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Prejunctional actions of some β-adrenoceptor antagonists in the vas deferens preparation of the guinea-pig

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Summary

1. The β-adrenoceptor antagonists propranolol, pronethalol, MJ 1999 and Ciba 39089-Ba reduced responses to field stimulation of the guinea-pig isolated vas deferens preparation without significantly affecting responses to exogenously added noradrenaline.

2. This prejunctional blocking action of the drugs cannot be correlated with their action as β-adrenoceptor antagonists or non-specific depressants.

3. The blockade produced was more pronounced at low (5 20 Hz) than at high (50 Hz) frequencies of stimulation.

4. The blockade was slow in onset, and once established was poorly reversed by washing the preparation over a period of 1 to 2 h.

5. The blockade produced could be reversed by dexamphetamine and cocaine.

6. These experiments suggest that the β-adrenoceptor antagonists may have some actions which closely resemble those of the adrenergic neurone blocking agent guanethidine.

Introduction

The β-adrenoceptor antagonists propranolol, pronethalol and Ciba 39089-Ba have been used in the treatment of hypertension (Prichard, 1964 ; Prichard & Gillam, 1969 ; Dorph & Binder, 1969), propranolol being the most widely used of these agents. Clinical reports have varied widely as to the effectiveness of propranolol as an antihypertensive agent (see Prichard & Gillam, 1969, for references). The mechanisms by which β-adrenoceptor antagonists may produce their antihypertensive activity are not clear. Prichard & Gillam (1964) and Dorph & Binder (1969) have suggested that the antihypertensive action may be due to a fall in cardiac output resulting from cardiac β-adrenoceptor blockade, whereas Waal (1966) has suggested that non-specific depressant or quinidine-like actions may be implicated. To explain the slow onset of the antihypertensive action, Prichard & Gillam (1966, 1969) later suggested that continuous cardiac β-adrenoceptor blockade resulted in a gradual re-setting of baroreceptors regulating the blood pressure at a lower level.

At body pH, propranolol will exist as an ionized molecule, with structural features closely resembling those necessary for attachment to the hypothetical receptor proposed for adrenergic neurone blocking drugs by Rand & Wilson (1967). Recent qualitative studies by Day, Owen & Warren (1968) have shown that, in

relatively high concentrations, propranolol and pronethalol possess prejunctional actions which in some respects resemble those of the adrenergic neurone blocking drug guanethidine.

In the present experiments, some actions of the β-adrenoceptor antagonists pronethalol, propranolol, Ciba 39089-Ba and MJ 1999 have been studied using the sympathetically innervated vas deferens preparation of the guinea-pig.

Methods

Guinea-pigs were killed by a blow on the head and the vasa deferentia removed. These were threaded through bipolar platinum electrodes and suspended in McEwen's (1956) solution at 37° C aerated with 95% oxygen and 5% carbon dioxide. Electrical stimulation of the intramural nerve endings was produced by square wave pulses of 1 ms duration using a supramaximal voltage. The preparation was stimulated at 5 min intervals for a period of 15 s at frequencies of 5 to 50 Hz. In some experiments stimulation was interrupted and noradrenaline was added to the organ bath. Isotonic contractions were recorded on smoked paper using a frontal writing lever.

The drugs used were propranolol hydrochloride and pronethalol hydrochloride (Imperial Chemical Industries); MJ 1999 (4-2-(isopropylamino-1-hydroxyethyl) methanesulphonanilide hydrochloride) (Mead Johnson); Ciba 39089-Ba (1-isopropylamino-2-hydroxy-3-(o-allyloxyphenoxy) propane hydrochloride) (Ciba); (−)-noradrenaline bitartrate (Winthrop); dexamphetamine sulphate (Smith, Kline & French) and cocaine hydrochloride (Drug Houses of Australia). Drugs were dissolved in 0·9% w/v sodium chloride solution before use. Concentrations of noradrenaline refer to the base and the remaining compounds to their salts.

