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Uptake of desipramine by the rat vas deferens

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Summary

1. Determinations of desipramine in the isolated rat vas deferens were carried out in order to study the uptake of the drug and to evaluate how this may correlate with the effect on noradrenaline (NA)-induced contraction.

2. Vasa deferentia, in contact with concentrations of desipramine of 1 ng−25 μg/ml for 10 min accumulated the drug about 6-fold in respect to the medium. When the time of contact was prolonged to 4 h, desipramine (200 ng/ml) was concentrated about 100-fold. The uptake was not saturable and it was not affected by the presence of imipramine, cocaine, ouabain, dinitrophenol and iodoacetate.

3. No metabolic process is involved although the accumulation of desipramine is temperature dependent. The release of desipramine from the vas deferens is exponential and it is not affected by the presence of plasma in the medium.

4. No clear correlation was found between tissue concentration of the drug and the potentiation of noradrenaline responses, probably because of the high non-specific binding of the drug to tissue components which may mask specific binding sites for NA resulting in potentiation. However, the concentration of desipramine seems to correlate with the inhibition of NA effect which occurs at high doses of desipramine.

Introduction

Tricyclic antidepressant drugs, and in particular desipramine (DMI), exert a dual action on noradrenaline activity on several isolated preparations such as renal artery (Bonaccorsi & Hrdina, 1967), tail artery (Bonaccorsi, Jespersen & Garattini, 1970), atria (Matsuo & Toda, 1968) and vas deferens (Ursillo & Jacobson, 1965). At low doses, usually 10 ng–10 μg/ml DMI increases the effect of noradrenaline several fold while at higher concentrations it exerts an inhibitory action on the response to noradrenaline (NA). In addition, a dose with potentiating activity may become inhibitory by prolonging the time of contact with the tissue. Conversely, after washing with DMI-free Krebs solution, a vas deferens which was previously incubated with an inhibitory concentration of desipramine may then show an enhancement of NA response. These observations suggested that tissue uptake of DMI may parallel the sensitivity to NA and that the drug may be accumulated by those structures able to take up NA.

The purpose of the work described in this paper was to determine to what extent DMI is taken up by a tissue rich in adrenergic nerve terminals. Since correlation between concentrations of DMI and sensitivity to NA was postulated, the rat vas deferens was chosen as one of the most suitable preparations for its high sensitivity

to drugs which potentiate NA responses. For this purpose a kinetic study of the accumulation and release of DMI from the isolated vas deferens was carried out.

Methods

Sprague Dawley male rats weighing 200 ± 10 g were used throughout the experiments. After killing, their vasa deferentia were removed, freed from mesenteric attachments and then suspended in 20 ml of Krebs bicarbonate solution of the following composition: NaCl, 6·9 g ; KCl, 0·35 g ; $CaCl_2 \cdot 2H_2O$, 0·37 g ; $MgSO_4 \cdot 7H_2O$, 0·29 g ; KH_2PO_4, 0·16 g ; glucose, 2 g ; $NAHCO_3$, 2·1 g ; per litre of distilled water.

The medium was kept at 37° C if not otherwise stated and gassed with 95% O_2 and 5% CO_2. The organs were attached to a glass holder and a tension of 0·5 g was applied to them. After equilibration for 15 min the organs were incubated for periods of up to 4 h in medium containing concentrations of DMI ranging from 1 ng to 10 μg/ml. After incubation the vasa deferentia were blotted between filter paper, weighed and left in dry ice until analysed. In order to study the release of DMI, vasa deferentia were incubated for 10 or 30 min with a given concentration of the drug, and transferred for various periods to a medium which did not contain DMI. This medium was changed every 15 minutes. In another set of experiments, DMI-free medium was diluted with an equal volume of human plasma. In those cases where drugs presumed to affect DMI uptake were studied, the drugs were added to the incubation solution 15 min before DMI so that during the subsequent incubation period both drugs were present in the medium. Unilateral denervation of the vasa deferentia was performed 8 days before the experiments as described by Birmingham (1970). DMI was determined in the vas deferens (average weight 35 mg) by the method of Hammer & Brodie (1967) after homogenization with 1·15% KCl.

In other experiments the organs were suspended in an isolated organ bath and connected to an isotonic lever writing on a kymograph. Cumulative dose-response curves to NA were obtained, using the method described by Van Rossum (1963).

In this second series of experiments, time of incubation and exposure to different concentrations of the drug were identical with those used for the determinations of DMI uptake or release.

