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A COMPARISON IN RABBIT ISOLATED HEARTS OF THE DYSRHYTHMOGENIC POTENTIAL OF AMITRIPTYLINE, MAPROTILINE AND MIANSERIN IN RELATION TO THEIR ABILITY TO BLOCK NORADRENALINE UPTAKE

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1 In isolated hearts of rabbits, perfusion with (−)-noradrenaline (0.0059 to 5.9 μM) resulted in chronotropic and inotropic responses and a shortening of the interval between peak atrial and peak ventricular tensions (the A–V contraction interval). No dysrhythmias developed but at higher concentrations (590 μM) 2 out of 7 hearts developed dysrhythmias (extrasystoles).

2 Perfusion with the antidepressants amitriptyline or maprotiline (4.8 μM) or mianserin (28.8 μM) reduced ventricular force, did not change heart rate and only amitriptyline reduced atrial force and lengthened the A–V contraction interval. At 4.8 μM mianserin produced only a marginal shortening of the A–V contraction interval.

3 At these concentrations no dysrhythmias developed but at higher concentrations (amitriptyline 8 μM, maprotiline 8 μM, mianserin 60 μM) all the agents produced dysrhythmias involving an interference with atrio-ventricular synchronization.

4 In the presence of mianserin (4.8 μM) perfusion with noradrenaline (0.0059 to 5.9 μM) shortened the A–V contraction interval and did not produce dysrhythmias. In the presence of amitriptyline or maprotiline (4.8 μM) or mianserin (28.8 μM) the A–V contraction interval generally lengthened and most hearts developed dysrhythmias (usually involving interference with atrio-ventricular synchronization).

5 [^{3}H]-(−)-Noradrenaline uptake in perfused rabbit hearts and in mouse isolated atria or vasa deferentia was inhibited by the antidepressants to a similar extent, amitriptyline being marginally most potent (molar potency taken as 1.0), maprotiline being less potent (1.5) and mianserin least potent (2.0).

6 It is concluded that of these three antidepressants, mianserin is least cardiotoxic in this preparation and that the ability of these antidepressants to predispose to noradrenaline-induced dysrhythmias is not related to blockade of noradrenaline uptake.

Introduction

Administration of tricyclic antidepressants is known to be associated with the development of a variety of cardiac changes both in poisoning by these agents and after therapeutic use in man (Freeman, Mundy, Beattie & Ryan, 1969; Fouron & Chicoine, 1971; Williams & Sherter, 1971; Roberts, Mueller & Lauer, 1973). Concurrent use of sympathomimetic agents or procedures known to increase the levels of endogenous catecholamines appear to exacerbate the cardiotoxicity of the tricyclic antidepressants especially with regard to changes in the ECG. Thus, ECG changes have been observed in response to exercise in patients taking antidepressants (Kristiansen, 1961; Åsberg, Cronholm, Sjöqvist & Tuck, 1970) and infusions of sympathomimetics in healthy volunteers treated with imipramine tended to induce dysrhythmias (Boakes, Laurence, Teoh, Barar, Benedikter & Prichard, 1973). In animals, administration of tricyclic antidepressants in conjunction with noradrenaline has also been shown to produce dysrhythmias both *in vivo* (Elonen & Mattila, 1972; Elonen, Mattila & Saarnivaara, 1974) and *in vitro* (perfused rabbit heart) with either exogenous or endogenous noradrenaline (Barth & Muscholl, 1974; Barth, Manns & Muscholl, 1975). The mechanism by which these effects are produced is not clear but it has been suggested that the undoubted ability of antidepressants to block the uptake of noradrenaline will sensitize the heart to the

dysrhythmogenic action of this catecholamine (Byck, 1976).

It was originally suggested by Barth & Muscholl in 1974 that the isolated perfused heart of the rabbit stimulated through associated sympathetic nerves might prove a satisfactory model on which to assess the relative cardiotoxicity of antidepressants. A technically simpler modification of this preparation involving administration of exogenous noradrenaline through the perfusate was later used successfully by Barth *et al.* (1975) in the assessment of the cardiotoxicity of several antidepressants and related drugs. They concluded that among tricyclic drugs the ability to inhibit noradrenaline uptake was related to the incidence of dysrhythmias evoked by noradrenaline but that the two parameters were not causally related. We have used this preparation with several modifications to assess the relative cardiotoxicity of the antidepressants amitriptyline, maprotiline (Mâitre, Waldmeier, Greengrass, Jaekel, Sedlacek & Delini-Stula, 1975; Reiss, Dubey, Fünfgeld, Imhof, Hürzeler, Matussek, Rajagopalan, Raschdorf & Schmid, 1975) and the tetracyclic antidepressant, mianserin (Itil, Polvan & Hsu, 1972; Fell, Quantok & van der Burg, 1973) with special reference to the ability of these compounds to inhibit the uptake of noradrenaline and to predispose to noradrenaline-induced dysrhythmias.

