuropharmac.* **1**, 23 (1962)

9. P. A. SHORE and H. S. ALPERS, *Life Sci.* **3,** 551 (1964).
10. J. J. SCHILDKRAUT, P. R. DRASKOCZY and P. S. LO, *Science, N.Y.* **172,** 587 (1971).
11. N. WEINER, *A. Rev. Pharmac.* **10,** 273 (1970).
12. K. Y. NG, T. N. CHASE, R. W. COLBURN and I. J. KOPIN, *Science, N.Y.* **172,** 487 (1971).
13. R. E. BARRETT and T. ST. BALCH, *Experientia* **27,** 663 (1971).