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THE EFFECTS OF AMIODARONE,* AN α AND β RECEPTOR ANTAGONIST, ON ADRENERGIC TRANSMISSION IN THE CAT SPLEEN

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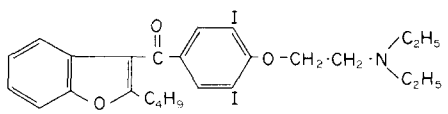
Abstract— The anti-anginal drug amiodarone produced a dose dependent reduction in the overflow of transmitter from the isolated blood perfused cat spleen following nerve stimulation at 30 Hz.

In the presence of phenoxybenzamine (30 μg/ml) the normal increase in overflow of transmitter, following 200 stimuli at 10 Hz was prevented. This effect occurred whatever the order of addition of phenoxybenzamine and amiodarone, indicating that amiodarone did not reduce the overflow by stimulation of inhibitory presynaptic α receptors.

In experiments in which the transmitter stores were labelled with [^{3}H](−)-noradrenaline, amiodarone inhibited the release of label following nerve stimulation but had no effect on release induced by tyramine. Responses of the spleen to both nerve stimulation and tyramine were reduced by amiodarone but uptake of [^{3}H](−)-noradrenaline given as injections (pulses) or as infusions, was not significantly affected. The effects of amiodarone on nerve evoked overflow of transmitter are not therefore related to changes in uptake of noradrenaline or to selective stimulation of presynaptic α receptors but probably reflect a neurone blocking action of the drug.

Amiodarone (2-butyl-3-(4-diethylamino-ethoxy-3, 5-diiodobenzoyl)-benzofuran hydrochloride) (see Fig. 1) has been reported to have beneficial actions in patients with angina pectoris [1]. It has been shown to decrease heart rate, vascular resistance and myocardial oxygen consumption and to increase myocardial blood flow [2]. Both α and β effects of catecholamines are antagonised in a non-competitive manner [3]. Amiodarone has no demonstrable effect on the catecholamine content of both heart and adrenals [4]. The antiarrythmic properties of the drug protect anaesthetised guinea pigs against ouabain induced ventricular fibrillation [5]. In isolated rabbit atria or ventricular muscle strips the drug prolongs the duration of the action potential but has no effect on the resting potential or action potential height [5]. The present study was undertaken to determine whether a drug known to antagonise the actions of catecholamines non-competitively would elevate the overflow of transmitter from the cat spleen following nerve stimulation in the same way as competitive α-receptor blockers such as hydergine [6] and phentolamine [13].



Amiodarone

Fig. 1.

METHODS

Overflow of endogenous transmitter. Cat spleens were perfused with blood *in vitro* [7, 9]. The splenic nerves were stimulated supramaximally with shielded bipolar platinum electrodes. In order to stabilise the transmitter overflow, 3 trains of 200 impulses at 10 Hz followed by 2 trains of 200 impulses at 30 Hz were given at 10 min intervals. Subsequent overflows of transmitter following 200 impulses at 30 Hz remained at a steady level [7]. Blood containing overflowing transmitter was collected in chilled tubes for 30 sec from the start of nerve stimulation.

Bioassay of transmitter. Blood samples containing transmitter were spun at 3100 *g* for 15 min at 0°. Plasma was removed from the buffy coat and packed red cells. The plasma samples were assayed against noradrenaline for pressor activity on the blood pressure of the pithed rat [8]. Amiodarone is rapidly taken up by the spleen and does not interfere with the assay.

Uptake of radioactively labelled noradrenaline. Uptake from 1 μg pulses of [7-^{3}H](−)-noradrenaline was measured as previously described [9]. Venous blood containing [^{3}H](−)-noradrenaline that had not been taken up and [^{3}H] metabolites was collected in chilled tubes during the 3 min following the injection. Uptake of [7-^{3}H](−)-noradrenaline was also measured from infusions [10]. In all these experiments uptake was taken as the difference between the amount of label given and the amount recovered in the venous blood. Evans Blue was added to the noradrenaline to act as an intravascular marker for the determination of the overall recovery of the injectate or infusate [10].