Methods

Cardiotoxicity of the antidepressants

Rabbits of either sex (1.5 to 4.0 kg; New Zealand or Californian White strains) were given heparin injection B.P. (2000 iu) through the ear vein 5 min before they were killed by a blow on the head. The heart was removed and placed in ice-cold physiological saline of the following composition, mM: NaCl 122, KCl 3.8, $CaCl_2$ 1.25, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 5.5, L-ascorbic acid 0.085; and aerated with 5% CO_2 in O_2. The heart was trimmed and mounted for perfusion through the coronary arteries by the method of Langendorff using a constant flow of 25 ml/min (Watson Marlow Flow inducer) at a temperature of 35.5°C. Atrial and ventricular tensions were recorded isometrically (Dynamometer UF1) from threads sewn into the heart tissue according to the 'transverse' method (Fozard & Muscholl, 1971; Barth & Muscholl, 1974). Heart rate was derived from the ventricular tension record with a Devices Instantaneous Rate-meter and perfusion pressure was monitored constantly. Ventricular tension, atrial tension, heart rate and perfusion pressure were displayed on Devices recorders (M2) and a Lan Oscilloscope (Lan-scope 419A) provided a visual monitor of atrial and ventricular tensions. The last two parameters were also displayed on a Mingograf 34 ink writer set to record at a fast speed on which periodic records were obtained and the time interval between the peak atrial and peak ventricular tensions determined (the A–V contraction interval).

Diastolic atrial and ventricular tensions were adjusted to 1.0 and 6.0 g respectively, the preparations were allowed to equilibrate for 15 min and the values of the above parameters were noted. Perfusion was then continued with physiological saline containing the drug under test for the rest of the experiment and after 15 min the values of the above parameters were again noted. Atrial and ventricular tensions were expressed as a percentage of the initial control values while changes in heart rate and A–V contraction interval were expressed in absolute terms. The preparations were then perfused for 5 min successively with each of several concentrations of (−)-noradrenaline (usually 0.0059, 0.019, 0.059, 0.19, 0.59, 1.9 and 5.9 μM) and any changes in the above parameters were noted 4 min after the start of perfusion. The concentrations of noradrenaline in the perfusion when any dysrhythmia was established was also noted. The experiments with the various drugs were performed in random order and each heart was used for one experiment only.

Uptake of [^{3}H]-(−)-noradrenaline in perfused rabbit heart

Rabbit hearts were prepared as above, allowed to equilibrate for 15 min and were then perfused for a further 15 min with physiological saline containing the drug under test. [^{3}H]-(−)-noradrenaline was then added to the perfusion (0.059 μM; 3.33 nCi/ml) and the effluent from the heart was collected in successive 2 min periods for at least 20 minutes. A 1.0 ml sample of the effluent obtained at each collection period (and 3 × 1.0 ml samples of the un-perfused solution) was mixed with 10 ml of a dioxan-based scintillator (naphthalene 100 g, PPO 7 g, POPOP 0.5 g, methanol 50 ml, dioxan to 1000 ml) and counted for tritium in a Packard 3320 Liquid Scintillation Spectrometer. Correction for quench was made by an external standard channels ratio method. The amount of tritium in the effluent leaving the heart was expressed as a percentage of the amount entering the heart and hence the percentage removal of tritium from the perfusate was obtained and taken as an index of noradrenaline uptake.

Uptake of [^{3}H]-(−)-noradrenaline in mouse tissues

Mature male mice (Tuck No. 1) were killed by a blow on the head; the heart and vasa deferentia were removed and placed in cold physiological saline (composition as above but L-ascorbic acid increased to 1.16 mM). Adherent tissue was carefully dissected away and the atria were separated from the ventricles. The atria and vasa deferentia were incubated in physiological saline (containing drugs when

appropriate) for 15 min at 36°C. After this time the tissues were incubated in the presence of drug when appropriate for a further 15 min with [^{3}H]-(–)-noradrenaline (0.059 μM; 167 nCi/ml). The tissues were removed from the incubation solution, washed briefly in drug-free physiological saline at 4°C and then washed in 100 ml of physiological saline at 4°C for 15 min in the case of atria and 20 min in the case of vasa deferentia. The tissues were blotted dry on filter paper, weighed and combusted on tissue paper in a Packard Tri-Carb Oxidiser (305). The resulting solutions were counted for tritium as above and the tritium content of the tissue was expressed as ng noradrenaline base per g wet weight of tissue. The results were not corrected for recovery from the combustion process which was 90.8 ± 2.7% (mean ± s.e., $n = 10$) as determined by combustion of known amounts of [^{3}H]-(–)noradrenaline on tissue paper. In one group of tissues all the incubations were carried out at 4°C.

Drugs

Amitriptyline hydrochloride (Laroxyl, Roche), L-ascorbic acid (BDH), maprotiline hydrochloride (Ludiomil, Ciba), mianserin hydrochloride (Bolvidon, Organon), (–)-noradrenaline bitartrate (Koch-Light) and [^{3}H](–)-noradrenaline (Radiochemical Centre, Amersham) were used. Stock solutions of noradrenaline were made up freshly each day in a solution of NaCl (153 mM), HCl (10 mM) and L-ascorbic acid (0.11 mM) and stored at 4°C. Dilutions in physiological saline were prepared immediately before use, the L-ascrobic acid being added immediately before the noradrenaline.

Statistical procedures

Where appropriate all results are expressed as means ± standard error and in tests for statistical significance Student's t test was used. Regression analysis was carried out according to Snedecor & Cochran (1967).

