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## Effects of desipramine on noradrenaline uptake into isolated nerve granules

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DESIPRAMINE (DMI) is one of the most potent inhibitors of noradrenaline (NA) uptake into sympathetically innervated tissues.[1] Considerable evidence indicates that the inhibitory effect of this drug on the uptake process is exerted on a NA transport mechanism located in the axonal membrane.[2,3] However, the concept that DMI may, in addition, affect amine uptake into the intraaxonal storage particles ("nerve granules ') has recently been proposed.[4]

The present experiments were prompted by the observation that DMI causes some inhibition of NA uptake into isolated whole bovine splenic nerves *in vitro*,[5] and were designed to determine to what extent the observed effect of DMI could be due to interference with NA uptake into the granules from such nerves.

### MATERIAL AND METHODS

Bovine splenic nerves were obtained at the slaughter house within 15–30 min post mortem, and were immediately chilled on ice. After careful removal of contaminating tissue, the nerves were

desheathed, minced and homogenized in 0·13 M potassium phosphate, pH 7·5, by means of an Ultra–Turrax apparatus. The coarse tissue particles were removed by centrifugation at 9000 *g* for 10 min, and the supernatant fraction obtained was diluted with phosphate so that the material derived from 60 mg of nerve was contained in each ml. Portions of this suspension were preincubated with DMI ($10^{-7}$–$10^{-4}$) for 30 min at 0° in order to avoid unnecessary release of endogenous amine, which occurs rapidly at 37°. At the end of this period 5-ml portions of preincubated and control low speed supernatant fractions were added to 10-ml Spinco centrifuge tubes containing unlabeled DL-NA ($1{\cdot}2 \times 10^{-5}$M or $9{\cdot}6 \times 10^{-5}$M) and $^{3}$H-DL-NA (New England Nuclear Corp., DL-NA-7-H$^{3}$ hydrochloride; sp. act., 4·8 m-mole) 0·25 or 2 $\mu c^{3}$ml respectively. All tubes were then incubated at 37° for 15 min. The reaction was stopped by transferring the tubes to ice and by adding 5 ml of ice-cold potassium phosphate. The tubes were then centrifuged at 105,000 *g* for 30 min, the supernatants were discarded, the tubes inverted to allow drainage of supernatant trapped by the sedimented pellets, the walls of the tubes wiped dry and the pellets resuspended in 10 ml of fresh potassium phosphate. After a second centrifugation at 105,000 *g* for 30 min, the above procedure was repeated and the washed pellets were extracted with 0·5 ml of 0·4 M perchloric acid. The extract was diluted with 2·5 ml water and 0·3-ml aliquots were added to vials with 20 ml of a 7:3 toluene–absolute ethanol mixture containing 4 g of 2,5-diphenyloxazole and 100 mg of 1,4-*bis*-2(5-phenyloxazolyl) benzene per liter toluene and were counted for 10 min in a Packard Tri-Carb liquid scintillation spectrometer. Quenching was monitored by internal standards. The uptake of NA at the two concentrations of exogenous amine studied represent about 25–56 percent of the starting endogenous total intragranular catecholamine level.[6] More than 90 per cent of the radioactivity found in the pellet has been shown chromatographically to be intact NA.

## RESULTS

The amounts of exogenous NA recovered from particles incubated in the presence of DMI ($10^{-7}$–$10^{-5}$ M) were not significantly different from those found in controls incubated in the absence of the drug (Fig. 1). However, particles incubated with DMI ($10^{-4}$ M) contained less (39–49 per cent) exogenous NA than the controls.

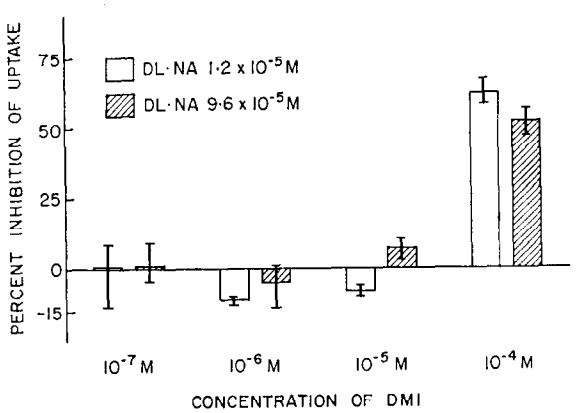


FIG. 1. Per cent inhibitory effect of DMI on uptake of exogenous DL-NA at concentrations in the medium of $1{\cdot}2 \times 10^{-5}$ M and $9{\cdot}6 \times 10^{-5}$ M. Means and range of 3 observations.

## DISCUSSION

The present results clearly demonstrate that DMI is, at most, a weak inhibitor of NA uptake into the isolated bovine splenic nerve granules. In this respect the results resemble those obtained on

incubation of bovine adrenomedullary granules, where DMI ($3 \times 10^{-5}$ M) was found to cause a 25 per cent inhibition of catecholamine uptake.[7] A comparison of the present results with those obtained when bovine splenic nerve granules were incubated with reserpine indicates that this drug is from 200–800 times more potent as an inhibitor of the NA uptake process.[6] Thus the $ID_{50}$ for reserpine on NA uptake into the nerve granules at NA concentrations in the medium of $1{\cdot}2 \times 10^{-5}$ M and $9{\cdot}6 \times 10^{-5}$ M was $10^{-7}$ M and $5 \times 10^{-7}$ M, respectively, while the $ID_{50}$ for DMI on the same process in the present experiments appears to be of the order of $8 \times 10^{-5}$ M and $10^{-4}$ M respectively.

By contrast, the $ID_{0}$ for DMI in NA uptake into the isolated perfused rat heart was as low as $1{\cdot}3 \times 10^{-8}$ M at NA concentrations in the perfusion medium within physiological and pharmacological range *in vivo* (less than $10^{-6}$ M, "uptake 1").[1] On the other hand, the uptake of NA into the rat heart at higher NA concentrations in the medium was far less efficiently inhibited by DMI. Thus, a $2 \times 10^{-4}$ M concentration of the drug was required to produce a 79 per cent inhibition of this uptake process ("uptake 2").[8] The closeness of this latter figure to that observed in the present experiments for the DMI effect at the granule level may of course be purely coincidental, but may also indicate that the inhibitory effect of DMI on "uptake 2" could be, to some extent, exerted at the intraaxonal storage granule level.

In the intact tissue it has been clearly demonstrated by fluorescence microscopy that DMI blocks the entry of NA across the axonal membrane into sympathetic neurons, as mentioned above.[2] The drastic difference in efficiency of DMI as an inhibitor of NA passage into the neuron at "physiological" NA concentrations in the periaxonal medium, and of the subsequent uptake of the exogenous NA into the intraaxonal storage particles is illustrated by the fact that the $ID_{50}$ for the latter process ("granule amine pump") is up to 8000 times higher than that for the former process ("axonal membrane amine pump").

Unless DMI is efficiently concentrated by the sympathetic neuron, the present results indicate that it is highly unlikely that the drug, in reasonable pharmacological doses, reaches intraaxonal concentrations sufficiently high to cause impairment of the "granule amine pump". Thus, the strong inhibitory effect of DMI on NA uptake into sympathetically innervated tissues appears to be almost exclusively exerted on the mechanism(s) responsible for transferring NA from the periaxonal area into the interior of the axon.

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