Labelling of transmitter stores. In order to label the transmitter stores [^{3}H](−)-noradrenaline (10·1 Ci/m-mole) was infused close arterially into the blood perfused spleen for 10 min at a rate of 45 ng/min. The spleen was then perfused with Krebs–Henseleit solution for 30 min during which two periods of stimu-

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lation of 200 impulses at 10 Hz were given. The total venous effluent during the infusion of label and for 30 sec afterwards was collected and counted to determine the amount of radioactivity retained by the spleen. The Krebs–Henseleit solution was replaced with fresh blood and perfusion continued for a further 30 min during which one train of stimuli was given (200 impulses at 10 Hz).

Release of label by nerve stimulation and tyramine. Initially two trains of stimuli of 200 impulses at 30 Hz were given at 10 min intervals followed by alternate periods of stimulation and the administration of tyramine (5 μg/g) close arterially to the spleen. Blood was collected for 1 min after stimulation and for 4 min following the administration of tyramine. Each of these collection periods was at least twice as long as the responses produced by the procedures. After centrifugation of the blood, plasma samples were assayed biologically for transmitter and counted for tritium.

Sample counting. 10 ml of scintillant comprising 5% Scintol 2 (Koch-Light Laboratories Ltd.), 33·3% Triton X 100 (Scintillation Grade) and 61·7% toluene were added to 0·1 ml of plasma and 1·0 ml distilled water. Samples were counted for ^3H on a Packard Tri-Carb Scintillation Counter. Blank and quench corrections were applied.

Amiodarone determination. Since amiodarone contains 37% by wt of iodine, the difference between the iodine content of control untreated spleen and spleens exposed to the drug was taken as an index of amiodarone content. Small pieces of spleen were freeze dried and assayed for iodine content by neutron activation analysis.

Drugs. [7-^3H](−)-Noradrenaline (10·1 Ci m-mole), Radiochemical Centre, Amersham; amiodarone hydrochloride, Labaz Laboratories; tyramin hydrochloride, Sigma; phenoxybenzamine hydrochloride, Smith, Kline & French Laboratories Ltd; Heparin (mucus), Boots Pure Drug Co.; Prostaglandin E_1, Dr. John E. Pike, Upjohn, Kalamazoo. Amiodarone and phenoxybenzamine were dissolved in a few drops of ethyl alcohol, diluted with about 10 ml Krebs solution and added to the blood as an opalescent solution. All doses of the amines are expressed as base.

Unless otherwise defined results are presented as means ± S.E.M.

RESULTS

(a) *Fate of amiodarone added to blood perfusing the spleen.* Almost all the amoidarone added to the perfusing blood was bound to the spleen. This can be seen in Fig. 2 which shows the amount of amiodarone (as iodine) recovered from the spleen at the end of the experiment plotted against the dose administered. If all the amiodarone is bound the points should fall along the line shown which has a gradient of 1·0. Since there was good agreement between the points and the line it can be assumed that almost all the amiodarone is bound within the spleen and for this reason all doses of amiodarone are expressed as mg/g (wet wt spleen).

(b) *Effect of amiodarone on overflow of transmitter following nerve stimulation.* Following the conditioning series of stimuli the overflow of transmitter collected in each of the four control periods after 200 impulses at 30 Hz remained essentially constant. Addition of amiodarone to the blood in the reservoir produced a dose-dependent inhibition of overflow of transmitter which reached a maximum 30–40 min after the addition of the drug (Fig. 3).

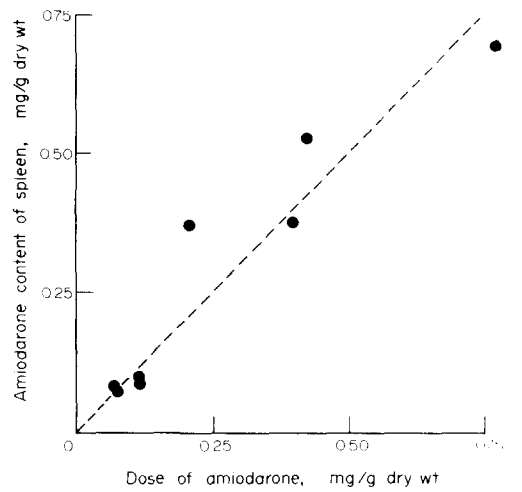

Fig. 2. The relationship between the dose of amiodarone administered to the spleen in the blood and the amount found in the spleen at the end of the experiment.