Results

Effects of the antidepressants amitriptyline, maprotiline and mianserin alone

At the end of the initial equilibration period the values for the recorded parameters in a group of 6 control hearts were: (mean ± s.e.) ventricular force 9.8 ± 0.8 g, atrial force 0.78 ± 0.13 g, heart rate 125.0 ± 2.2 beats/min, perfusion pressure 31.8 ± 2.1 mmHg (4.23 ± 0.28 kPa) and A–V contraction interval (the interval between peak atrial and peak ventricular force) 168.6 ± 10.6 milliseconds. The groups of hearts later to be perfused with drug-containing solutions showed no statistically significant differences from the above values.

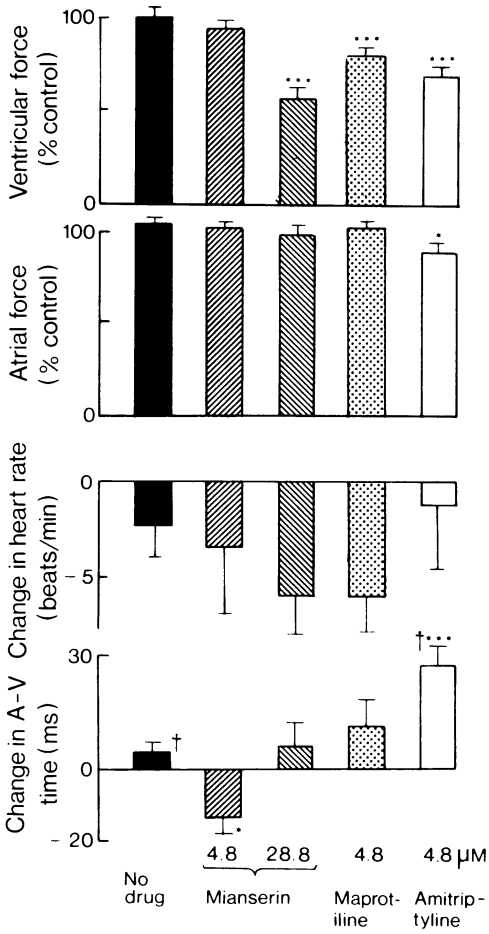

Figure 1 The changes produced in the performance of isolated perfused hearts of rabbits (6 per group) by 15 min perfusion with mianserin (4.8 and 28.8 μM), maprotiline (4.8 μM), amitriptyline (4.8 μM) or physiological saline alone (no drug). Atrial and ventricular force are expressed as a percentage of the initial values obtained immediately before the start of this perfusion. The changes in heart rate and in A–V contraction interval (the interval between peak atrial and peak ventricular tensions) are given in absolute terms. The error bars represent ± s.e. mean and the asterisks indicate statistically significant differences from perfusion with physiological saline alone (* $P < 0.05$ but > 0.02; *** $P < 0.01$). †, only 5 values contributed to this observation in this group.

After a further 15 min perfusion with physiological saline alone, control hearts showed no significant changes in these parameters (Figure 1). Perfusion with amitriptyline (4.8 μM) reduced ventricular force and atrial force and lengthened the A–V contraction

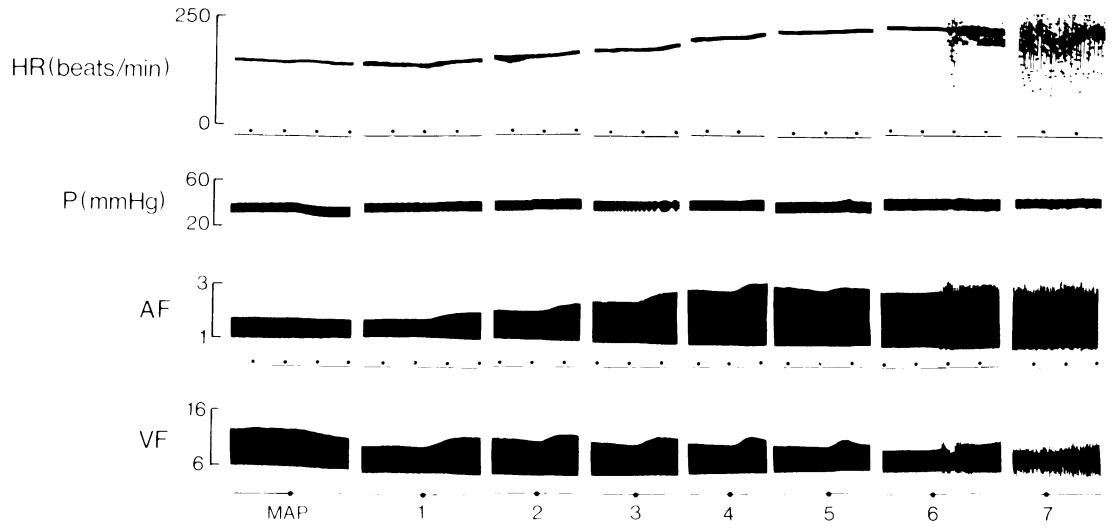

Figure 2 Records of heart rate (HR, beats/min), perfusion pressure (P, mmHg), atrial force (AF, g) and ventricular force (VF, g) from an isolated perfused heart of rabbit which was exposed to maprotiline (MAP, 4.0 μM) for 15 min and then perfused for 5 min successively with each of various concentrations of (—)-noradrenaline (1–7, 0.0059, 0.019, 0.059, 0.19, 0.59, 1.9 and 5.9 μM respectively). Note the initial chronotropic and inotropic responses to noradrenaline and the dysrhythmia developing after 30 s perfusion with noradrenaline at a concentration of 1.9 μM. Time marker 60 seconds.

interval but did not change heart rate. Maprotiline (4.8 μM) also reduced ventricular force but did not change the other parameters in a statistically significant manner. Mianserin (4.8 μM) had no significant effect on ventricular force, atrial force or heart rate but produced a small but statistically significant shortening of the A–V contraction interval. At a higher concentration (28.8 μM), ventricular force was reduced significantly and a marginal increase in the A–V contraction interval was observed though this latter effect was not statistically significant. All these changes are presented in histogram form in Figure 1.