In each experiment, two isolated organs dissected from the same animal were run together, one being used as a control and the other as the experimental organ. DMI was added to the experimental organ after having established the first dose-response curve to NA and a second dose-response curve to NA was determined after exposure for a fixed time to DMI.

The control vas deferens was treated in exactly the same way as the experimental organ except that it was not exposed to DMI. The absolute contraction values were transformed into percentage values with the maximal response of the first dose-response curve to NA being taken as 100.

ED50's were read off from the graph for each curve in which percentage contraction was plotted against the log of the dose. The mean ED50 values were calculated from the corrected data ± S.E.M. (Patil, LaPidus & Tye, 1967). When no change in sensitivity occurred in the control tissue, the ED50's were read off from the curves obtained from the treated organ before and after the addition of DMI. (This

happened when the time between the first and the second dose-response curve to NA was not more than 20 min).

When changes in sensitivity occurred in the control, the ED50's were read off from the second curve obtained simultaneously in the control and in the experimental organ.

Statistical significance between the means was calculated by Student's *t* test.

Drugs

(−)-Noradrenaline bitartrate was dissolved in 0·01 NHCl and kept in the refrigerator. Dilutions were made each day with added ascorbic acid (10 μg/ml) as an antioxidant. DMI concentrations were expressed as the free base. Desipramine hydrochloride was kindly supplied by Geigy Milan.

TABLE 1. *Effect of desipramine (DMI) on the sensitivity to noradrenaline in the rat vas deferens*

DMI concentration (10 min contact time)	Log ED50±S.E.			Potentiation		DMI concentrations (μg/g of tissue±S.E.)
	Before DMI	After DMI	*P* values	In log units*	In dose ratio	
1 ng/ml	5·29±0·02	5·74±0·07	<0·01	0·45	2·75	<0·2
50 ng/ml	5·19±0·07	5·90±0·11	<0·01	0·71	5·13	0·617±0·077
100 ng/ml	5·28±0·02	5·96±0·06	<0·01	0·68	4·80	0·819±0·10
200 ng/ml	5·11±0·03	5·80±0·06	<0·01	0·69	4·90	1·69 ±0·09
500 ng/ml	5·20±0·04	5·52±0·02	<0·01	0·32	2·04	4·54 ±0·12
1 μg/ml	5·24±0·03	5·50±0·02	<0·01	0·26	1·82	6·10 ±0·54
2 μg/ml	5·02±0·05	5·13±0·08	>0·05	0·11	1·28	13·20 ±1·32

*Difference in negative log molar ED50 of (−)-noradrenaline before and after desipramine. Number of observations ranges from 5 to 10.

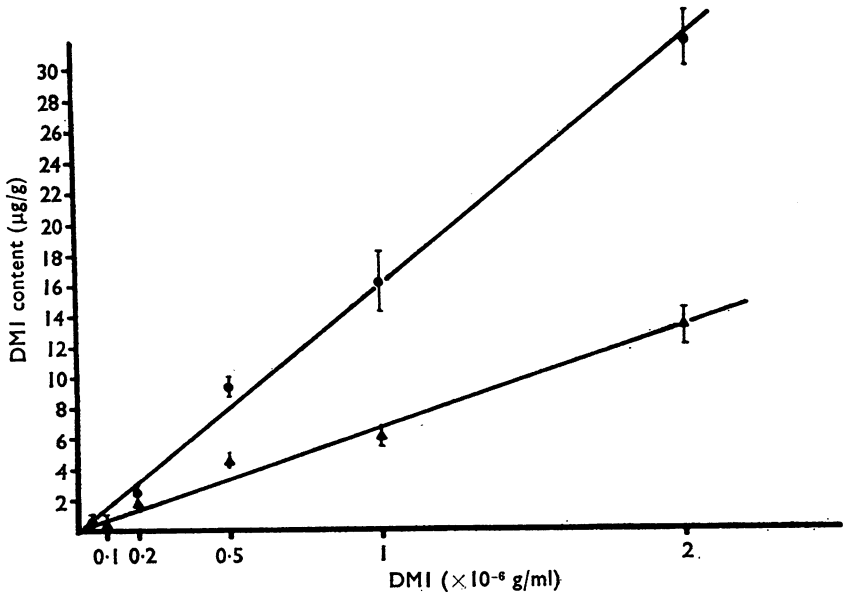

FIG. 1. Uptake of desipramine (DMI) by the rat vas deferens at various concentrations in the medium. Incubation period was 10 min (▲, $y=6{\cdot}63{\cdot}x$) or 30 min (●, $y=16{\cdot}3{\cdot}x$).

