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Correlation between desipramine levels and (−)-noradrenaline uptake and chronotropic effect in isolated atria of rats

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Summary

1. The uptake of unlabelled and [^{14}C]-desipramine was studied in rat isolated atria incubated in a medium containing concentrations of desipramine ranging from 200 pg/ml to 2 μg/ml. The uptake was found to be dose- and time-dependent. Equilibrium was not reached after 2–3 h of incubation unless concentrations of desipramine higher than 1 μg/ml were used.

2. The washout curves of atria previously loaded with desipramine showed that the drug is slowly released and that this release is not influenced by its initial tissue concentration.

3. The binding appeared to be at non-specific sites and not at sites where noradrenaline is stored since atria taken from rats treated with 6-hydroxydopamine accumulated the drug at the same rate as control atria.

4. The inhibition of (−)-noradrenaline uptake and the potentiation of the chronotropic response to (−)-noradrenaline is correlated with the concentration of desipramine in atria for tissue levels of the drug ranging from 0·01 to 1 μg/g. Higher tissue levels show less potentiation of the effect of (−)-noradrenaline or even inhibition of the maximal response to (−)-noradrenaline. These concentrations of desipramine (>7 μg/g) markedly depressed the atrial rate.

5. The results show that despite the accumulation of desipramine by unspecific sites, concentrations of desipramine in the tissue are correlated with the pharmacological response. Furthermore a gradual shift from potentiation to inhibition of noradrenaline response can be obtained with the same bath concentrations of desipramine by increasing the time of incubation.

Introduction

A number of studies have contributed information regarding the kinetics of the uptake of noradrenaline in isolated organs as well as the influence of several drugs on this uptake (Callingham, 1967; Iversen, 1967; Foster, 1968; Maxwell, Keenan, Chaplin, Roth & Eckardt, 1969; Starke, Montel & Wagner, 1971). However, relatively little is known of the relationship between the concentration of a drug in an isolated organ and its effect on the uptake and the pharmacological responses to noradrenaline.

Previous studies from this laboratory have attempted to relate the accumulation of desipramine, a tricyclic antidepressant agent known to be one of the most potent inhibitors of noradrenaline uptake by peripheral adrenergic nerves, to the potentiation of noradrenaline response in the vas deferens (Binini, Bonaccorsi, Garattini,

Morselli & Muscettola, 1972). However, in the vas deferens the α-adrenoceptor blocking activity of desipramine may have obscured the interaction of this drug with noradrenaline. Furthermore, the analytical method for desipramine was not sensitive enough to cover the range of tissue concentrations having pharmacological activity. In order to avoid these problems in the present study we have used the rat atria, where the α-adrenoceptor activity is of no importance to the response, and a more sensitive method for determination of desipramine ([^{14}C]-desipramine) so that measurement of the drug in the lower range was possible.

Methods

Animals

Male Sprague Dawley rats (Charles River) weighing 220–250 g were used.

Some animals were pretreated with 6-hydroxydopamine 100 mg/kg i.v. for two consecutive days and were killed 5 days later. 6-Hydroxydopamine was dissolved in 0·01 N HCl gassed with nitrogen for 10 min just before injection.

Incubation technique

Paired atria (50 mg $\pm$2) were dissected and placed in tubes (uptake studies) or baths for isolated organs (physiological studies) containing 10 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. In some of the uptake experiments 4 pooled atria were used. The bathing solution was kept at 32° C and gassed with a mixture of 95% O_2 and 5% CO_2.

The tissue was allowed to equilibrate for 30–45 min before any drug was added. When noradrenaline was given, the incubation mixture also contained 10 μg/ml of the disodium salt of ethylene diamine tetra-acetic acid (EDTA) to protect the added catecholamine from oxidation.

Determination of the potentiation of the chronotropic response to (−)-noradrenaline

Atria suspended in 10 ml Krebs bicarbonate solution were attached to a force displacement transducer under the minimal tension (150 mg) suitable to record the contraction rate on a pen recorder (Grass 5D). The tissue was allowed to equilibrate for 45 min and during this period it was washed 3 or 4 times. (−)-Noradrenaline was then added; the concentration was increased 3–3·3 fold with each addition.