In none of these experiments was cardiac dysrhythmia observed with the concentrations of the drugs under test. In single experiments however it was found that all three agents were capable of producing cardiac dysrhythmias. When hearts were exposed to step-wise increasing concentrations of the anti-depressants (2, 4, 8, 20, 40 and 60 μM) a progressive lengthening of the A–V contraction interval was noted which culminated in a dysrhythmia (amitriptyline 8 μM; maprotiline 8 μM; mianserin 60 μM).

Effects of noradrenaline in the presence and absence of the antidepressants

When perfused with noradrenaline at concentrations of up to 5.9 μM (the highest used routinely) none of the control hearts developed dysrhythmias but in the presence of the antidepressants, dysrhythmias often developed during perfusion with low concentrations of noradrenaline and, once developed, were maintained for the remainder of the perfusion period. A typical record from an experiment is shown in Figure 2. Note the initial inotropic and chronotropic responses to low concentrations of noradrenaline and the maintained dysrhythmias developing after 30 s perfusion with noradrenaline at a concentration of 1.9 μM. Six hearts were tested in this way at each of the concentrations of the drugs examined and Figure 3 presents the numbers of hearts which showed dysrhythmias at the various concentrations of noradrenaline used. Control hearts and those perfused with mianserin (4.8 μM) did not develop noradrenaline-induced dysrhythmias, neither did those perfused with low concentrations of amitriptyline (1.0 μM, 3 expts.; 2.0 μM, 1 expt.) but at 4.8 μM, 2 of the hearts developed a dysrhythmia at the lowest concentration of noradrenaline (0.0059 μM) and all 6 were eventually affected. Maprotiline appeared to be marginally less effective in predisposing to noradrenaline-induced dysrhythmias since only 1 of the 6 hearts became dysrhythmic with noradrenaline concentrations up to 0.059 μM but at higher noradrenaline concentrations all were eventually affected. With mianserin (28.8 μM) 4 of the hearts became dysrhythmic eventually but the remaining 2 did not develop a dysrhythmia even at the highest concentration of noradrenaline used routinely (5.9 μM).

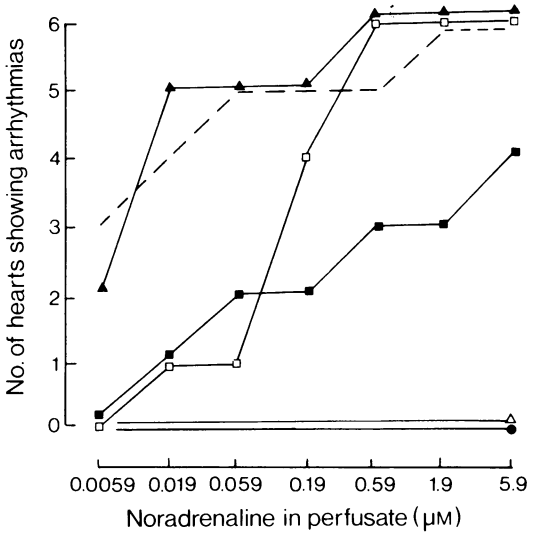

Figure 3 The number (out of 6) of rabbit isolated perfused hearts exhibiting dysrhythmias during perfusion with various concentrations of noradrenaline alone (control, ●) or in the presence of amitriptyline (▲, 4.8 μM), maprotiline (□, 4.8 μM) or mianserin (△, 4.8 μM; ■, 28.8 μM). For clarity some points have been marginally off-set from their correct values on the abscissa scale and all points have been omitted from the control and mianserin (4.8 μM) lines. The broken line represents the results obtained for amitriptyline (4.8 μM) by Barth *et al.* (1975).

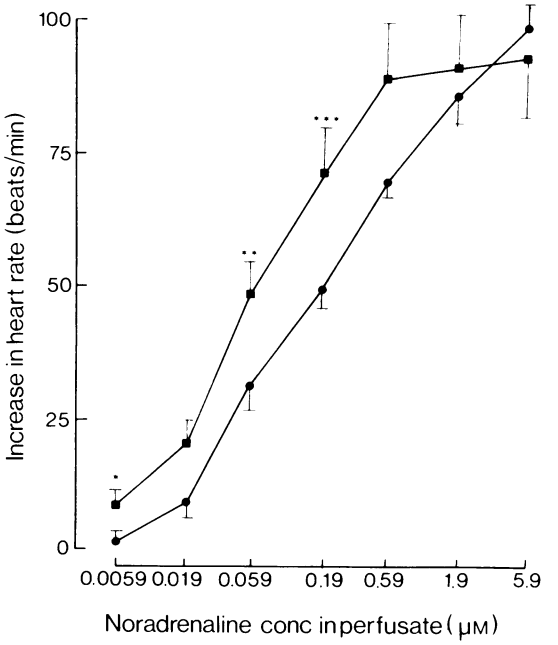

Figure 4 Increases in heart rate (beats/min) in rabbit isolated hearts in response to perfusion with various concentrations of (−)-noradrenaline alone (●, $n=6$) or in the presence of mianserin (■, 4.8 μM; $n=6$). The resting heart rates in the control group (125.0 ± 2.2 beats/min) and the group perfused with mianserin (121.3 ± 8.7 beats/min) were not significantly different ($P > 0.8$). Mean values are shown; bars indicate s.e. means. The asterisks indicate statistically significant differences from control responses at corresponding noradrenaline concentrations: * $P < 0.05$ but >0.02; ** $P < 0.02$ but >0.01; *** $P < 0.01$.

