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DESIPRAMINE-INDUCED RELEASE OF NOREPINEPHRINE FROM HEART

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Abstract—Desipramine (DMI) in low concentrations inhibits the uptake of norepinephrine (NE) by sympathetic nerve endings but does not release the amine. In high concentrations, however, DMI releases NE. The rate of NE release from heart slices depends on the DMI concentration; at 25 μg/ml, the NE stores are depleted by 50 per cent in 4 hr; at 50 μg/ml, they are depleted by 50 per cent in 1 hr. In the isolated heart, DMI releases the NE mainly in the form of deaminated metabolites, indicating that the drug acts within the neuron, possibly on the storage granules.

AN IMPORTANT characteristic of desipramine (DMI) and other tricyclic antidepressants is that they impair the uptake of norepinephrine (NE) and structurally related amines, metaraminol, guanethidine and tyramine, into sympathetic nerve endings.[1–3] In addition, DMI inhibits the ability of the latter drugs to release NE from peripheral tissues by an action that is commonly attributed solely to the reduced uptake of these substances by sympathetic neurons.[4, 5] However, accumulating evidence suggests that DMI counteracts the release of NE by these drugs by more than one action. For example, a given amount of metaraminol taken up within the adrenergic neuron releases as much NE from control heart slices as from slices incubated in the presence of amphetamine, but only one-third as much NE is released by this amount of metaraminol in the presence of DMI. It was concluded, therefore, that DMI counteracts the release of NE in two ways: (1) by reducing the uptake of metaraminol into nerve endings; (2) by interfering with the ability of metaraminol taken up by nerve endings to release endogenous NE.[6] Similar conclusions were drawn from studies with tyramine *in vivo*.[3] Further support of the view that DMI has an effect on granules was based on studies showing that reserpine, which releases NE from granules, did so at a slower rate in rats pretreated with DMI.[7,†]

In preliminary studies, Titus *et al.*[8] showed that high concentrations of DMI (75–125 μg/ml) released considerable amounts of NE from sympathetic nerve endings in the isolated rabbit heart. The present report concerns the possible mechanism by which DMI released NE from sympathetic nerve endings in the heart.

METHODS AND MATERIALS

Male Sprague–Dawley rats (200 g) were injected with 2 μg/kg *dl*-^{3}H-NE (9 c/m-mole)

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† U. Zor and D. F. Bogdanski, in preparation.

and killed 18 hr later. At this time the labelled amine was evenly distributed throughout the endogenous NE stores in the heart.[9] Ventricle slices about 0·5 mm thick were prepared with a Stadie–Riggs microtome. Approximately 200 mg of slices were placed in 30-ml beakers containing 10 ml of a modified Krebs–Ringer solution[10] and incubated at 37° for 30 min in a Dubnoff shaker in an atmosphere of 95% O_2–5% CO_2. The slices were then transferred to beakers containing fresh media with different concentrations of DMI, and the incubation continued for 4 hr. Radioactivity was measured in samples of incubation fluid taken at various times and in slices taken at the end of the experiments. Rates of ^{3}H-NE efflux from slices were measured as previously described.[11]

The metabolic fate of NE released by DMI, metaraminol and RO 4-1284 (2 H-Benzo[a]quinolizine-2-ol, 2 ethyl-1,3,4,6,7,11b-hexahydro-3-isobutyl-9,10-dimethoxy), a synthetic benzoisoquinolizine[12] with reserpine-like effects, was determined using isolated rat hearts. The hearts were perfused for 20 min at 37° with Tyrode's solution (3·5 ml/min) containing 10 ng/ml *dl*-^{3}H-NE and then for 20 min with NE-free solution. This was followed by a 5-min perfusion with the drug to be tested and finally by a 5-min perfusion with a drug-free solution.