Results

The potentiation of NA responses after incubation of the vas deferens with DMI (1 ng/ml–2 μg/ml) for 10 min is shown in Table 1. Maximal increase in sensitivity to noradrenaline was obtained in the concentration range 50 ng–200 ng/ml. Increase of the concentration of DMI above these values caused a decrease in the potentiation of the NA effect.

The concentrations of DMI measured in the vas deferens (Table 1) show a certain correlation with the pharmacological activity, although at the lowest doses used the amount of the drug in the tissue could not be detected by our method. At higher concentrations, the concentrations of DMI in the vas deferens increased while the potentiating effect on NA was diminishing.

TABLE 2. *Effect of increased contact time on desipramine (DMI) concentrations and on the sensitivity to noradrenaline in the rat vas deferens*

Contact time (min) of DMI (200 ng/ml)	Log ED50±S.E.			Potentiation		DMI concentrations (μg/g of tissue±S.E.)
	Controls	In the presence of DMI	*P* values	In log units*	In dose ratio	
10	5·11±0·03	5·80±0·06	<0·01	0·69	4·90	1·54±0·12
20	5·14±0·07	5·65±0·06	<0·01	0·51	3·20	2·11±0·16
30	4·92±0·04	5·38±0·14	<0·05	0·46	2·89	3·39±0·20
60	5·12±0·06	5·53±0·07	<0·01	0·41	2·61	5·13±0·22
90	5·39±0·05	5·56±0·10	>0·05	0·17	1·49	9·00±0·36

*Difference in negative log molar ED50 of (+)-noradrenaline before and after desipramine. Number of observations ranges from 5 to 10.

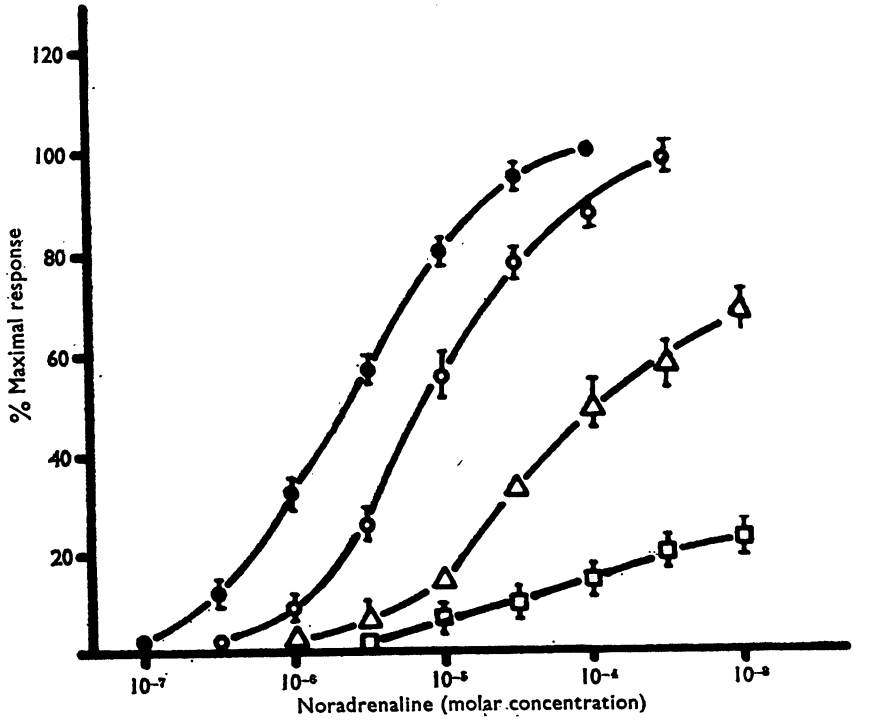

FIG. 2. Dose-response curves of the isolated rat vas deferens to (−)-noradrenaline before and after incubation for 10 min with desipramine (DMI) at three different concentrations: ●——●, control; ○——○, 5 μg/ml; △——△, 7·5 μg/ml and □——□, 25 μg/ml. Vertical lines show the S.E.M.