In addition to producing dysrhythmias, noradrenaline perfusions in the presence of the antidepressants also produced inotropic responses. These could not readily be quantitated since dysrhythmias occurred in many cases and because steady-state responses were rarely seen. The chronotropic response however usually did reach a steady-state and it can be seen that in the case of mianserin (4.8 μM; the only solution tested that did not provoke dysrhythmias in the presence of noradrenaline) there was a potentiation of the chronotropic response to noradrenaline in comparison with control tissues (Figure 4).

Noradrenaline perfusions also affected the A–V contraction interval and the values obtained for this parameter in each heart during perfusion with the various concentrations of noradrenaline are shown in Figure 5. No attempt has been made to calculate means but it is clear that in control hearts noradrenaline produced a shortening of the A–V contraction interval.

In contrast to this effect, in the presence of amitriptyline (4.8 μM) or maprotiline (4.8 μM), a lengthening of the A–V contraction interval was observed. In the presence of mianserin (4.8 μM) the normal action of noradrenaline (to shorten the A–V contraction interval) was still seen, although possibly to a lesser extent, but at 28.8 μM (as with amitriptyline and maprotiline at 4.8 μM) a progressive lengthening of the A–V contraction interval was usually seen, terminating in a dysrhythmia. In 2 hearts the A–V contraction interval was not markedly lengthened and neither of these hearts developed dysrhythmias.

The dysrhythmias seen in response to noradrenaline in the presence of the antidepressants were somewhat variable in nature but usually involved an apparent interference with atrio-ventricular conduction; missed ventricular beats were common as was atrio-ventricular dissociation. In the absence of the antidepressants even at extremely high noradrenaline concentrations (590 μM) only 2 out of 7 hearts showed dysrhythmias. In both cases the dysrhythmias involved extrasystoles and in neither case was any lengthening of the A–V contraction interval seen.

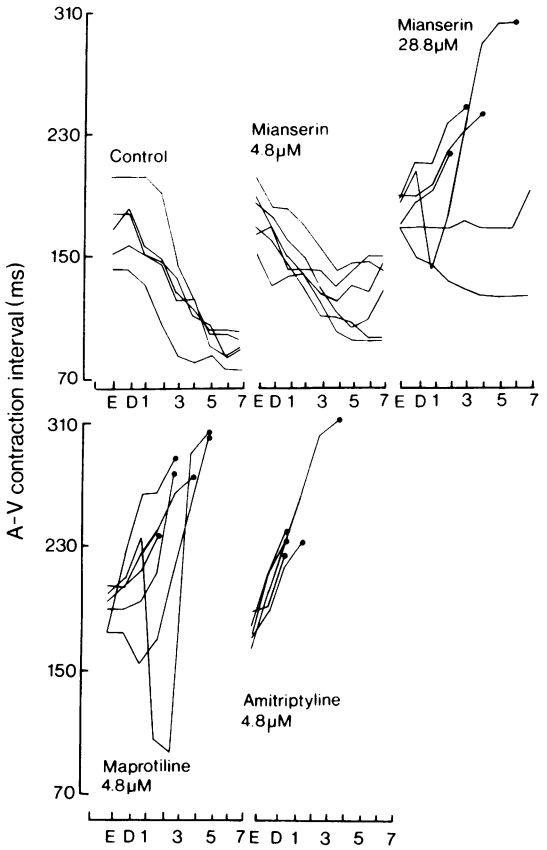

Figure 5 Effect of noradrenaline on the A–V contraction interval (interval between peak atrial and peak ventricular tensions; ms) in the presence and absence of the antidepressants. Each line represents an isolated perfused heart of a rabbit and measurements were taken: E, at the end of an equilibration period; D, after 15 min perfusion with a drug when appropriate; 1–7, during perfusion with (−)-noradrenaline (0.0059, 0.019, 0.059, 0.19, 0.59, 1.9 and 5.9 μM respectively). Lines ending in closed circles indicate that a dysrhythmia developed before the next measurement of the A–V contraction interval could be made.

