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# STUDIES *IN VITRO* OF AMINE UPTAKE MECHANISMS IN HEART*

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**Abstract**—Amine uptake mechanisms of rabbit and rat heart slices were studied; *l*- and *d*-metaraminol, *dl*-α-methyl-*m*-tyramine, and *l*- and *d*-*m*-octopamine were accumulated by slices. The α-ethyl analog of metaraminol was taken up only slightly. Immunosympathectomy greatly inhibited metaraminol uptake, as did preheating slides, incubation in the cold, or presence of imipramine, cocaine, guanethidine, ouabain, or norepinephrine. Rabbit heart slices were much more sensitive to ouabain action than were rat heart slices. Reserpine or tetrabenazine had no effect on metaraminol uptake, but tetrabenazine or pretreatment with reserpine blocked *m*-octopamine accumulation. Treatment with a monamine oxidase (MAO) inhibitor reversed the effect of reserpine and allowed *m*-tyramine, normally not accumulated, to be taken up. Comparison of these results with findings *in vivo* provide evidence for the existence of two amine concentrating mechanisms: a relatively nonspecific mechanism in the neuronal membrane, which is blocked by a variety of drugs; and an intracellular mechanism of higher specificity which is blocked by reserpine or tetrabenazine. The results also emphasize the important role of MAO in regulation of amine stores and in the action of amine-depleting drugs.

PREVIOUS studies *in vivo* have demonstrated that certain compounds chemically related to norepinephrine are taken up by adrenergic neurons in heart and other tissues where they displace norepinephrine and then act as false adrenergic transmitters. Such substances include *l*-metaraminol (*l*-MA), *l*-α-methyl-norpinephrine and *m*- and *p*-octopamine.[1–7] Other closely related substances such as *d*-metaraminol (*d*-MA), or *dl*-α-methyl-*m*-tyramine (MMTA) do not persist in tissues and do not displace norepinephrine, indicating considerable stereospecificity.[1,8] In the present study, the uptake of some of these substances into heart slices was examined in an attempt to learn more of the mechanisms involved in amine uptake by adrenergic neurons. Evidence is presented to support the existence of two concentrating mechanisms: a primary membrane mechanism of rather low specificity and a secondary intracellular mechanism of high specificity. The findings also demonstrate that the action of monoamine oxidase (MAO) is of great importance in regulation of amine storage and in the action of amine-depleting drugs.

## METHODS

Adult albino rabbits or Sprague-Dawley rats were killed by air embolism, a blow on the head, or by chloroform inhalation. Hearts were excised quickly, washed with

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ice-cold saline, and atria and fat removed. Ventricular tissue slices of about 0·3-mm thickness were prepared at 5°. About 75 to 100 mg of slice was placed in 3 ml cold Krebs-Ringer phosphate buffer, (pH 7·4) in 20-ml beakers which were then placed in a Dubnoff metabolic shaker at 37° and gassed with $O_2$. After 15-min preincubation, the amine to be studied was added to the beakers and incubation continued. The slices were then removed, rinsed briefly with saline, blotted with filter paper, weighed, and homogenized in 0·4 N $HClO_4$ in a glass homogenizer. The amines were measured fluorometrically by a method previously described for metaraminol and related compounds.[9] In those cases where drugs affecting amine uptake were studied, the drugs were added at the start of the preincubation period. Variations of these techniques are described under Results.

Values for amine uptake are expressed as net uptake ($\mu$g/ml slice water). These values were calculated on the basis that 85% of the slice weight is water and the assumption that a concentration equal to that added to the incubation medium would have been passively transported into slice water. Thus net uptake ($\mu$g/ml slice water) = {[*C*] slice ($\mu$g/mg)}/0·85 — [*C*] external. All concentrations refer to free base unless otherwise specified.

## RESULTS

### *Uptake of l-MA and other amines by heart slice and effect of preheating or incubation in the cold*

*l*-Metaraminol added to the incubation medium was rapidly taken up into rabbit heart slices (Fig. 1). At an external concentration of 0·1 $\mu$g/ml, net uptake was still