When the overflow of transmitter at the fourth stimulus period after the drug was compared to the overflow following the last stimulus before the drug (●) and plotted against the log concentration of drug measured as mg/g (wet wt of the spleen) and the points tested by linear regression analysis, it was found that the points fell along a straight line ($P < 0{\cdot}001$) (Fig. 4). The dose of drug required to produce 50 per cent inhibition of the overflow of transmitter (ED_{50}) was 0·16 mg/g (wet wt spleen) (95% C.L. 0·10–0·24 mg/g).

(c) *Effect of phenoxybenzamine on overflow of transmitter in the presence of amiodarone.* Forty min after amiodarone, phenoxybenzamine (30 μg ml) was

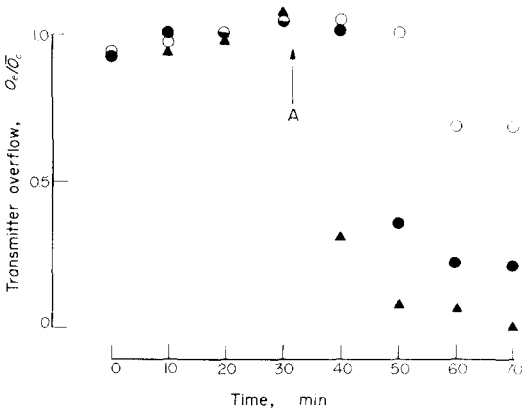

Fig. 3. The effect of amiodarone (A): 71 μg/g (wet wt spleen) (○); 270 μg/g (●); and 857 μg/g (▲), on transmitter overflow from the cat spleen in three typical experiments. Splenic nerves were stimulated at 10-min intervals with trains of 200 supramaximal stimuli at 30 Hz. Transmitter overflow is expressed as a fraction of the mean overflow of the pre-drug stimulations in each experiments.

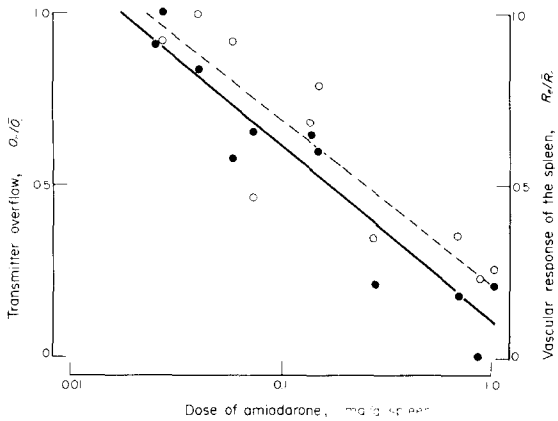

Fig. 4. Effect of amiodarone on overflow of transmitter (●) and vascular response (○) following 200 stimuli at 30 Hz. Overflow (O_c) and response (R_c) are expressed in terms of the mean pre-drug values ($\bar{O}_c$ and $\bar{R}_c$).

added to the blood and allowed to act for 30 min without nerve stimulation. The overflow of transmitter following 200 stimuli at 10 Hz was then collected for two successive periods of 40 sec. It was found that amiodarone was able to prevent the increase of transmitter normally produced by phenoxybenzamine [11] and that the effect was dose dependent. The log dose/inhibition line is plotted in Fig. 5. (In the absence of other treatment the overflow of transmitter from 200 stimuli at 10 Hz in the presence of phenoxybenzamine is 3·15 times that with stimuli at 30 Hz in the absence of drugs). The dose of amiodarone required to reduce the overflow following phenoxybenzamine to 50 per cent of the normal value was 0·30 mg/g (95% C.L. 0·13–0·68 mg/g).

(d) *Receptor protection experiments.* The results of the previous section illustrate that amiodarone can prevent the increase in transmitter overflow produced by phenoxybenzamine. Since it is possible that amiodarone may produce its effect by selective stimulation of presynaptic α-receptors, experiments were performed in which the order of addition of phenoxybenzamine and amiodarone was altered. In each case the drug was allowed 30 min to act before the addition of the second drug. Since phenoxybenzamine produces an irreversible blockade of α-receptors, addition before amiodarone should prevent amiodarone's action if it acts through this mechanism. However, as shown in Fig. 6, the overflow of transmitter following stimulation of the nerves at 10 Hz was significantly reduced by amiodarone (0·93 mg/g) compared with the overflow with phenoxybenzamine alone, whether amiodarone was given before or after phenoxybenzamine.