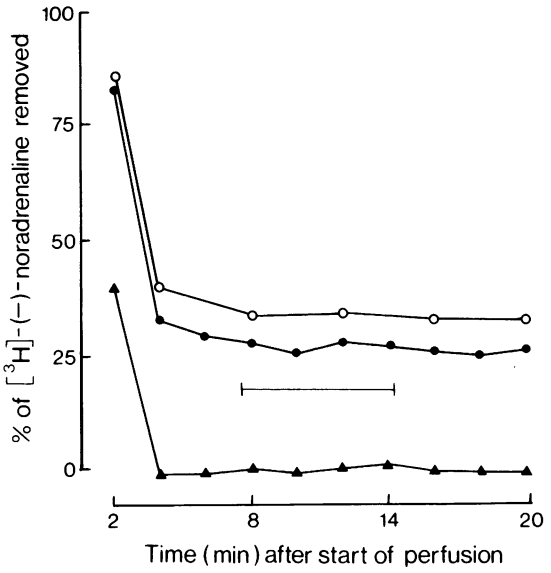

Figure 6 The percentage of [^{3}H]-(−)-noradrenaline removed from the perfusate by a single passage through an isolated perfused heart of rabbit. Assessment of the noradrenaline content of the effluent was made either fluorimetrically (open symbols) or isotopically (closed symbols) in control hearts (○●) or in the presence of mianserin (▲, 4.8 μM). The ordinate scale shows the time (min) from the start of the perfusion with [^{3}H]-(−)-noradrenaline. To calculate the percentage removal by each heart (see Figure 7) the average was taken of the points included by the solid bar.

Effects of the antidepressants on [^{3}H]-(−)-noradrenaline uptake

Measurement of the ^{3}H-content of the effluent from hearts perfused with [^{3}H]-(−)-noradrenaline (0.059 μM) showed that the amounts of tritium apparently 'removed' from the perfusate were very high initially as the H^3-content of the perfusate was diluted by mixing with the physiological saline already in the 'dead space' of the apparatus (28 ml). However, in both control and treated hearts a plateau was quickly reached which usually remained constant for the remainder of the experiment (20 minutes). In a single control experiment the amount of noradrenaline in the effluent from the heart was also measured fluorimetrically by a modified trihydroxyindole method applied directly to the perfusate. There was general agreement between the fluorimetric and isotopic methods though the fluorimetric method consistently indicated a greater removal of noradrenaline by the heart than did the isotopic method (Figure 6).

The percentage removal of noradrenaline for each control or treated heart was calculated as the average removal over 4 samples (each involving a 2 min collection period) obtained between 6 and 14 min after starting the perfusion with [^{3}H]-(−)-noradrenaline. The percentage removed by control hearts ($28.4 \pm 0.7\%$; $n=7$) was taken as 100% uptake and the amounts removed by treated hearts were expressed as a percentage of this uptake.

In single experiments (at 4.8 μM; the concentration used in investigation of the dysrhythmogenic potential of the agents) all three antidepressants effectively eliminated uptake of [^{3}H]-(−)-noradrenaline by the

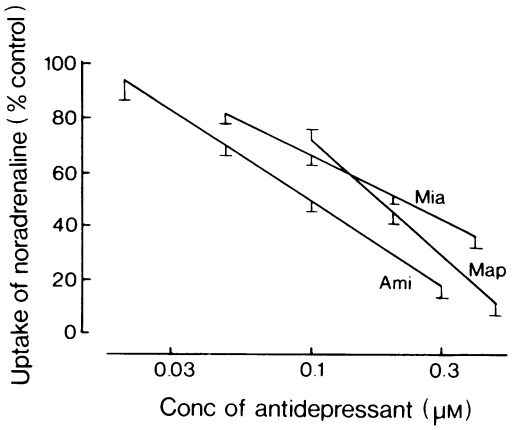

Figure 7 The relationship in rabbit isolated hearts between the concentration of antidepressant in the perfusate and the uptake of [^{3}H]-(−)-noradrenaline (expressed as a percentage of the control uptake which was 28.4 ± 0.7% (n=7) of the noradrenaline (0.059 μM) delivered to the heart). The lines shown (mianserin (Mia), n=11; maprotiline (Map), n=8; amitriptyline (Ami), n=9) are regression lines calculated from results obtained in several experiments at various concentrations. The error bars show the standard error of regression calculated at the concentrations at which the experiments were performed.

rabbit heart and the relationship between the concentration of antidepressant in the perfusate and the uptake of noradrenaline (as a percentage of control) is shown in Figure 7. It may appear that the lines are not parallel but the errors are such that neither the slope of the maprotiline line (−72.3 ± 8.0, n=8) nor the slope of the mianserin line (−45.1 ± 5.1, n=11) differ significantly (P>0.05), from the slope of the line determined for amitriptyline (−64.1 ± 8.8, n=9). Calculation from the regression equations of the concentration of the antidepressants required to produce 50% blockade of the uptake of noradrenaline yields the values shown in Table 1. Isolated atria and vasa deferentia of mice incubated with [^{3}H]-(−)-noradrenaline in the absence of other drugs retained some 57.4 ± 4.3 (n=8) and 45.2 ± 1.7 (n=20) ng noradrenaline base per g wet weight of tissue respectively. In the presence of cocaine (30 μM) the amount of noradrenaline retained was reduced to 15% of control in both cases while in tissues incubated at 4°C only 3% and 5% of normal control uptake occurred in atria and vasa deferentia respectively. The relationship between the uptake of noradrenaline and the concentration of antidepressant in the incubation fluid is shown in Figure 8 and calculation of the concentration of antidepressant required to produce 50% blockade of the uptake of noradrenaline yields the values shown in Table 1.