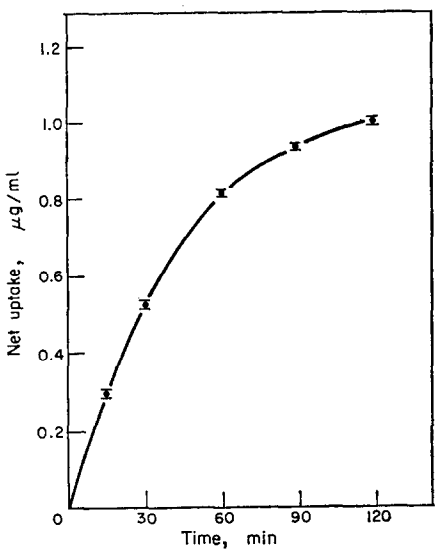


FIG. 1. Uptake of *l*-MA by rabbit heart slices at various incubation periods. Concentration of *l*-MA in medium was 0·1 $\mu$g/ml. Values denote mean ± S.E. of at least 4 experiments.

increasing after 120 min of incubation, although the rate of uptake was decreasing. Incubation with various concentrations of *l*-MA for 60 min showed that net uptake increased with increasing external concentration but that the efficiency of the concentrating mechanism, as defined by the ratio of slice amine concentration to external

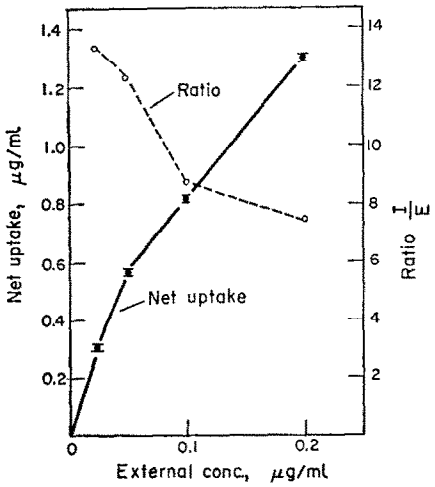


FIG. 2. Uptake of *l*-MA by rabbit heart slices at various amine concentrations in medium. Incubation period was 60 min. Ratio, *I*/*E*, denotes ratio of amine concentration in slice water (internal) to that in medium (external). Values for net uptake denote mean ± S.E. of at least 4 experiments.

amine concentration, was greatest at the lower external concentrations (Fig. 2).

Heart slices preheated at 55° for 10 min before being placed in the incubation medium showed no amine-concentrating ability. Amine uptake was also blocked by incubation at 5° (Table 1).

TABLE 1. INHIBITION OF ACCUMULATION OF *l*-METARAMINOL AND RELATED AMINES IN RABBIT HEART SLICES BY PREHEATING, COLD, AND BY VARIOUS DRUGS

| Treatment | Per cent inhibition of uptake | | | |
|---|---|---|---|---|
| | *l*-MA | *d*-MA | *dl*-MMTA | *l-m*-octopamine |
| Preheat, 55°, 10 min | 94 | | | |
| Cold, 5° | 97 | | | |
| Imipramine, $10^{-5}$ M | 88 | 85 | | 100 |
| Ouabain, $10^{-5}$ M | 78 | 100 | 84 | 100 |
| Cocaine, $10^{-5}$ M | 75 | | | |
| Guanethidine, $10^{-4}$ M | 85 | 100 | | 100 |
| *l*-NE, $1{\cdot}7 \times 10^{-5}$ M | 69 | | | |
| Reserpine, $10^{-5}$ M | 0 | 0 | 0 | ? |
| Tetrabenazine, $10^{-5}$ M | 0 | | | 100 |

Rabbit heart slices were prepared and incubated as described in Methods. In preheated slices, the tissue was cooled to 37° before being transferred to the incubation mixture. The various drugs were present during the preincubation period. Results show average of at least four experiments with each treatment. Concentration of *l*-MA and related amines in the incubation mixture was 0·1 μg/ml. Effect of reserpine on *l-m*-octopamine was highly variable.

*Effect of immunosympathectomy on uptake by rat heart slices*

Slices of heart from immunosympathectomized rats were compared with those from control rats of the same age and stock. As shown in Table 2, *l*-MA was accumulated by the normal hearts, whereas immunosympathectomy greatly inhibited uptake.

*Uptake by rabbit heart slices of amines related to l-MA*

Rabbit heart slices readily accumulated *d*-MA and MMTA, although uptake of these amines was significantly less than that of *l*-MA (Fig. 3 and Table 3). The α-ethyl analog of MA (*dl*-1-(3-hydroxyphenyl-2-aminobutane) (WIN 513) was taken up only slightly (Table 3). A significant uptake of *l*- and *d*-*m*-octopamine occured, although

TABLE 2. ACCUMULATION OF *l*-METARAMINOL BY RAT HEART SLICES AND EFFECT OF IMMUNOSYMPATHECTOMY

| Rats | Net uptake (μg/ml ± S.E.) |
|---|---|
| Normal | 0·31 ± 0·02 |
| Immunosympathectomized | 0·09 ± 0·03 |

Figures represent results from four normal and four immunosympathectomized rats. Concentration of *l*-MA in medium was 0·1 μg/ml. Incubations were of 60-min duration.

the accumulation was much less than with *l*-MA. With these amines, increasing the external concentration above 0·1 μg/ml caused no increase in net uptake (Fig. 3). No *m*-tyramine could be detected in slices after incubation.