The amount of NE and its metabolites was compared in the 5-min periods before and after drug perfusion. To minimize the oxidation of NE and its metabolites, the perfusates were collected at 0° in the presence of 10 mg EDTA and 25 mg Na_2SO_3, and then acidified with perchloric acid. To each perfusate, 25 μg of unlabelled NE and each metabolite were added. The recoveries of radioactive substances were based on the assay of unlabelled materials by ultraviolet adsorption. The samples were stored at −10° until analyzed. NE and its metabolites were separated into two fractions by means of an alumina column[13] and each fraction was adjusted to pH 6·5. Fraction 1 contained the *O*-methylated metabolites including normetanephrine, vanillylmandelic acid, and 3-methoxy-4-hydroxyphenylglycol. Fraction 2 contained the catechols including NE, 3,4-dihydroxymandelic acid and 3,4-dihydroxyphenylglycol. Fraction 1 was chromatographed on a Dowex 50 column (sodium form), and the *O*-methylated deaminated products were eluted with 0·1 M phosphate buffer, pH 6·5. Normetanephrine, which was retained on the column, was eluted with a 50% (v/v) ethanol and 6 N HCl solution. Fraction 2 was chromatographed on another Dowex 50 column, and the catechol deaminated compounds were eluted with phosphate buffer. NE, which was retained on the column, was eluted with 2 N HCl. Radioactivity was measured by liquid scintillation counting and corrected for quenching by means of internal ^{3}H-toluene standards.

RESULTS

Figure 1 shows the effect of DMI on the efflux of ^{3}H-NE from heart slices. DMI in a concentration (1 μg/ml) that markedly inhibited the uptake of NE by adrenergic neurons[8] elicited no detectable change in the rate of ^{3}H-NE efflux. When the concentration was increased to 25 μg/ml, the ^{3}H-NE content of the slices declined by about 50 per cent in 4 hr; at 50 μg/ml only about 1 hr was required for the ^{3}H-NE to decline by 50 per cent. This rate was not further increased on doubling the concentration of drug. The data in Table 1 show that after 3-hr incubation, 72 per cent of the labelled amine and 70 per cent of the endogenous amine were lost, indicating the efflux of ^{3}H-NE is an accurate measure of the loss of endogenous NE.

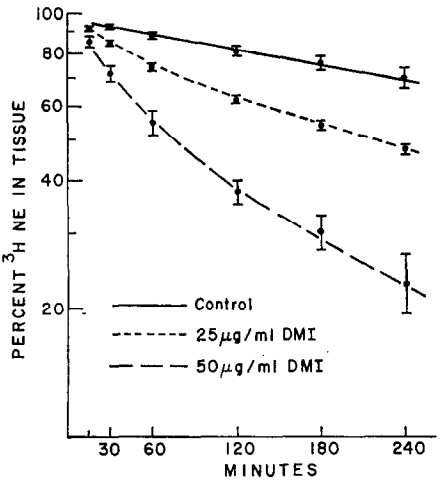

FIG. 1. Effect of DMI on the efflux of ^{3}H-NE from rat ventricle slices. Tissue slices were prepared from hearts of rats in which the NE was labelled by the intravenous injection of *dl*-^{3}H-NE 18 hr previously. Each point represents the mean of five experiments ± S.D.

TABLE 1. DEPLETION OF ENDOGENOUS AND ^{3}H-NOREPINEPHRINE FROM HEART SLICES INCUBATED AT 37° FOR 3 hr WITH 50 μg/ml DESIPRAMINE*

Control (ng/g)	Endogenous DMI (ng/g)	Per cent remaining	Control (dpm/g)	^{3}H-NE DMI (dpm/g)	Per cent remaining
826 ± 24	267 ± 28	32	12,920 ± 1970	3910 ± 810	30

* Values are the means of five determinations ± S. D. ^{3}H-NE was given (100 μc/kg, i.v.) 18 hr before sacrifice.

TABLE 2. ^{3}H-NOREPINEPHRINE AND METABOLITES RELEASED BY DESIPRAMINE FROM THE PERFUSED RAT HEART*

Treatment	Total radioactivity (dpm × 10³)	Amines: Catechols (dpm × 10³)	Amines: *O*-methylated derivative (dpm × 10³)	Deaminated products: Catechols (dpm × 10³)	Deaminated products: *O*-methylated derivative (dpm × 10³)
Control efflux	9·5	1·3	1·8	1·9	3·5
DMI efflux	59·3	6·1	1·2	42·5	5·8
Difference	49·7 ± 12†	4·8 ± 6†	(−0·6)	40·6 ± 7†	2·2 ± 0·7†

* Heart NE stores were labelled by perfusion for 20 min with Tyrode's media containing 10 ng/ml *dl*-^{3}H-NE, and then perfused for 20 min with NE-free Tyrode's media. The hearts were next perfused with Tyrode's media containing DMI (100 μg/ml) for 5 min and finally perfused with DMI-free Tyrode's media for 5 min. The distribution of NE and its metabolites was determined in the perfusate collected in the 5-min period immediately before (control efflux) and after (DMI efflux) the DMI perfusion. Values are the means of six experiments. Differences were treated statistically by the method of paired observations.[14] No measure of range is given for control and DMI means, because such a measure would include differences between hearts which play no part in the reliability of the *t* values obtained using the method of paired observations.