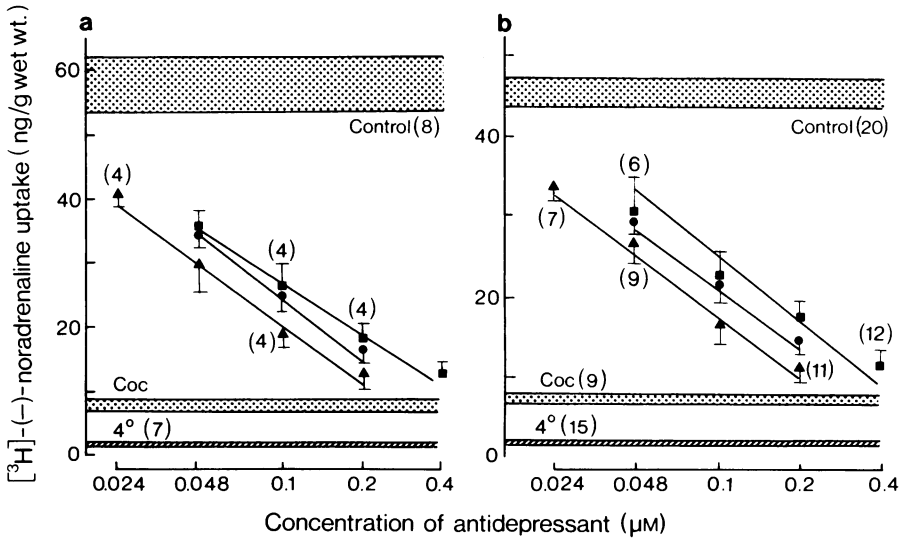

Figure 8 The amounts of [^{3}H]-(−)-noradrenaline (ng per g wet weight of tissue) taken up by (a) isolated atria and (b) vasa deferentia of mice after incubation for 15 min with [^{3}H]-(−)-noradrenaline in the presence of various concentrations of mianserin (■), maprotiline (●) or amitriptyline (▲). Points are mean values; bars show s.e. means. The lines are calculated regression lines based on the individual results from which the means were derived. Also shown is the uptake in the absence of drugs (control), in tissues incubated in the presence of cocaine (Coc, 30 μM) or at 4°C. In each case the upper and lower limits of the shaded areas show the mean plus and minus one s.e. respectively. Five tissues contributed to each group in the case of the atria (10 in the case of vas deferens) except where indicated (figures in parentheses).

Discussion

The use of the isolated perfused heart of the rabbit in investigations of antidepressant cardiotoxicity was established by Barth *et al.* (1975) who investigated the effects of a number of antidepressants in this preparation and the results described here for amitriptyline are in agreement with those obtained by these previous workers. We also found that at 4.8 μM, amitriptyline reduced the force of atrial and ventricular systole but had no effect on heart rate and that perfusion with low concentrations of noradrenaline in the presence of amitriptyline gave rise to dysrhythmias.

At equimolar concentrations (4.8 μM) amitriptyline reduced the force of ventricular systole to a greater extent than did maprotiline while mianserin produced no significant change. At 28.8 μM the effects of mianserin on ventricular force were slightly greater than those of amitriptyline and it would appear therefore that mianserin is less potent than either amitriptyline or maprotiline in this respect.

The relative ability of the three antidepressants to predispose to noradrenaline-induced dysrhythmias is more difficult to quantitate exactly because of the nature of the experimental design. At equimolar concentrations (4.8 μM) amitriptyline appeared more likely to precipitate dysrhythmias at low concentrations of noradrenaline than did maprotiline and no dysrhythmias developed with mianserin. Even when the concentration of mianserin was increased 6-fold only 4 out of the 6 hearts developed dysrhythmias when perfused with noradrenaline. This is not because mianserin prevents the actions of noradrenaline on the heart as inotropic responses were still seen and the chronotropic response was significantly potentiated compared to control tissues. This latter observation suggests the possibility that mianserin, in common with other antidepressants, can interfere with the uptake of noradrenaline in the periphery as it does in rat brain synaptosomes (Raiteri, Angelini & Bertollini, 1976).

This possibility was confirmed in the experiments investigating the uptake of [^{3}H]-(−)-noradrenaline in various tissues. Throughout this work it has been assumed that the ^{3}H-content of various solutions and tissues can be taken as an index of their noradrenaline content or uptake respectively. Although the percentage of noradrenaline removed from the perfusate was consistently higher when assessed fluorimetrically than when assessed by the isotopic method, correspondence was generally good. A higher result might in any case be expected since any noradrenaline metabolized will show as a loss of noradrenaline (i.e. uptake) in the fluorimetric method while this is not so in the isotopic method. With regard to uptake of noradrenaline in mouse tissues we have no evidence to suggest that the tritium in the tissue is present as noradrenaline. However, given the selectivity of the uptake process and the fact that [^{3}H]-(−)-noradrenaline is chemically stable in the incubation solution the ^{3}H-content of the tissue must have derived from uptake of noradrenaline and the form in which it exists in the tissue after uptake is irrelevant in this context.

In perfused rabbit hearts the concentration of amitriptyline required to produce 50% inhibition of [^{3}H]-(−)-noradrenaline uptake (100 nM) agrees well with the value found by Barth and his co-workers (128 nM), who used a fluorimetric assay. The uptake blocking potencies of the compounds in the three preparations show reasonable agreement; in each case amitriptyline is the most potent, maprotiline marginally less so and mianserin approximately 2-fold less potent than amitriptyline. At 4.8 μM all the compounds produced complete blockade of the uptake of [^{3}H]-(−)-noradrenaline in perfused hearts although their relative liability to predispose to noradrenaline-induced arrhythmias was very different. This would suggest that predisposition to noradrenaline-induced dysrhythmias is not directly associated with an ability to block uptake of noradrenaline. This suggestion is supported by several other aspects of the results described here.