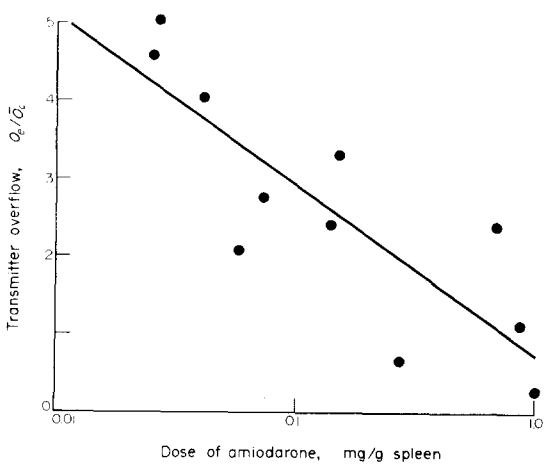

Fig. 5. Effect of amiodarone on overflow of transmitter in the presence of phenoxybenzamine (30 μg/ml) following 200 stimuli at 10 Hz. Transmitter overflow (O_c) is expressed in terms of the mean pre-drug overflow from 200 stimuli at 30 Hz ($\bar{O}_c$).

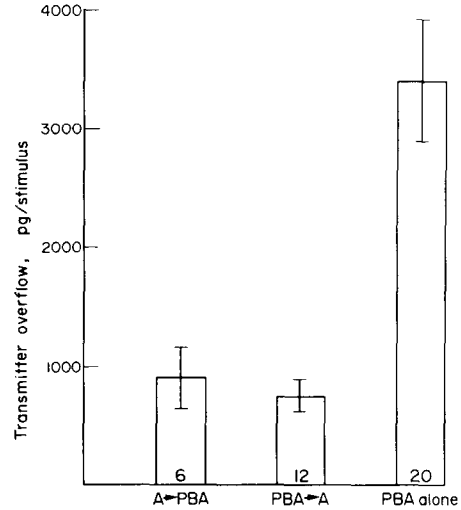

Fig. 6. The effect of amiodarone (A) (1 mg/g spleen) and phenoxybenzamine (PBA) (25 μg/ml) upon transmitter overflow from the spleen following 200 stimuli at 10 Hz. (A → PBA) A followed by PBA, n = 6. (PBA → A) PBA followed by A, n = 12, and PBA alone, n = 20.

(e) *Effect of amiodarone on overflow of transmitter and tritium following nerve stimulation and tyramine.* Amiodarone (1·33 mg/g) significantly reduced the overflow of both endogenous transmitter and tritium following stimulation of the nerves with 200 impulses at 30 Hz ($P < 0.001$; $n = 9$) but had no significant effect on the overflow produced by tyramine (5 μg/g) injected close arterially ($P > 0.6$; $n = 8$) as shown in Fig. 7a. The administration of the amiodarone solvent (ethanol in saline) (Fig. 7b) had no significant effect on the overflow of transmitter ($P > 0.8$; $n = 3$) or tritium ($P > 0.2$; $n = 3$) following nerve stimulation. In the control experiments tyramine evoked release was also unchanged.

(f) *Responses of the spleen to nerve stimulation and tyramine.* The spleen responded to stimulation at 30 Hz with increases in perfusion pressure (vascular responses) and reductions in spleen volume (capsular responses). Amiodarone produced inhibition of the vascular responses of the spleen which were dose-dependent but the responses were never abolished even with the highest doses used in these experiments (Fig. 4); this confirms what has been observed with other vascular beds [2]. The graph of splenic vascular response expressed as a percentage of the control responses against log dose of drug (mg/g wet wt spleen) shows a linear relationship ($P < 0.001$) and the ED_{50} is 0·24 mg/g (95% C.L. 0·13–0·66 mg/g). The effects of amiodarone on the capsular responses of the spleen