A dose-response curve to (−)-noradrenaline was determined before and after exposure to desipramine at different concentrations and times of contact. Cumulative doses were plotted on a log scale against the chronotropic responses, calculated as the increase in atrial rate over the resting rate (220 beats/minute). From such a plot obtained before and after adding desipramine a dose-ratio was calculated from the ED50 values. Control atria were always run together with the treated ones in order to correct for changes in sensitivity independent of desipramine (Furchgott, 1967).

Tissue determinations of (−)-[^{3}H]-noradrenaline

The uptake of (−)-[^{3}H]-noradrenaline was studied by the following procedure: the tissues were exposed to 6·1 μCi/ml of (−)-[^{3}H]-noradrenaline for 10 min and then washed for another 10 minutes. Labelled noradrenaline was diluted with unlabelled (−)-noradrenaline to give a final concentration of 50 ng/ml. This concentration was chosen because it produced 50–70% of the maximal response of the atria.

The atria were then removed, blotted with filter paper, weighed and homogenized in 3 ml of 0·4 N $HClO_4$ in a glass homogenizer, after addition of 0·2 ml of EDTA (10%), 0·1 ml of sodium bisulphite (5%) and 0·1 ml of unlabelled (−)-noradrenaline (50 μg/ml). After centrifugation, 2·5 ml of the clear supernatant was adjusted to pH 8·9 with 5 ml of tris buffer (0·1 M pH 8·9) and noradrenaline was adsorbed on 500 mg of alumina, by gentle shaking. The alumina was then washed twice with distilled water and noradrenaline eluted with 3 ml of HCl (0·2 N). One ml of the eluate was transferred to a vial containing 10 ml of a liquid scintillation solution (toluene-triton X-100-butyl-PBD) and counted. All samples were corrected for quenching. The overall recovery of noradrenaline was 65–70% and the efficiency of the counting system 38%.

Endogenous heart noradrenaline of 6-hydroxydopamine pretreated and control rats was extracted by the same procedure and determined spectrofluorimetrically according to Häggendal (1963).

Tissue determinations of desipramine

The accumulation of desipramine in the isolated atria was studied by adding to the incubation medium concentrations of the drug ranging from 200 pg to 2 μg/ml.

After various periods of incubation the atria were removed, blotted on filter paper, weighed and homogenized in 2·5 ml of 0·1 N HCl with a glass homogenizer. The homogenates were kept frozen (−20° C) until analyses were performed. The washout curve was studied on samples incubated with desipramine for 30 min and transferred to 10 ml of Krebs bicarbonate solution free of drug. The washing incubation medium was changed every 10 min and after various periods of time atria were removed and processed as before.

Unlabelled desipramine was determined according to the method of Hammer & Brodie (1967) with minor modifications. When the concentrations of desipramine in the incubation medium were lower than 20 ng/ml, ^{14}C-labelled desipramine was used and determined by the following procedure. The homogenates were made alkaline with 0·5 ml of 5 N NaOH and then extracted three times with 5 ml of n-hexane. Fourteen ml of the organic phase was then transferred into a glass liquid scintillation vial and brought to dryness at 60° C on a water bath under a gentle stream of nitrogen. The drug residue was then redissolved in 10 ml of a liquid scintillation counting solution (7 g of butyl-PBD/l of toluene) and counted. A similar procedure was followed for the determination of total extractable radioactivity in the incubation and washing medium.

There was practically no quenching and the counting efficiency was 90%. The overall recovery for [^{14}C]-desipramine total extractable radioactivity with the described procedure was 98 ± 1%. (No radioactivity was present in the homogenate after the three hexane extractions.)

In order to prevent adhesion of desipramine to the glass surface all the glassware used was first treated with 1% solution of trimethylchlorosilane.

Desipramine was not metabolized by the atria as shown by thin layer chromatography determinations. In duplicate experiments the concentrations of desipramine in atria obtained with either labelled or unlabelled desipramine were identical.

Concentrations of desipramine are always expressed as the free base.

Drugs

(−)-Noradrenaline bitartrate was provided by Recordati, Milano. (−)-Noradrenaline-7-[^{3}H] (6·95 Ci/mM) was obtained from New England Nuclear Corporation, Frankfurt, Germany. Desipramine 10-[^{14}C] (14·6 μCi/mg) and desipramine hydrochloride were provided by Ciba-Geigy, Basel, Switzerland. 6-Hydroxydopamine hydrochloride was provided by Dr. C. S. Stone, Merck, Sharp and Dohme, West Point, Pa., USA.