Firstly, noradrenaline by itself, even in concentrations some 10,000-fold greater than those needed to produce dysrhythmias in the presence of amitriptyline or maprotiline, only produced dysrhythmias in 2 out of 7 hearts. Complete blockade of noradrenaline uptake by minaserin produced only a 4-fold potentiation in the chronotropic response to noradrenaline and in other tissues complete blockade

Table 1 Concentrations (nM) of amitriptyline, maprotiline and mianserin required to produce 50% inhibition of the uptake of [^{3}H]-(−)-noradrenaline in isolated organs of rabbit and mouse

Drug	*Rabbit heart*	*Mouse atria*	*Mouse vas deferens*
Amitriptyline	100	53.6	64.9
Maprotiline	152 (1.52)	75.5 (1.41)	89.5 (1.38)
Mianserin	201 (2.01)	88.9 (1.66)	131.0 (2.02)

The figures in parentheses show the equi-active molar potency ratios relative to amitriptyline.

of uptake rarely produces more than a 30 to 50-fold potentiation in the response (Trendelenberg, 1972). If the predisposition to noradrenaline-induced dysrhythmias were solely due to blockade of noradrenaline uptake it would be expected that a 50 to 100-fold increase in the concentration of noradrenaline perfused through the heart would, by itself, produce dysrhythmias in all hearts but no such effect was seen.

Secondly, in the two hearts which did become dysrhythmic in the presence of high concentrations of noradrenaline, the dysrhythmias involved the production of extrasystoles and there was no evidence of any interference with atrio-ventricular synchronization or lengthening of the A–V contraction interval. In contrast, in hearts exposed to noradrenaline as well as an antidepressant, extrasystoles were rarely seen. Usually the dysrhythmias involved an interference with atrio-ventricular conduction. It appears therefore that the type of dysrhythmia induced by noradrenaline alone is different from that seen with noradrenaline in the presence of an antidepressant, a feature that would not be expected if the dysrhythmia were simply due to potentiation of the normal effects of noradrenaline. In this context it is interesting to note that the dysrhythmias induced by antidepressants in conscious rabbits were also characterized by atrio-ventricular conduction defects (Elonen *et al.*, 1974) and it may be conceptually more useful to think of noradrenaline predisposing to an antidepressant type of dysrhythmia rather than antidepressants predisposing to noradrenaline dysrhythmias.

The third factor which suggests that blockade of noradrenaline uptake is not solely responsible for the precipitation of dysrhythmias comes from a consideration of the effects on A–V contraction interval. The use of this measurement is fraught with difficulties since it represents the time interval between two mechanical events which may, through alterations in rise time for example, bring about a change in the measured A–V contraction interval which bears little relationship to changes in electrical conduction that may have taken place. Nevertheless noradrenaline shortened the A–V contraction interval in normal hearts and is known to speed atrio-ventricular conduction. A–V contraction interval was increased by amitriptyline alone and this agent is known to increase the P–Q interval in the ECG of the conscious rabbit (Elonen *et al.*, 1974).

Noradrenaline shortened the A–V contraction interval in normal hearts and also in the presence of mianserin (4.8 μM) though the effect was possibly less marked. In contrast, with both maprotiline and amitriptyline a progressive lengthening of the A–V contraction interval was seen as more concentrated solutions of noradrenaline were applied. Indeed, so long did the A–V contraction interval become in some hearts that the atrial contraction immediately preceding a ventricular contraction did not in fact trigger that contraction, but the next ventricular event. Thus a very long A–V contraction interval could easily be mistaken for a very short A–V contraction interval unless the changes are constantly monitored. In the presence of mianserin (28.8 μM) 4 hearts showed a lengthening of the A–V contraction interval and developed dysrhythmias while the remaining 2 did not develop dysrhythmias or show any marked lengthening of the A–V contraction interval.

It seems likely therefore that the dysrhythmogenic action of noradrenaline in the presence of the antidepressants cannot be due simply to a blockade of noradrenaline uptake. The primary cause in this preparation would appear to be the ability of these compounds to interfere with the normal effects of noradrenaline on the A–V contraction interval which may be related to the intrinsic ability of the molecular structures involved to produce the effect. The presence of an additional ability to block the uptake of noradrenaline would exacerbate this action as the effective concentration of noradrenaline would be raised but this is not the primary cause of the ability of these compounds to predispose to noradrenaline-induced dysrhythmias.

Any extrapolation of these results to the clinical situation in man must be done with extreme caution. Quite apart from any species variation and the fact that the experiments described above do not involve chronic administration, the effects of vagal and other influences on the heart *in vivo* may grossly modify the effects observed. Furthermore, plasma levels of amitriptyline may approach 300 ng/ml in clinical use (Braithwaite & Widdop, 1971; Jørgensen, 1975) and may be much higher in poisoning but allowing for 95% plasma binding (Borgå, Azarnoff, Forshell & Sjövist, 1969) this represents a free drug concentration of 50 nM, some 100-fold below the concentration used to predispose to dysrhythmias in these experiments.

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