*Inhibition of uptake by drugs*

Preincubation of rabbit heart slices with guanethidine, cocaine, imipramine, norepinephrine, or ouabain greatly inhibited uptake of *l*-MA (Table 1). Further investigation of the effect of ouabain showed that this agent also blocked uptake of

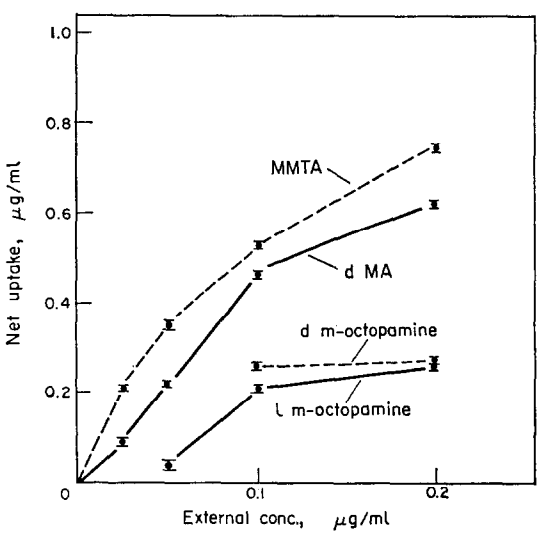


FIG. 3. Uptake of *d*-MA, MMTA, and *d*- and *l*-*m*-octopamine by rabbit heart slices after 60-min incubation with various amine concentrations in the medium. Values denote mean ± S.E. of at least 4 experiments.

*d*-MA, MMTA, and *m*-octopamine. *l*-Metaraminol uptake by rat heart slices was also inhibited by ouabain, but only at a much higher concentration ($10^{-3}$ M). Hearts taken from rabbits given reserpine (5 mg/kg i.v.) 2 or 16 hr previously showed no diminution of uptake of *l*- or *d*-MA (Table 4). Furthermore, preincubation with

TABLE 3. ACCUMULATION OF *l*-METARAMINOL AND RELATED AMINES BY RABBIT HEART SLICES

| Amine | Net uptake ($\mu$g/ml slice water $\pm$ S.E.) |
|---|---|
| *l*MA | 0·82 $\pm$ 0·03 |
| *d*-MA | 0·46 $\pm$ 0·03 |
| *dl*-MMTA | 0·53 $\pm$ 0·06 |
| *l*-*m*-octopamine | 0·21 $\pm$ 0·02 |
| *d*-*m*-octopamine | 0·26 $\pm$ 0·02 |
| *m*-tyramine | 0·0 |
| WIN 513 | 0·06 $\pm$ 0·01 |

Experiments were carried out as described in Methods. Amine concentration in medium was 0·1 $\mu$g/ml. Incubation period was 60 min. Figures represent results from at least four experiments with each amine.

TABLE 4. EFFECT OF PRETREATMENT OF RABBITS WITH RESERPINE, A MONOAMINE OXIDASE INHIBITOR, OR THEIR COMBINATION ON ACCUMULATION OF VARIOUS AMINES BY HEART SLICES

| Treatment | Net uptake ($\mu$g/ml $\pm$ S.E.) | | |
|---|---|---|---|
| | *l*-MA | *l*-*m*-octopamine | *m*-tyramine |
| None | 0·82 $\pm$ 0·03 | 0·21 $\pm$ 0·02 | 0 |
| Reserpine | 0·73 $\pm$ 0·04 | 0 | 0 |
| Iproniazid | 0·65 $\pm$ 0·07 | 0·57 $\pm$ 0·06 | 0·50 $\pm$ 0·17 |
| Pheniprazine | 1·00 $\pm$ 0·07 | 0·44 $\pm$ 0·01 | 0·22 $\pm$ 0·03 |
| Reserpine + iproniazid | 0·74 $\pm$ 0·06 | 0·27 $\pm$ 0·05 | 0·40 $\pm$ 0·04 |
| Reserpine + pheniprazine | | 0·34 $\pm$ 0·04 | 0·34 $\pm$ 0·06 |

Reserpine (5 mg/kg i.v.) was given 2 or 16 hr before killing. Iproniazid phosphate (150 mg/kg) or pheniprazine hydrochloride (5 mg/kg) was given 16 hr before killing. In the combination experiments, the MAO inhibitors were given 5 hr after reserpine, and rabbits were killed 16 hr later. Concentration of amines in medium was 0·1 $\mu$g/ml except for *m*-tyramine which was 0·2 $\mu$g/ml. Incubation period was 60 min.