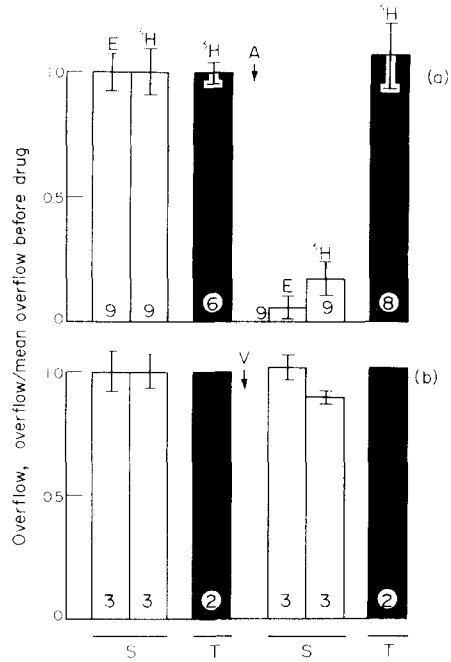

Fig. 7. (a) Effect of amiodarone (A) 1·3 mg/g spleen and (b) effect of vehicle (V) on release of endogenous transmitter (E) and ^3H (taken up previously as [^3H](−)-noradrenaline) by nerve stimulation (S) and tyramine (T). The numbers in the columns are the number of observations.

to nerve stimulation were variable. With high doses of amiodarone (0·6–1 mg/g) the response was inhibited whereas with low doses no consistent effect was observed. The reduction in response is due to the combined effect of a reduction in transmitter release and a post-synaptic α-blocking action. Responses of the spleen to tyramine were also inhibited but not to the same extent as those to nerve stimulation. This inhibition can be explained in terms of post-synaptic α-blockade alone.

(g) *Effect of amiodarone on uptake of* [^3H](−)-*noradrenaline.* (i) Uptake from pulses of [^3H](−)-noradrenaline. In the blood perfused cat spleen it has been shown that the characteristics of uptake of [^3H](−)-noradrenaline given as an infusion varies from that of [^3H](−)-noradrenaline given as pulses [10]. 1 μg pulses of [^3H](−)-noradrenaline were given over a period of 6 sec to the spleen before and 40 min after addition of amiodarone (1 mg/g). Uptake (corrected for Evans Blue recovery) from the first pulse of [^3H](−)-noradrenaline was 366 ± 10 ng and from the second, 40 min after amiodarone (1 mg/g), 455 ± 36 ng ($n = 3$; $P > 0{\cdot}1$). In control experiments uptake from the first pulse was 400 ± 32 ng and from the second, 40 min after the addition of amiodarone vehicle, 363 ± 24 ng ($n = 4$; $P > 0{\cdot}3$). Amiodarone, therefore, had no significant effect on uptake of [^3H](−)-noradrenaline from pulses. Overall recovery of the Evans Blue intravascular marker in these experiments was 96·2 ± 2·3 per cent. (ii) Uptake from infusions. On infusion of [^3H](−)-noradrenaline into the isolated blood perfused cat spleen a steady-state condition is reached within 2–3 min of the start of the infusion [10]. In the absence of drugs the spleen is able to take up about half the [^3H](−)-noradrenaline presented to it at a rate of 345 ng/min (spleens were perfused at a mean rate of 8·0 ± 0·4 ml/min). The remaining label overflows into the venous circulation to be collected and counted. As shown in Fig. 8 the pattern of overflow of label in the spleens treated for 40 min with amiodarone (▲ 1 mg/g) was not significantly different from that observed in spleens treated with the amiodarone vehicle (△). The overflow of the intravascular marker Evans Blue in the plasma during these experiments (●) rose to about 93% of the amount given, the losses being due to small leaks and trapping of Evans Blue in the extracellular space of the loosely packed red cells (3·5%). Amiodarone, therefore, has no demonstrable effect on uptake of noradrenaline from infusions.