Results

In guinea-pig vas deferens preparations, responses to electrical stimulation at frequencies producing submaximal responses (5, 10 and 20 Hz) were reduced by low concentrations (0·05 to 0·5 μg/ml) of the β-adrenoceptor antagonists propranolol, pronethalol, Ciba 39089-Ba and MJ 1999. At a higher frequency which produced maximal responses (50 Hz), higher concentrations of the drugs were generally required (1 or 2 μg/ml).

The first signs of the reduction in size of responses to sympathetic stimulation occurred 5 to 15 min after administration of the drugs and thereafter the blockade increased, reaching a steady level within 40 to 120 min. Low concentrations of the β-adrenoceptor antagonists (0·05 and 0·1 μg/ml) frequently produced a small potentiation of the responses before blockade occurred. The blockade, once established, was persistent, little or no reversal of it being obtained after repeated washing of the preparation for a period of 1 to 2 hours (Fig. 1). At the height of the blockade produced by concentrations of 0·1 to 0·5 μg/ml of all four β-adrenoceptor antagonists, responses to noradrenaline were essentially unchanged or even slightly potentiated when compared with control responses (Fig. 1).

These results confirm and extend observations obtained by Day *et al.* (1968), who studied the effects of higher concentrations of propranolol and pronethalol (1 to 5 μg/ml) on the Finkleman preparation of the rabbit ileum, the rat isolated vas deferens preparation and the isolated perfused central ear artery of the rabbit.

A series of experiments was carried out in which the actions of the four β-adrenoceptor antagonists were studied quantitatively. The basic frequency of stimulation used was 10 Hz. After stabilization, responses were also obtained to frequencies of stimulation of 5, 20 and 50 Hz. A β-adrenoceptor antagonist was then added in a concentration of 0·05 μg/ml, and when the maximum degree of blockade at a frequency of 10 Hz was established, responses were obtained to the other frequencies of stimulation. The bath was then washed, refilled, the next higher dose of the β-adrenoceptor antagonist added, and the process repeated. The four β-adrenoceptor antagonists were used at concentrations of 0·05, 0·1, 0·2, 0·5, 1·0 and 2·0 μg/ml. Four to six experiments were performed with each drug. In five control experiments, the same regime was followed without the addition of drugs. Figure 2 shows a tracing of results obtained from an experiment using MJ 1999. With a low concentration (0·2 μg/ml), responses to low frequencies of stimulation (5 and

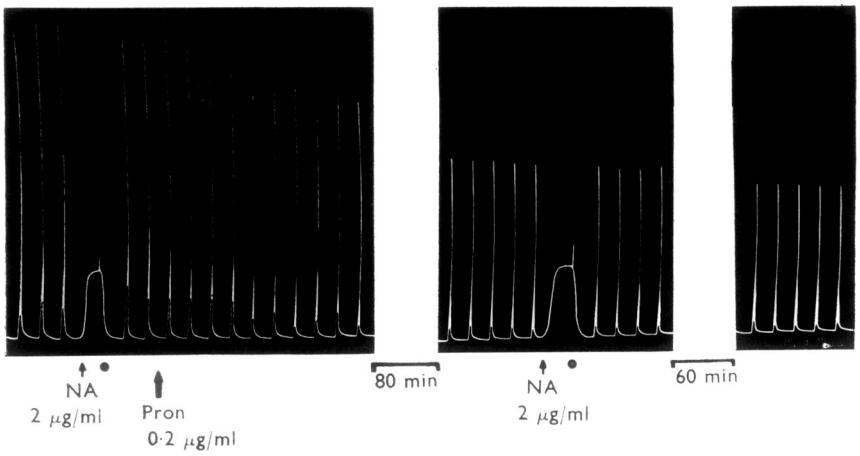

FIG. 1. Guinea-pig isolated vas deferens stimulated transmurally at 5 min intervals for 15 s at a frequency of 10 Hz. At Pron, pronethalol 0·2 μg/ml was administered. At NA, the stimulation was stopped and noradrenaline (2 μg/ml) was administered. This was washed out at ●. Between the second and third panels three further washes were given.