Figure 1 shows that the concentrations of DMI in the vas deferens are directly proportional to the concentration added to the bath when the period of incubation was 10 or 30 minutes. The tissue/medium ratio is several fold in favour of the tissue. Prolongation of the time of contact of DMI with the tissue results in a further increase in the tissue concentration of the drug. These observations are extended in Table 2 where the concentrations of DMI are reported as a function of the time of contact. In this case too, the increased DMI concentration in the vas deferens results in a decrease of NA potentiation. Further determinating of the concentrations of DMI and the tissue/medium ratio in the vas deferens after several periods of incubation with a concentration of 200 ng/ml of the drug in the medium, gave a linear relationship with ratios ranging from 7·7 after 10 min of incubation up to 111 after 4 h, indicating that at the later times about 20% of DMI present in the medium (20 ml) was taken up by vas deferens. The data reported above also show that the

TABLE 3. *Effect of washing on desipramine (DMI) concentrations and on the sensitivity to noradrenaline in the rat vas deferens*

Concentration of DMI (μg/ml)	Duration of contact (min)	Duration of wash (min)	Log ED50±S.E.			Potentiation		DMI concentrations (μg/g of tissue±S.E.)
			Before DMI	After DMI	*P* values	In log units*	In dose ratio	
0·2	10	30	5·21±0·03	6·09±0·02	0·01	0·88	7·58	0·56±0·02
0·5	10	30	5·22±0·0	5·99±0·08	0·01	0·77	5·91	2·63±0·40
1	10	30	5·12±0·12	5·74±0·06	0·01	0·62	4·20	3·60±0·20
0·2	30	0	4·92±0·04	5·38±0·14	0·05	0·46	2·89	3·33±0·09
0·2	30	15	4·88±0·05	5·76±0·10	0·01	0·88	7·48	2·37±0·19
0·2	30	30	5·10±0·04	5·88±0·05	0·01	0·78	5·97	1·51±0·08
0·2	30	60	5·13±0·05	5·63±0·05	0·01	0·50	3·20	0·89±0·10

* Difference in negative log molar ED50 of (−)-noradrenaline before and after desipramine. Number of observations ranges from 5 to 10.

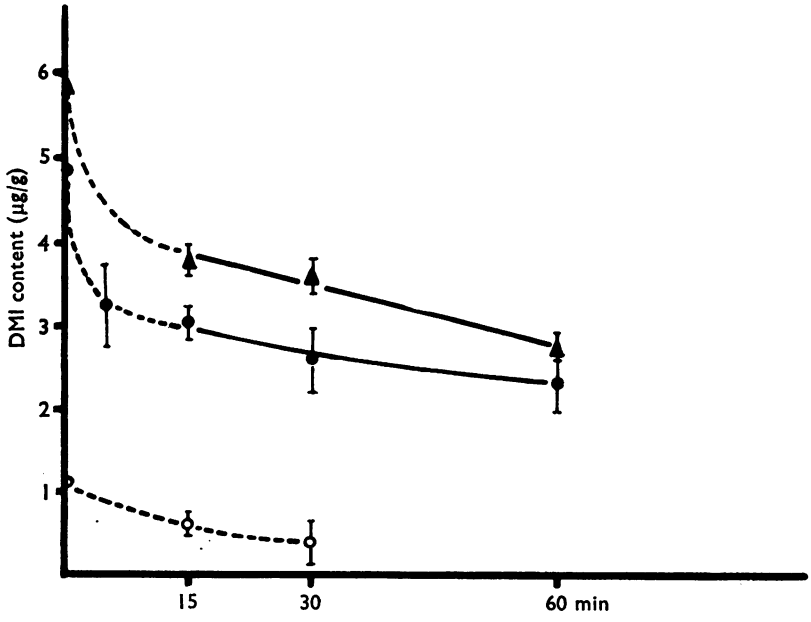

FIG. 3. Release of accumulated desipramine (DMI) after incubation in DMI-free medium. DMI concentration in the vas deferens is plotted against time. Vasa deferentia were incubated for 10 min in medium containing three concentrations of DMI. Release from the vas deferens is expressed by the equation $y=A(1-e^{-kt})$, except for the dashed part of the curves. ○, 0·2 μg/ml; ●, 0·5 μg/ml ($y=3{\cdot}22e^{-0{\cdot}0055{\cdot}x}$); ▲, 1 μg/ml ($y=4{\cdot}34e^{-0{\cdot}0074{\cdot}x}$).

accumulation of DMI in the vas deferens did not reach saturation by increasing the dose up to 2×10^{-6} g/ml or by prolonging the time of incubation up to 4 hours. In other experiments DMI was tested at concentrations higher than 2 μg/ml in order to investigate its antagonistic activity toward NA. Figure 2 indicates that by increasing the concentration of DMI from 5 μg/ml to 25 μg/ml (incubation time 10 min) the inhibition of NA maximal response reached about 80%. The concentration of DMI in the vas deferens was from 41·8 to 155 μg/g.