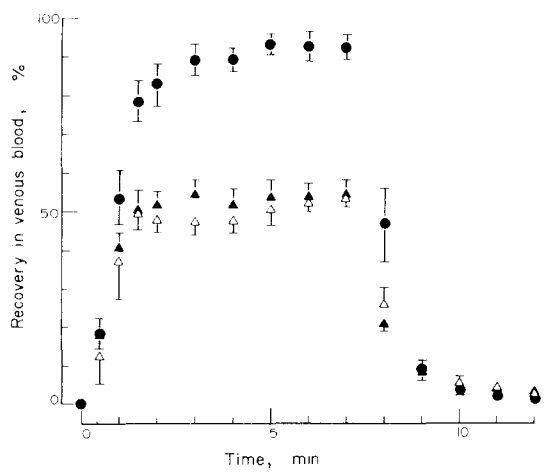

Fig. 8. Recovery of ^3H in splenic venous blood following 7 min infusion of [^3H](−)-noradrenaline close arterially at 345 ng/min in the presence of (▲) and absence (△) of amiodarone (1 mg/g). (●) recovery of Evans Blue intravascular marker ($n = 4$).

DISCUSSION

The effect of amiodarone, a non-competitive α and β antagonist upon the overflow of endogenous transmitter from the isolated blood perfused cat spleen, is opposite to that of all tested pure α antagonists [12]. A reduction in overflow after amiodarone could be due to several factors: increased inactivation of transmitter, a depletion of the nerve terminal, a reduction in transmitter liberation, either directly or indirectly via an α agonist effect upon postulated presynaptic α receptors, or to a local anaesthetic effect.

The reduction in overflow does not appear to be due to increased inactivation of transmitter by uptake since spleens treated with amiodarone showed no increase in their ability to remove noradrenaline given as infusions or pulses. Since the transmitter overflow is measured by a bioassay method which detects only noradrenaline in the overflowing venous blood, a reduction in overflow could be brought about by increased metabolism. However, in the experiments in which the transmitter stores were labelled with radioactive noradrenaline, the overflow of label following nerve stimulation, which includes both noradrenaline and metabolites, was reduced by amiodarone to the same extent as the bioassayed noradrenaline.

An effect of amiodarone on metabolism could not therefore explain its effect on overflow. Catecholamine depletion also seems unlikely as a mode of action since no response was seen following injection of amiodarone which might have indicated release of amines, and in addition, other workers have looked for but not found, a catecholamine-depleting effect [2]. Amiodarone has also been tested for local anaesthetic effects using the guinea pig wheal test [5]. No effect was seen even in concentrations as high as 50 mg/ml. The most likely explanation for the reduction of overflow produced by the drug is, therefore, an effect on liberation of transmitter. It is interesting to note that in the past overflows from spleens treated with phenoxybenzamine have been used as an index of transmitter liberation [7] and in the present experiments the increase in overflow usually seen after phenoxybenzamine was antagonised by amiodarone. The drug, therefore, could be producing its effect by acting on the release mechanism. The experiments in which nerve stimulation evoked release has been compared with that produced by tyramine provide support for this conclusion. Since stimulation evoked release is calcium-dependent unlike that produced by tyramine and only stimulation evoked release is reduced by amiodarone it would indicate that the drug may act on calcium metabolism in the nerve endings. The effect is unlikely to be mediated through presynaptic α receptors since the irreversible α antagonist phenoxybenzamine did not protect against the action of amiodarone. In addition, even in high concentrations the drug failed to produce any response of the splenic musculature indicating that it has no stimulating effect on post-synaptic α receptors. Amiodarone also blocks β as well as α receptors [2] and this must be considered as a possible site of action since it has recently been suggested that presynaptic β-receptor stimulants increase and β-antagonists decrease release of transmitter in guinea pig auricles [13]. However, the effect on noradrenaline overflow of the β antagonist propranolol is too small to be considered as an explanation for the almost total abolition of transmitter liberation in the presence of large doses of amiodarone.

In conclusion, amiodarone reduces transmitter overflow from the cat spleen following nerve stimulation. The effect is not related to changes in uptake or metabolism of noradrenaline or to selective stimulation of presynaptic α receptors but probably reflects a neurone blocking action of the drug. In this amiodarone appears to resemble adrenergic blocking drugs such as guanethidine or bretylium. However, unlike these drugs it does not block uptake of noradrenaline and its effects are not reversed by amphetamine (Blakeley and Summers, unpublished observations).

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