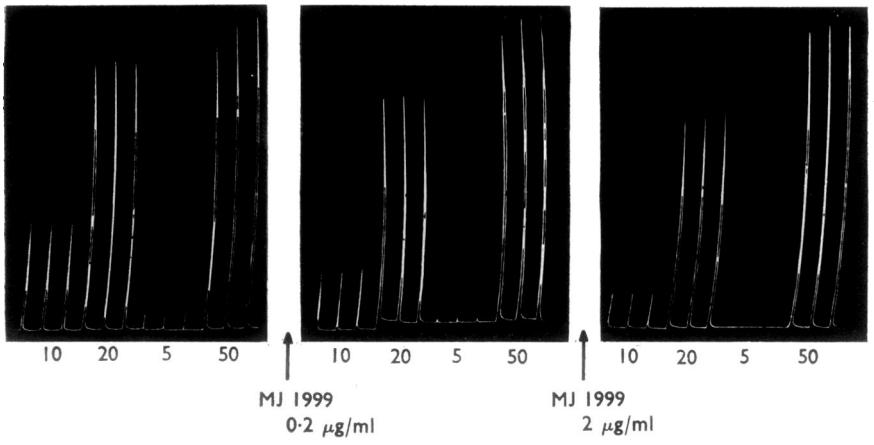

FIG. 2. Guinea-pig isolated vas deferens stimulated transmurally at frequencies of 5, 10, 20 and 50 Hz as indicated at 5 min intervals for 15 s. Control responses and responses after the cumulative addition of total concentrations of 0·2 and 2·0 μg/ml of MJ 1999 are shown.

10 Hz) were reduced to a much greater extent than those to the higher frequencies (20 and 50 Hz). With the higher concentration of 2 μg/ml, responses to frequencies of stimulation of 5, 10 and 20 Hz were inhibited, but responses to 50 Hz were not markedly affected. A similar frequency-dependent depression of responses of the guinea-pig vas deferens was seen with pronethalol, propranolol and Ciba 39089-Ba. Figure 3a shows graphically the frequency-dependent reduction in the contractions produced by propranolol. A marked inhibition of those responses elicited by submaximal frequencies of stimulation (5, 10 and 20 Hz) was obtained with the lower concentrations (0·05 to 0·5 μg/ml) of propranolol; higher concentrations (1 and 2 μg/ml) produced little further effect. In these experiments there was little or no significant difference between the blockade produced by 0·5 μg/ml or 2·0 μg/ml of propranolol ($P>0·05$). However, the responses of the vas deferens to stimulation