By washing the vas deferens for different periods after contact for 30 min with DMI (200 ng/ml) it was observed that there was a potentiation of the NA response which was highest after a washing period of 15 minutes (Table 3).

When three different concentrations of DMI (200 ng/ml, 500 ng/ml and 1 μg/ml) were left in contact with the tissue for 10 min and then washed for 30 min the concentrations of DMI were inversely related to the degree of NA potentiation, being maximal with the lowest concentration (Table 3).

The kinetics of DMI release on washing the vas deferens are presented in Fig. 3. The decrease is exponential and is expressed by the equation $y=x+e^{-kt}$. This rate of decrease is strikingly similar to that obtained in the vas deferens *in vivo* after

TABLE 4. *Effect of various drugs and experimental conditions on the accumulation of desipramine (DM) in the rat vas deferens*

Experimental condition	Temperature °C	DMI (μg/g±S.E.) Vas deferens
Control	37	3·7±0·3 (10)
	18	1·0±0·2 (4)
	0	0·1±0·03 (4)
Surgical denervation	37	4·0±0·6 (3)
Ouabain (1×10^{-5}M)	37	3·0±0·3 (5)
Cocaine (1×10^{-4}M)	37	3·6±0·6 (5)
Imipramine (1×10^{-5}M)	37	3·4±0·2 (5)
Dinitrophenol (3×10^{-4}M+) Iodoacetate (5×10^{-4}M)	37	3·8±0·4 (5)

Rat vasa deferentia were equilibrated with desipramine (200 ng/ml) for 30 minutes. Drugs were incubated with the tissue for 15 min before adding desipramine and were present during the following 30 minutes.

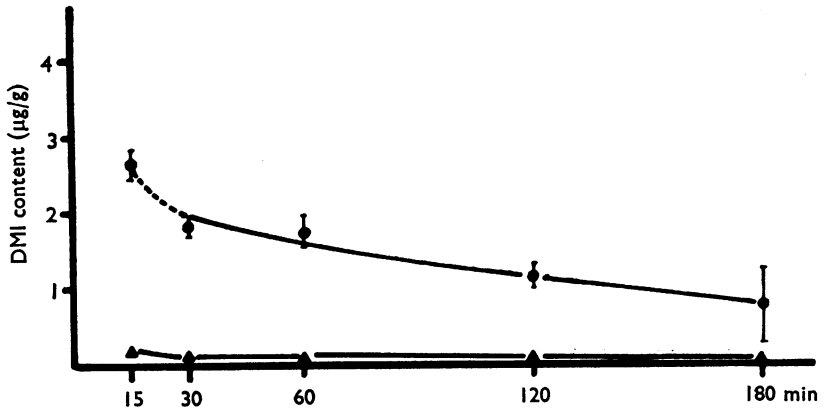

FIG. 4. *In vivo* disappearance of desipramine (DMI) from the vas deferens after an intravenous injection of DMI (5 mg/kg). Except for the dashed part, the curve was calculated using the equation $y=A(1-e^{-kt})$. Plasma concentrations at the times indicated are also shown. (●), Vas deferens ($y=2{\cdot}35e^{-0{\cdot}0062x}$); ▲, plasma.

administration of 5 mg/kg desipramine intravenously (Fig. 4). The presence of human plasma (50%) in the medium did not change the rate of decline of desipramine in the vas deferens after incubation with 500 ng/ml.

The effect of temperature, surgical denervation and addition of dinitrophenol + iodoacetate, ouabain, cocaine and imipramine on the uptake of desipramine by the vas deferens *in vitro* is shown in Table 4. No effects were noted except the marked temperature dependence of the accumulation of DMI. A decrease of 70% at 18° C and 99% at 4° C was observed.