The radiochemical purity of the labelled desipramine was checked by thin layer chromatography followed by scanning on a Packard Radiochromatoscanner. All the radioactivity was counted on a Nuclear Chicago Isocap, 3000-L.S.C.

Results

Desipramine-induced potentiation of the chronotropic effect of (−)-noradrenaline on isolated spontaneously beating atria

Cumulative dose-response curves to (−)-noradrenaline showed a maximal increase of 120 beats/min in atrial rate.

Desipramine (200 pg–20 ng/ml) caused a dose-dependent increase in the sensitivity to (−)-noradrenaline. The maximal potentiation, obtained with 20 ng/ml of desipramine was about 70 times (Fig. 1 and Table 1). A further 10–100 fold increase in the concentrations of desipramine in the bath resulted in a lesser degree of potentiation and even in a decrease of the maximal response to (−)-noradrenaline.

The basal spontaneous atrial rate was not affected by desipramine at concentrations as high as 2 ng/ml. However, higher concentrations led to a small increase (at 20–200 ng/ml) or decrease (at 2 μg/ml) in the atrial rate (Table 1). This effect was inhibited by 10^{-7}M propranolol. In order to verify further whether the observed increase in the spontaneous rate induced by desipramine was mediated by endogenous noradrenaline, atria from animals pretreated with 6-hydroxydopamine were used. Endogenous noradrenaline content in these hearts was very low, accounting for only 9% of the concentration found in control hearts (Table 3). The spontaneous rate of 6-hydroxydopamine pretreated atria was actually decreased by desipramine (Table 2) and, as expected, no potentiation of the chronotropic response to noradrenaline was observed.

Accumulation of desipramine in isolated spontaneously beating rat atria

The time-course of desipramine accumulation in rat atria obtained after incubation with various concentrations of the drug is shown in Figures 2 and 3. It is evident that the tissue concentrations of desipramine are directly proportional to

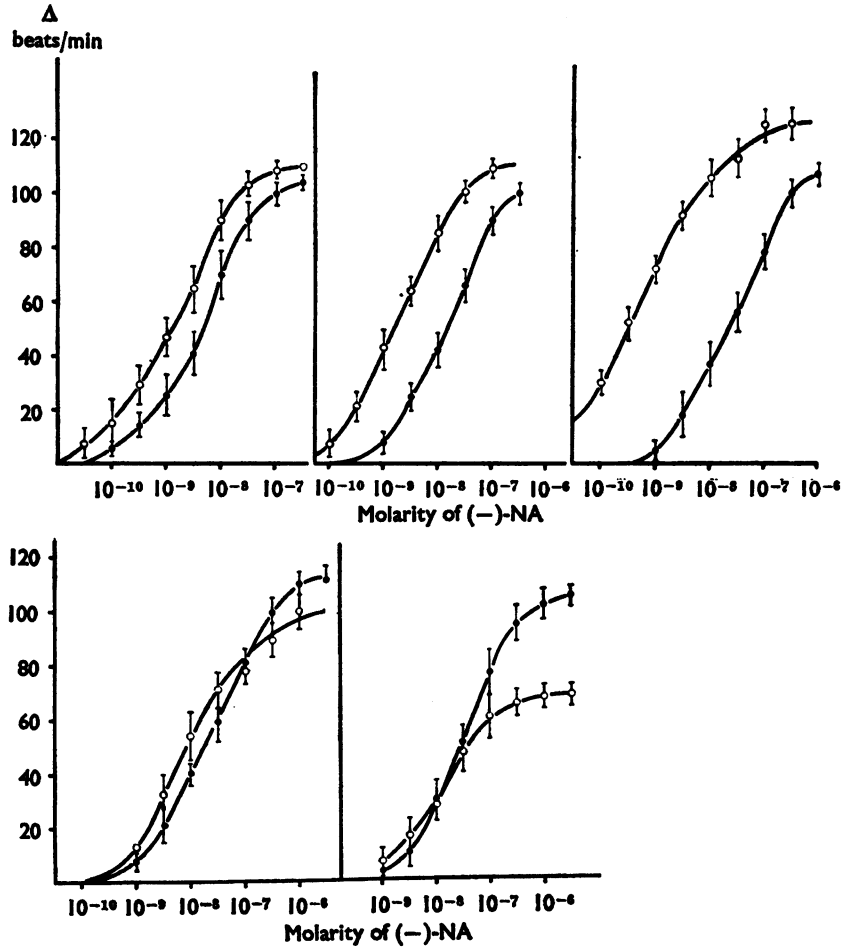

FIG. 1. Cumulative dose-response curves to noradrenaline ((−)-NA) in rat isolated atria before and 30 min after exposure to different concentrations of desipramine. On the abscissae: (−)-noradrenaline expressed as molar concentration; on the ordinates: increase in atrial rate expressed as beats/minute. (●——●) Before, (○——○) after desipramine; from the left to the right 200 pg, 2 ng; 20 ng; 200 ng; 2 μg/ml.