Figures represent results from at least four experiments with each treatment.

reserpine ($10^{-5}$ M) had no effect on uptake of *l*-MA, *d*-MA, or MMTA (Table 1). Pretreatment of rabbits with reserpine, however, completely abolished accumulation of *m*-octopamine by heart slices (Table 4). Reserpine added *in vitro* also tended to diminish *m*-octopamine uptake, but the results were highly variable. Tetrabenazine had no effect on uptake of *l*-MA, but blocked accumulation of *m*-octopamine (Table 1).

*Effect of monoamine oxidase inhibition on amine uptake in normal and reserpine-treated hearts*

Rabbits were given reserpine (5 mg/kg i.v.) followed 5 hr later by either iproniazid phosphate (150 mg/kg) or pheniprazine hydrochloride (5 mg/kg). The animals were killed 16 hr later, and heart slices were prepared. Incubation with *m*-octopamine now resulted in accumulation of this amine to about the extent observed in untreated heart slices; furthermore, *m*-tyramine was now accumulated by the slice (Table 4). In other rabbits given only a MAO inhibitor, considerable amounts of both *m*-octopamine and *m*-tyramine were taken up (Table 4). Monoamine oxidase inhibition had no significant effect on uptake of *l*-MA in either reserpine-treated or nonreserpinized animals.

## DISCUSSION

The marked uptake of *l*- and *d*-MA and MMTA is similar to, but more pronounced than the uptake of norepinephrine noted by other workers using similar techniques.[10] Thus the marked accumulation, the greater efficiency of the uptake mechanism at the lower external amine concentrations, and the marked inhibition of uptake by heat treatment of the slice or by incubation in the cold suggest that an active process is operant. Uptake of *l*-MA by rat heart slices has also been noted by other investigators.[11] The striking deficiency in uptake by heart slices from immunosympathectomized animals, similar to that observed *in vivo*,[1] demonstrates that uptake occurs in the adrenergic neuron, known to be degenerated in these animals. Furthermore, inhibition of uptake of these amines by the same drugs (cocaine, imipramine, ouabain, etc.) known to inhibit norepinephrine uptake[10] indicates that the same mechanism is involved. This conclusion is strengthened by the observation that *l*-MA uptake is inhibited by the addition of norepinephrine to the incubation mixture (Table 1). It is of interest that rabbit heart is much more sensitive to the blockade of amine uptake by ouabain than is heart of rat, a species known to be highly resistant to the toxic action of cardiac glycosides.

The relative nonspecificity of uptake in the slice differs greatly from results obtained in the whole animal.[1] In the studies *in vivo*, it was found that *l*-MA but not *d*-MA or MMTA is taken up and bound persistently, while in the studies *in vitro* reported here, each of these amines was concentrated, although *l*-MA was accumulated to a greater extent than were the other amines. WIN 513, the α-ethyl analog of MA, however, was taken up neither *in vivo*[8] nor *in vitro*.

A marked difference was also seen in the effect of reserpine. Pretreatment with this drug greatly inhibits persistent binding of *l*-MA *in vivo* and even releases stored *l*-MA,[1] but had no effect on uptake of this amine *in vitro*. These discrepancies are readily resolved, however, by a consideration of the dual amine-concentrating mechanisms thought to operate in adrenergic neurons.[12, 13] This view presupposes a primary pump at the neuronal membrane and a secondary intracellular mechanism acting, perhaps, at the granular level. It would seem, then, that the membrane pump is relatively nonspecific and tends to concentrate a variety of amines in the cell. As *d*- and *l*-MA and MMTA are not substrates for MAO (or catechol-O-methyltransferase), in the slice they remain in high concentration, while *in vivo*, a washing-out process occurs unless the amine (e.g. *l*-MA) is highly bound by the intracellular