Discussion

Considerable data are available on the uptake of adrenergic mediators or substances sharing the same physiological mechanism of uptake or storage, but relatively little information has been collected concerning the accumulation of other drugs in isolated organs. Drugs which have been studied include propranolol (Potter, 1967), dibenamine (Morgan, May, Kinielberg & Triggle, 1967, and Lewis & Miller, 1966), digoxin (Kuschinsky, Lahrtz, Lüllmann & Van Zwieten, 1967), atropine (Paton & Rang, 1965) and cocaine (Marks, Dutta & Hoffman, 1967). In the present study the accumulation and release of DMI, a drug which acts as inhibitor of NA uptake at the sympathetic nerve terminals (Iversen, 1967) was investigated in the isolated vas deferens. The process of accumulation was linear in relation to the concentration of DMI in the medium and in relation to the time of exposure to a fixed concentration of the drug. The uptake did not reach saturation in a range of DMI concentrations from 1 ng to 10 μg/ml and for a time of up to 4 h when DMI was added to the medium at a concentration of 200 ng/ml. About 20% of DMI added to the medium could be taken up by the tissue giving a ratio between the concentration in the tissue and the medium of over 100.

The process by which DMI is taken up in the tissue is not known but it may result from the lipid solubility of the non-ionized drug in aqueous solution. Since the pK_a of DMI is 9·5 (Sjöqvist, Baglund, Borgå, Hammer, Andersson & Thorstrand, 1969) this drug has a ratio of 1 to 111 at pH 7·4 in terms of non-ionized form. Although DMI is very lipid soluble it is difficult to explain the high ratio between tissue and medium solely on the basis of its partition between heptane and water at pH 7·4 (Dingell, Sulser & Gillette, 1964). It is possible that a non-specific binding to lipids takes place in a manner similar to that shown by Gillette in liver subfractions (Gillette, 1966). This large, non-saturable non-specific binding may mask other types of more specific binding such as the one postulated at the level of the adrenergic postsynaptic membrane to explain the inhibition of NA uptake. The supersensitivity to NA is dependent on the concentration of DMI. Maximal potentiation is reached with a concentration of DMI ranging from 50 ng to 200 ng/ml being less for lower or higher concentrations. Since the activity of DMI measured in the vas deferens depends on the concentration of DMI, a correlation between levels and activity may exist even though we could not follow it for smaller concentrations (less than 50 ng/ml).

The increase in the concentration of DMI over 200 ng/ml reduced the potentiation, suggesting the presence of an inhibitory component. This was clearly observed with concentrations of desipramine in the vas deferens higher than 40 μg/g. Attempts were made to disclose the specific binding sites presumably present in the

adrenergic nerve terminals, by denervation or by previous incubation with another drug like cocaine acting at the same site as DMI.

Dinitrophenol+iodoacetate or ouabain were utilized in an attempt to demonstrate an active transport of DMI, while imipramine was incubated with DMI to diminish the binding to the non-saturable compartment. All these experiments were unsuccessful and therefore we cannot draw any conclusions except to suggest again that the specific binding sites, if any, must account for a small fraction of the total DMI present in the vas deferens. The temperature dependent uptake of DMI probably does not involve any active transport, since the partition coefficient of DMI between hexane and water is temperature dependent being 0·65 at 37° C and 0·17 at 0° C, at pH 7·4.

Since Krebs bicarbonate saturated with carbogen (95% oxygen+5% carbon dioxide) has a lower pH (6·9) at 0° C, than at 37° C (7·4), and since the partition coefficient decreases from 0·17 to 0·13 by decreasing the pH from 7·4 to 6·9 it can be concluded that both factors play a role in reducing the uptake of DMI.

Other experiments were performed in an attempt to differentiate non-specific from specific binding, by washing in a medium free of DMI. The curve of DMI release from the vas deferens is biphasic. There is an initial fast component, including presumably the washout from the extracellular compartment, and an exponential component which is similar to the one observed *in vivo* after a single intravenous administration of DMI. When attempts were made to correlate levels of desipramine with the ED50 of NA in the washout experiments, maximal potentiation was reached after 15 min of washing corresponding to concentrations of 2·37 μg/g, while with longer periods of washout there was a decrease in sensitivity although the concentrations were not so low as to justify the decreased potentiation. Potentiation of the NA effect was obtained even with unmeasurable concentrations of DMI in the vas deferens (Table 1). These findings are consistent with the idea that the pool of the drug responsible for NA potentiation is too small in relation to the total amount of DMI to be unmasked by the presence of plasma in the medium although it is known that desipramine is 90% bound to plasma proteins (Borgå, Azarnoff & Sjöqvist, 1968). Further studies with different techniques are required in order to measure the size of the binding of DMI to those adrenergic structures which may be linked to the site of action of this tricyclic antidepressant drug.

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