TABLE 1. *Effect of desipramine (DMI) on spontaneous or (−)-noradrenaline (NA)-stimulated atria rate of rat isolated atria*

Concentration of DMI (per ml) (30 min exposure)	No. of experiments	Change in rate after DMI (beats/min ± S.E.)	Potentiation of the chronotropic response to NA (log dose-ratio)
200 pg	4	−5±2	0·5
2 ng	4	+3±0·7	0·95
20 ng	6	+23±6*	1·85
200 ng	6	+14±4*	0·3
2 μg	4	−7±7	0

* $P<0.05$.

the amount of the drug added to the incubation medium. The drug continued to accumulate in the atria with a constant rate of entry and equilibration was not attained after 120 min of incubation, even when the tissue concentrated the drug about 100 fold (Figure 2). Only with the highest concentration (2 μg/ml), which

TABLE 2. *Effect of desipramine (DMI) on the spontaneous atrial rate of rat isolated atria, in controls and 6-hydroxydopamine (6-OHDA)-treated rats*

Concentration of DMI per ml (10 min exposure)	No. of experiments	Change in atrial rate (beats/min±S.E.) Controls	6-OHDA*	*P*
200 ng	4	+20± 7	−8±5	<0·05
2 μg	4	+9±11	−55±8	<0·01

* Atria from 6-hydroxydopamine pretreated rats were hypersensitive to (−)-noradrenaline.

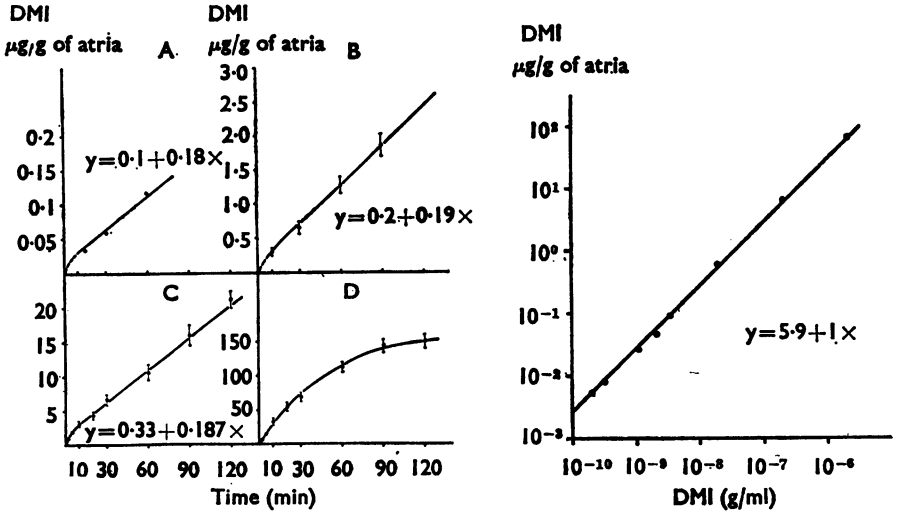

FIG. 2. (Above left.) Accumulation of desipramine (DMI) in rat isolated atria at different concentrations and times of incubation. On the abscissae: time in minutes. On the ordinates: concentrations of desipramine expressed as μg/g of atria. Notice the different scales for the ordinates. The concentrations of desipramine in the medium were: 2 ng/ml (A); 20 ng/ml (B); 200 ng/ml (C); 2 μg/ml (D).

FIG. 3. (Above right.) Accumulation of desipramine (DMI) in rat isolated atria after 30 min of incubation. On the abscissae: concentration of desipramine in the medium expressed as g/ml; on the ordinates: concentrations of desipramine in the atria expressed as μg/g.

decreased the maximal response to (−)-noradrenaline, was an equilibrium approached at 90 min of incubation.

