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EFFECTS OF RESERPINE AND TYRAMINE ON RELEASE OF NOREPINEPHRINE FROM SYNAPTOSOMES

ROBERT W. COLBURN and IRWIN J. KOPIN

Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Md. 20014, U.S.A.

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Abstract—Synaptosomes incubated with norepinephrine-^{14}C and subsequently treated with reserpine released labeled deaminated products of the catecholamine. Tyramine, however, resulted in release of unmetabolized norepinephrine-^{14}C. These findings are similar to those found in peripheral organs and suggest that in brain, as well as in peripheral sympathetic nerves, intraneuronal release of norepinephrine is attended by deamination.

NOREPINEPHRINE (NE) administered into the cerebrospinal fluid can be metabolized by either catechol-*O*-methyltransferase (COMT) or monoamine oxidase (MAO). Glowinski and Baldessarini[1] reviewed the evidence supporting the view that the initial metabolic route of catecholamines in brain is similar to that in the peripheral sympathetic nervous system but concluded that the importance of *O*-methylation versus deamination was not yet clear. In the sympathetic nervous system, increased release of deaminated products after treatment with reserpine is the basis for concluding that deamination is the major route of metabolism of NE within the neuron.[2–4]

O-methylation appears to be the major route of metabolic inactivation of catecholamines which have been administered intravenously or released by sympathomimetic amines. After intracisternal injection of NE-^{3}H, a similar metabolic pattern has been found.[5] Such studies, however, have not examined directly the fate of NE released in brain since metabolites formed *in vivo* are removed by the circulation. NE taken up by brain slices[6,7] or synaptosomes[8] appears to enter preferentially those nerve endings which normally contain this catecholamine. Synaptosomes were used here to study the effects of reserpine and tyramine on the metabolism of catecholamines.

MATERIALS AND METHODS

Synaptosomes were prepared from whole rat brain by the method of Whittaker *et al.*[9] except that the centrifugation to remove microsomes was omitted because it was not essential for the present studies. Synaptosomes recovered from the 0·8 M sucrose layer were transferred to 5 ml of incubation medium/50 mg of whole brain used. The incubation medium contained 118 mM sodium chloride, 4·7 mM potassium chloride, 2·2 mM calcium chloride, 1·18 mM magnesium sulfate, 11 mM dextrose, 25 mM sodium phosphate at pH 7·0, and NE-^{14}C (New England Nuclear Corporation, Boston, Mass.) at 4×10^{-7} M (20 nc/ml). Uptake was determined by incubating the synaptosome suspension with labeled NE for 20 min at 37°; uptake was stopped by

cooling the tubes in ice. The synaptosome suspension was then layered over 5 ml of 0·6 M sucrose and centrifuged at 50,000 *g* for 10 min in a swinging-bucket rotor. The supernatant fluid was removed by aspiration, and the pellet was resuspended in 3 ml of incubation medium/50 mg of whole brain originally used.

The synaptosome suspension was pre-incubated for 5 min at 37° to allow equilibration. Aliquots (3 ml) were then transferred to 6-ml ultracentrifuge tubes, and 0·1 ml of incubation medium containing drugs was added. After incubation for 2, 5, 10 and 20 min at 37°, the samples were cooled in ice and centrifuged at 16,000 *g* for 10 min. The supernatant incubation medium was removed for subsequent analysis. The pellets were homogenized with 250 ml of 0·4 N perchloric acid and centrifuged; then 200 ml of the supernatant was transferred to vials for assay of total radioactivity in a liquid scintillation spectrometer.

The total radioactivity released into the incubation medium was also determined by assay of an aliquot of the medium. The labeled amines present in 1·2-ml aliquots of medium were adsorbed by a suspension of 150 mg of AG 50 (Bio-Rad Laboratories, New York, N.Y.) in the Na^+ form. After centrifugation the radioactivity remaining in solution was measured. The radioactivity present in the form of amines was calculated from the difference between total radioactivity and deaminated products which were not adsorbed on the ion-exchange resin.

Non-catechol metabolic products in another aliquot of the medium were separated by adsorption of catechols on chromatographic columns of alumina (Woelm) (Alupharm Chemicals, New Orleans, La.) prepared as described by Anton and Sayre.[10] Sodium metabisulfite (0·1 mg) and EDTA (2·0 mg disodium salt) were added in a volume of 0·2 ml to 1·2 ml-aliquots of supernatant. The samples were adjusted to pH 8·6 and passed over small (5 × 20 mm) alumina columns followed by 3 ml of distilled water, and the combination effluent/wash was assayed for radioactivity. Catecholamines were eluted with 3 ml of 0·2 N acetic acid, and the radioactivity was assayed as described above.