mechanism. An amine metabolized by MAO would be destroyed unless sequestered by the intracellular mechanism in a protected site. Cocaine, imipramine, guanethidine, and ouabain may be pictured as inhibiting the membrane pump, thus blocking uptake of the several amines. The discrepancy between the actions of reserpine *in vivo* and *in vitro* is thus understandable. Reserpine, acting only on the intracellular mechanism, does not affect uptake by the membrane pump, but disallows persistent intracellular binding, thus, *in vivo*, preventing persistent uptake of MA. In the static system of the slice experiments, despite the action of reserpine on the intracellular mechanism, no washout or metabolism of the α-methyl amines concentrated by the membrane pump can occur. Other experiments support this likelihood. Carlsson, while agreeing that both desipramine and reserpine block persistent uptake of *l*-MA, nonetheless found definite differences, *l*-MA uptake persisting longer after reserpine pretreatment than after desipramine pretreatment.[12] These conclusions agree closely with those of Lindmar and Muscholl[13] who, on the basis of measurement of norepinephrine taken up and retained by perfused isolated rat heart in the presence of various drugs, concluded that cocaine and guanethidine inhibit norepinephrine transport across the cell membrane, while reserpine (and guanethidine) blocks norepinephrine uptake into intracellular storage sites. Similar conclusions have been reached on the basis of fluorescent histochemical studies.[12, 14]

If this picture is correct, then studies on the uptake *in vitro* of an amine which is a MAO substrate should show a lessened total uptake compared with *l*-MA and also should allow an inhibitory effect of reserpine to be seen. Accordingly, in the experiments with *m*-octopamine, a MAO substrate, reserpine pretreatment blocked uptake of this amine. It would seem likely that *m*-octopamine is taken up by the normal slice, but, except for that portion which is protected by intracellular binding, is rapidly metabolized. Reserpine, by blocking the intracellular mechanism, allows more of the amine to be destroyed by MAO, thus apparently lowering uptake, but in actuality allowing access of more amine to MAO. The same processes explain the inability of tetrabenazine to affect *l*-MA uptake while blocking *m*-octopamine accumulation. They also explain the action of reserpine on norepinephrine uptake by organ slices.[10]

The results with *m*-tyramine further substantiate this picture. This amine is not granule-bound, whereas *m*-octopamine is, in part, granule-bound in heart tissue.[4] Accordingly, any *m*-tyramine acted upon the membrane pump is promptly destroyed by MAO. Blockade of MAO allows accumulation of *m*-tyramine and also allows a reserpine-treated slice to accumulate *m*-octopamine or *m*-tyramine (Table 4). Presumably, the *m*-octopamine in a normal slice should be in a different compartment than that in a reserpine-treated, MAO-blocked slice. Differential centrifugation studies have not revealed a significant difference, but this may be misleading in view of previous work showing that *l*-MA, persistently bound *in vivo*, and *d*-MA, fleetingly bound, have essentially identical differential centrifugation patterns.[15]

From these experiments several conclusions may be drawn. (1) The primary or membrane pump is relatively nonspecific, although some stereochemical factors are important, for example the α-ethyl analog of MA not being taken up. (2) The intracellular binding mechanism, possibly a granule pump, is quite specific, witnessed by the finding that *m*-octopamine, but not *m*-tyramine, accumulates in heart slices. This conclusion is in agreement with the findings of Musacchio *et al.* that *m*-octo-

pamine, but not *m*-tyramine, is particle bound.[4] (3) Various drugs, such as guanethidine, imipramine, cocaine, ouabain, etc., inhibit the membrane pump, while reserpine and tetrabenazine act only on the intracellular mechanism. Guanethidine presumably acts on the intracellular mechanism also, since it is an amine-depleting drug. (4) The findings emphasize the key role of MAO in the regulation of overall cell stores of amines and in the mechanism of action of certain amine-releasing drugs such as reserpine.

These findings allow a convenient method for differentiation between the two amine-concentrating mechanisms and the effect of drugs on these mechanisms. Thus, a substance inhibiting uptake of MA or MMTA into heart slices may be presumed to act on the membrane pump, while a drug not inhibiting uptake of these amines, but blocking uptake of *m*-octopamine, may be presumed to act on the intracellular storage mechanism.

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## NOTE ADDED IN PROOF:

After submission of this paper, we discovered that due to some confusion as to the salt form of the *d-m*-octopamine used in these experiments, the data in Fig. 3 and Table 3 showing accumulation of this amine are not correct and should be disregarded. This correction in no way changes the conclusions reached in the paper. Data showing accumulation of the *l* isomer and other amines remain correct.