By plotting the concentration of desipramine in the medium against desipramine levels in the tissue determined after 30 min of incubation, a linear relationship was obtained with a tissue/medium ratio of 30·8 (Figure 3). Pretreatment with

TABLE 3. *Accumulation of desipramine (DMI) in rat isolated atria from control and 6-hydroxydopamine-pretreated rats*

Experimental group	Heart noradrenaline (μg/g±S.E.)	Desipramine concentration in atria (μg/g) after 30 min incubation of 3 different concentrations in the medium +30 min washing		
		2 ng/ml	20 ng/ml	200 ng/ml
Saline	0·91 ±0·056	0·034	0·361	3·55
6-OHDA	0·082±0·008	0·026	0·330	4·22

Each value was obtained from 2 atria. 6-Hydroxydopamine was given i.v. for 2 days at a dose of 100 mg/kg and atria dissected 5 days after the last dose.

6-hydroxydopamine did not affect the desipramine accumulation in rat atria, as shown in Table 3.

Observations on the washout of desipramine indicated that approximately 60% of the radioactivity was retained by the tissue after 15 min of washing and little release occurred after 30 or 60 min of additional washing (Figure 4). Table 4 shows

TABLE 4. *Release of desipramine (DMI) from isolated atria of rats after washing with a drug-free medium*

DMI concentration per ml of medium	DMI concentrations in atria (μg/g) after 30 min incubation	DMI concentrations in atria (μg/g) after 30 min incubation +30 min washing	% Release
200 pg	0·005	0·003	40
2 ng	0·049	0·031	38
20 ng	0·66	0·323	40
200 ng	6·69	3·99	41
2 μg	72·20	43·50	40

Each value was obtained from 2 atria except for the 200 pg/ml desipramine concentrations where 4 atria were pooled.

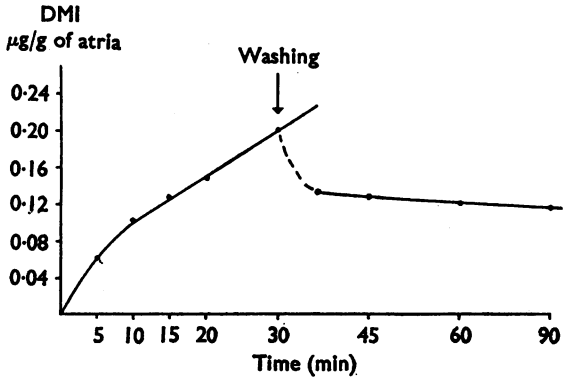

FIG. 4. Accumulation and release of desipramine (DMI) from isolated rat atria incubated with 5 ng/ml of desipramine. On the abscissae: time in min; on the ordinates: desipramine concentrations in atria (μg/g). The arrow indicates the beginning of washing which was continued for 60 min, changing the Krebs solution every 10 minutes.

that even when the initial concentration of desipramine in the tissue was within a range of 10^4 fold μg/g the washout from the tissue was always about 40%, suggesting a very strong binding of the drug to tissue components. When the tissue was washed with a continuous flow or with a medium enriched with bovine serum albumin (3%) or plasma (50%) the washout curve was not altered. Similar results were obtained with the atria of 6-hydroxydopamine pretreated animals.

Effect of desipramine on (−)-[^{3}H]-noradrenaline uptake in rat isolated atria

Desipramine effectively inhibited (−)-[^{3}H]-noradrenaline uptake by atria. As shown in Fig. 5 the inhibition of uptake was dependent on the bath concentration of desipramine. The ID_{50} calculated after 30 min of incubation with desipramine was 3·6 ng/ml. The concentration-inhibition curve was shifted to the right when the incubation with desipramine (30 min) was followed by 30 min of washing.