† $P < 0{\cdot}01$.

Table 2 describes the metabolic fate of the ^{3}H-NE released by DMI from the isolated perfused heart. The results show that about two-thirds of the ^{3}H-NE spontaneously released from control hearts consisted of deaminated products and about one-third consisted of bases (NE + normetanephrine). Perfusion with DMI (100 μg/ml) increased the efflux of radioactivity by more than 6-fold; almost all of the ^{3}H-NE released by DMI was in the form of deaminated catechol compounds. The marked increase in the efflux of ^{3}H-NE elicited by RO 4-1284 (50 μg/ml) also consisted mainly of deaminated products. In contrast, the increased efflux elicited by metaraminol (1 μg/ml) consisted largely of bases (FIG. 2).

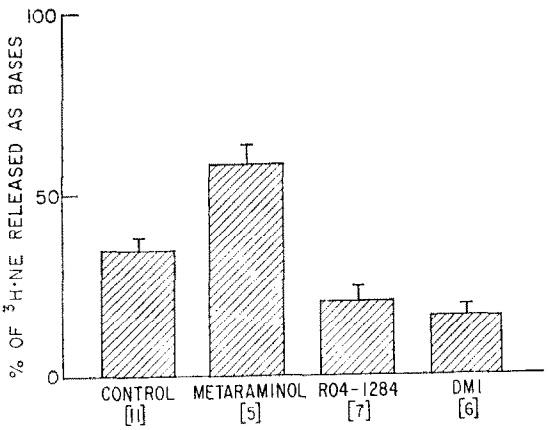

FIG. 2. Heart NE stores were labelled by perfusion for 20 min with Tyrode's media containing 10 ng/ml *dl*-^{3}H-NE and then perfused for 20 min with NE-free Tyrode's media. The hearts were next perfused with Tyrode's media containing drug for 5 min and finally perfused with drug-free Tyrode's media for 5 min. The distribution of NE and its metabolites was determined in the perfusate collected in the 5-min period immediately before (control efflux) and immediately after the drug perfusion (drug efflux). Verticle bars indicate the standard error, and the number in brackets indicates the number of experiments. The following concentrations of drugs were used: metaraminol, 1 μg/ml; RO 4-1284, 50 μg/ml; DMI, 100 μg/ml.

DISCUSSION

Previous studies have shown that DMI in doses that block the uptake of NE by sympathetic nerve endings also decreases the spontaneous efflux of the catecholamine from the rat heart.[15] In contrast, as shown in the present report, higher concentrations of DMI actually accelerate the efflux of catecholamines from heart slices and from the isolated heart. In agreement, recent histochemical studies by Hamberger have shown that comparable concentrations of DMI also release NE from rat brain slices and the vas deferens.[16]

The metabolic fate of the NE released by drugs has been used as an indication of the site of action of these drugs. Drugs, such as reserpine and RO 4-1284, interfere with the storage of NE, releasing the amine from binding sites into the cytoplasm, where it undergoes deamination by monoamine oxidase.[12, 17, 18] Our observation that DMI released NE mainly as deaminated metabolites indicates that this drug also releases NE from the storage granule into the neuronal cytoplasm.

The maximal depleting effect in slices is elicited by 50 μg/ml DMI, which depletes the NE stores by about 50 per cent in 1 hr. Since DMI is highly bound to tissue protein, it is unlikely that the plasma levels of the free drug could approach the concentrations necessary to achieve these maximum depleting effects.[19] However, there is evidence that administration of DMI or its precursor imipramine can, in fact, lower the levels of brain NE. For example, Schildkraut reported that imipramine (10 mg/kg, twice daily for 3 weeks) lowered brain NE.[20] Similar observations were reported after the administration of DMI and protriptyline.[16]

Recent reports provide convincing evidence that DMI possesses more than a single action on the adrenergic neuron. It interferes not only with the specific amine transport mechanism at the neuronal membrane but also has a second action on the NE storage granule. Evidence for this granular action are experiments in which DMI prevents neuronal metaraminol from releasing NE[6] which blocks the exchange of endogenous NE with large doses of administered amine which have overcome the membrane blockade[21] and slows the spontaneous efflux of neurotransmitter.[15] Our finding that DMI depletes NE stores is considered to be an extension of this granular action. It is suggested that the granular action of DMI may be related to its antidepressant action.

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