RESULTS

The total radioactivity released spontaneously in the presence of reserpine (10^{-7} M) or tyramine (10^{-4} M) at various times of incubation is shown in Fig. 1. There was an exponential decrease, which was more rapid when drugs were present, in radioactivity remaining in the particulate fraction. A 3-fold increase in reserpine (3×10^{-7} M) increased release of total radioactivity from synaptosomes so that reserpine-induced release was equivalent to tyramine-induced release (about 80 per cent in 20 min). With reserpine added, nearly a 2-fold increase in deaminated products was found over control levels, but labeled amines increased by only 12 per cent (Table 1). The increased release of radioactivity produced by tyramine was due mainly to an increase in the amine fraction. There was a slight, insignificant decrease in the amount of deaminated products. With or without drug applications, only very little radioactivity was recovered in the non-catechol fraction.

DISCUSSION

Both tyramine and reserpine induce release of NE from synaptosomes previously incubated with the labeled amine (Fig. 1). Although the release rates of the amines are about equal, differences in the metabolites of the catecholamine are apparent. Most of

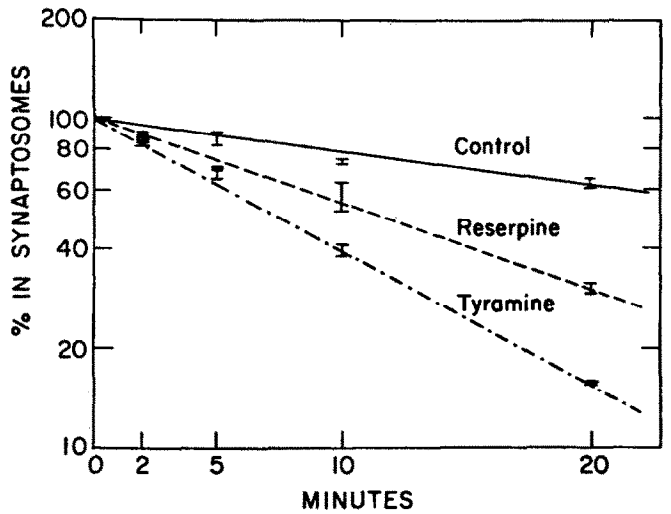

FIG. 1. Total radioactivity remaining in synaptosomes following uptake of norepinephrine-^{14}C and varying times of incubation without drugs and with reserpine (10^{-7} M) or tyramine (10^{-4} M) as described in the section on Methods. The radioactivity at zero time (100 per cent) was 2038 ± 68 counts/min/sample.

the radioactivity released from synaptosomes by reserpine treatment was identified as deaminated products. This is consistent with the concepts that reserpine acts at the vesicular storage site and that NE released by this drug is metabolized intraneuronally by monoamine oxidase (MAO). The fact that tyramine increases release of labeled products (mainly amines) from the synaptosomes clearly indicates that it releases stored NE to a site where MAO is not active.

Perhaps some decrease in the action of MAO may also be the result of competitive inhibition of the enzyme by tyramine.[11] *O*-methylation, however, does not appear to be a major means of inactivation of the catecholamines in the synaptosome preparation. The lack of *O*-methylation may be a consequence of separation of the nerve endings from the cells containing COMT and suggests that COMT levels in the nerve endings

TABLE 1. RELEASE OF TOTAL RADIOACTIVITY AND METABOLIC PRODUCTS OF NOREPINEPHRINE-^{14}C FROM SYNAPTOSOMES BY RESERPINE OR TYRAMINE*

	Total carbon-14	Non-amine	Amine
Control	1394 ± 124	708 ± 155	686 ± 198
Reserpine (3×10^{-7} M)	2083 ± 151	1308 ± 103	775 ± 182
Increment†	689	600	89
Tyramine (10^{-4} M)	2019 ± 100	547 ± 31	1472 ± 104
Increment†	625	−161	786

* Results are expressed as counts per minute of carbon-14 (± S.E.M.) released during a 20-min incubation for groups of 4–6 synaptosome preparations.

† Increment refers to the difference between control values and drug treatment.

are low or inactive. In brain slices where architecture is preserved, COMT appears to play a more important role in the metabolism of extraneuronal catecholamines. These findings are consistent with the view that central noradrenergic neurons are similar to peripheral sympathetic neurons in their drug effects on binding, storage and release of norepinephrine.

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