A clear relationship between desipramine tissue levels and inhibition of (−)-[^{3}H]-noradrenaline uptake was found. Figure 6 shows that by plotting the % inhibition

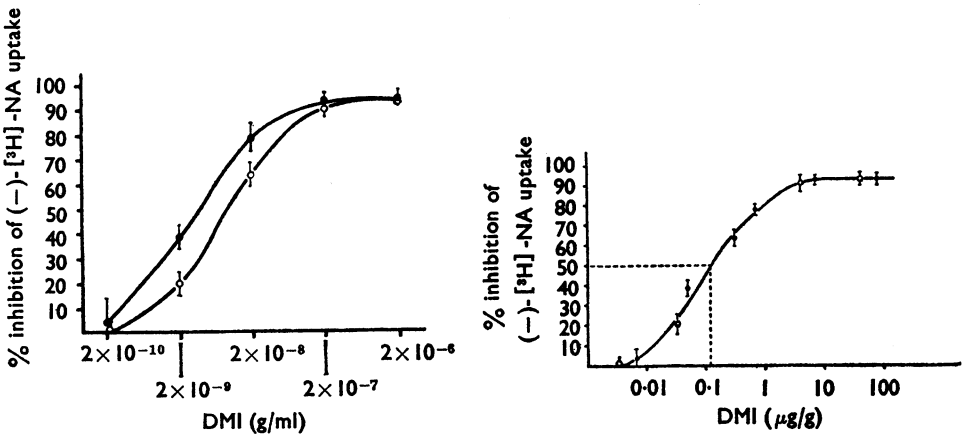

FIG. 5. (Above left.) Relationship between desipramine (DMI) (g/ml) concentration in the medium and % inhibition of (−)-noradrenaline ((−)-[³H]-NA) uptake by isolated rat atria. ●——● 30 min incubation with desipramine; ○——○ 30 min incubation with desipramine followed by 30 min wash with drug-free medium.

FIG. 6. (Above right.) Relationship between concentration of desipramine (DMI) in the atria (μg/g) (abscissae) and % inhibition of (−)-noradrenaline ((−)-[³H]-NA) uptake (ordinates) by isolated rat atria. ●——● 30 min incubation with desipramine; ○——○ 30 min incubation with desipramine followed by 30 min wash with drug-free medium.

of noradrenaline uptake (obtained with and without washing after 30 min of exposure to desipramine) against the tissue desipramine concentrations, a single concentration-response curve is obtained. The calculated IC_{50} was in this case 0·13 μg/g and the ratio between IC_{50}/ID_{50} is 36 $\left(\frac{0{\cdot}13\ \mu g/g\ \text{atria}}{0{\cdot}0036\ \mu g/ml\ \text{medium}}\right)$.

Maximal inhibition was attained with a tissue concentration of about 5 μg/g and the minimal one with 0·01 μg/g.

Relationship between desipramine tissue levels, inhibition of (−)-[³H]-noradrenaline uptake and potentiation of chronotropic responses

One of the main purposes of this study was to evaluate the relationship among the observed variables. Figure 7 summarizes al lthe data on the uptake of (−)-[³H]-

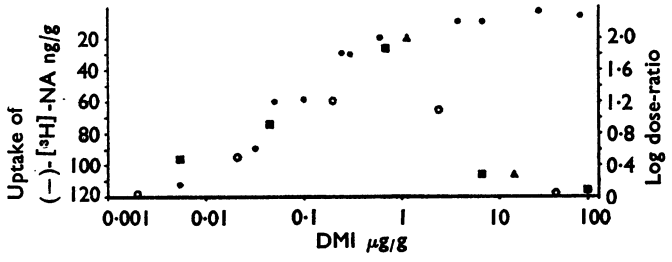

FIG. 7. Multiple relationship between desipramine (DMI) concentration, (−)-noradrenaline uptake and potentiation of chronotropic responses to (−)-noradrenaline in rat isolated atria. On the abscissae: desipramine as μg/g of atria. Left ordinates: uptake of (−)-noradrenaline expressed as ng/g of atria. Right ordinates: increase of chronotropic responses induced by (−)-noradrenaline expressed as the log dose-ratio inducing 50% of the maximal response before and after desipramine. ● Uptake of (−)-[³H]-noradrenaline in relation to desipramine concentration. The other points refer to the relation between desipramine concentration and the chronotropic response to (−)-noradrenaline obtained after incubation with desipramine for 10 min (○), 30 min (■), and 60 min (▲).

noradrenaline obtained under various experimental conditions together with comparable information on the potentiation of the chronotropic effect of (−)-noradrenaline and the levels of desipramine in the tissue.

It is evident that both the degree of potentiation of responses to (−)-noradrenaline and the degree of inhibition of (−)-[^{3}H]-noradrenaline uptake are related to the actual concentration of desipramine in the tissue ranging from 0·006 μg/g to 1 μg/g. Higher tissue levels of desipramine cause a clear dissociation of the two effects: while the inhibition of uptake is increased by increasing the concentration of desipramine up to 5 μg/g, the potentiation of the noradrenaline effect is reduced and an inhibition of the maximal response to noradrenaline appears when desipramine concentrations of 7–40 μg/g of atria are reached.

Discussion

In agreement with the data previously obtained on the isolated rat vas deferens (Binini *et al.*, 1972), the accumulation of desipramine in the rat atria is linearly related to the concentration of the drug in the medium. The uptake tended to saturation only with a concentration of desipramine as high as 2 μg/ml which already showed cardiotoxic activity. These data indicate that desipramine accumulation in the rat atria is, as in the vas deferens, a passive diffusion phenomenon, in agreement with the physico-chemical characteristics of the drug (lipid solubility and pKa) and with previous data reported by Mitchell & Oates (1970) in rat heart slices. There is only one difference between the accumulation of desipramine in the vas deferens and atria. In comparing the uptake curves for desipramine in the two organs, higher levels were found in the atria than in the vas deferens suggesting that a problem of surface may be involved, with the atria having a surface larger than that of the vas deferens.

In both vas deferens and atria the washout curves for desipramine do not follow first order kinetics. After 30 min of washing, 60% of the initial concentration is still retained in the tissue, indicating a large non-specific binding to tissue constituents as suggested by Gillette for the liver subfractions (Gillette, 1966). The concentration of desipramine bound to the adrenergic terminals must be a very small portion of the total concentration since the destruction of the sympathetic innervation by 6-hydroxydopamine does not modify the desipramine accumulation in atria. Stabilization of the potentiating effect of desipramine on responses to (−)-noradrenaline is hard to obtain even after a long period of exposure to the drug. Concentrations of desipramine in the medium that cause potentiation of (−)-noradrenaline effects after short periods of exposure become less effective after long periods of exposure. This finding is strongly related to the continuous accumulation of desipramine. However, a significant relationship was obtained between the inhibition of (−)-[^{3}H]-noradrenaline uptake and the potentiation of the response to (−)-noradrenaline at desipramine levels ranging from 0·01 to 1 μg/g. The potentiation of the (−)-noradrenaline response was decreased by concentrations in the tissue ranging from 2–7 μg/g. The same concentrations gave similar effects in the vas deferens. At tissue levels of desipramine over 7 μg/g there was also an inhibition of the maximal response to (−)-noradrenaline.

Previous studies have shown that desipramine inhibits the responses to noradrenaline mediated by α-adrenoceptors (Scriabine, 1969; Türker & Khairallah, 1967) and probably some steps involving the excitation-contraction sequence

(Hrdina & Ling, 1970), while it does not affect responses mediated by β-adrenoceptors in the guinea-pig trachea (Foster, 1967) or in the isolated guinea-pig atria (Greeff & Wagner, 1969). In normal rat atria 0·1–1 μg/ml of desipramine caused a small increase in rate which is probably mediated by β-adrenoceptors since it is inhibited by propranolol. In atria taken from rats pretreated with 6-hydroxydopamine, desipramine induces a decrease in heart rate.

In the light of these observations the inhibition of the maximal response to (−)-noradrenaline is not likely to be due to blockade of β-receptors but could rather be caused by a quinidine-like effect already shown for other tricyclic antidepressant agents (Schmitt, Cheymol & Gilbert, 1970; Auclair, Gulda & Lechat, 1969). A quinidine-like effect is probably responsible for the dissociation of the inhibition of (−)-[^{3}H]-noradrenaline uptake and potentiation of the (−)-noradrenaline responses.

This study underlines the need to determine drug tissue concentrations when seeking for relationships among pharmacological and biochemical effects if the physico-chemical characteristics of the drug do not allow it to reach an equilibrium between bath and tissues.

Whether the relationships observed *in vitro* are also present *in vivo* remains to be established and further studies along this line are in progress.

It is hoped that this study may ultimately also have significance in interpreting the cardiac side effects observed during tricyclic antidepressant treatment in depressed patients (Edwards, 1964; Freyschuss, Sjöqvist, Tuck & Asberg, 1970; Coull, Crooks, Dingwall-Fordyce, Scott & Weir, 1970; Moir, Cornwell, Dingwall-Fordyce, Crooks, O'Malley, Turnbull & Weir, 1972).

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