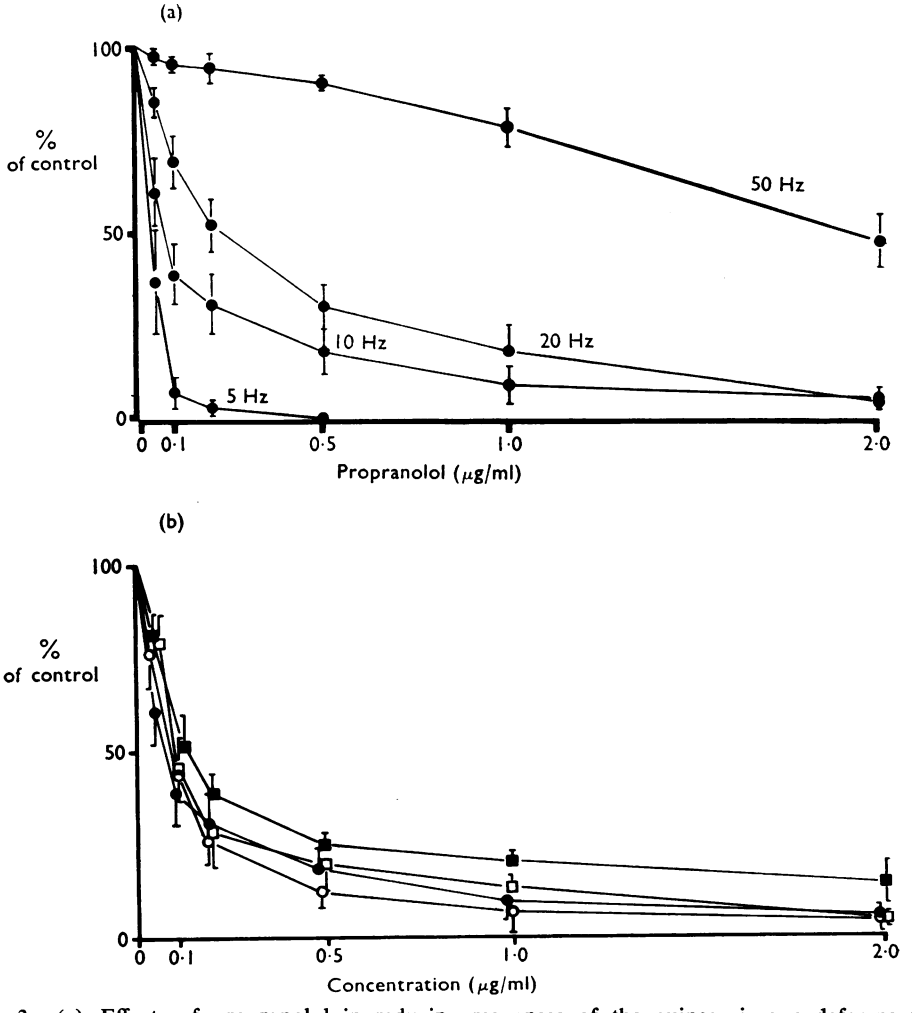

FIG. 3. (a), Effects of propranolol in reducing responses of the guinea-pig vas deferens to transmural stimulation at the indicated frequencies. Each point represents the mean (±S.E.) from six experiments, expressed as a percentage of control values. (b), As in (a) showing effects of the four β-adrenoceptor antagonists on responses elicited at a frequency of 10 Hz. ■, MJ 1999; ●, propranolol; □, pronethalol; ○, Ciba 39089. Each point represents the mean (±S.E.) of the responses from four to six experiments.

at the supramaximal frequency of 50 Hz were little affected by low concentrations of propranolol (0·05 to 0·5 μg/ml), and the inhibition caused by higher concentrations (1 and 2 μg/ml) was much less than that observed when the lower frequencies of stimulation were applied. Similar results were obtained with pronethalol, Ciba 39089-Ba and MJ 1999.

Figure 3b shows the effects of various concentrations of the four β-adrenoceptor antagonists on responses of the vas deferens elicited by stimulation at a frequency of 10 Hz. With the lowest concentrations of the antagonists used (0·05 μg/ml), the mean reduction in the response was lower than that found in control experiments carried out in the absence of drugs. However, at this concentration only propranolol caused a significant reduction ($P<0·05$). With higher concentrations (0·1 to 2·0 μg/ml), all responses were significantly less than those of controls.

The ratio of the concentrations of the β-adrenoceptor antagonists required to produce a 50% reduction in responses to stimulation at a frequency of 10 Hz was propranolol, 1·0 ; pronethalol, 1·14 ; Ciba 39089-Ba, 1·16 and MJ 1999, 1·39. There was little or no significant difference in the degree of blockade produced by the four β-adrenoceptor antagonists at each concentration used. On a molar basis the relative potencies were propranolol, 1·0 ; Ciba 39089-Ba, 0·88 ; pronethalol, 0·79 ; and MJ 1999, 0·75. At frequencies of 5 and 20 Hz, similar results were obtained, but at the frequency of 50 Hz MJ 1999 produced significantly less inhibition than the other drugs in concentrations of 1 and 2 μg/ml ($P<0·05$).

The adrenergic neurone blockade produced by guanethidine may be reversed by dexamphetamine (Day & Rand, 1963 ; Gerkens, McCulloch & Wilson, 1969 ;

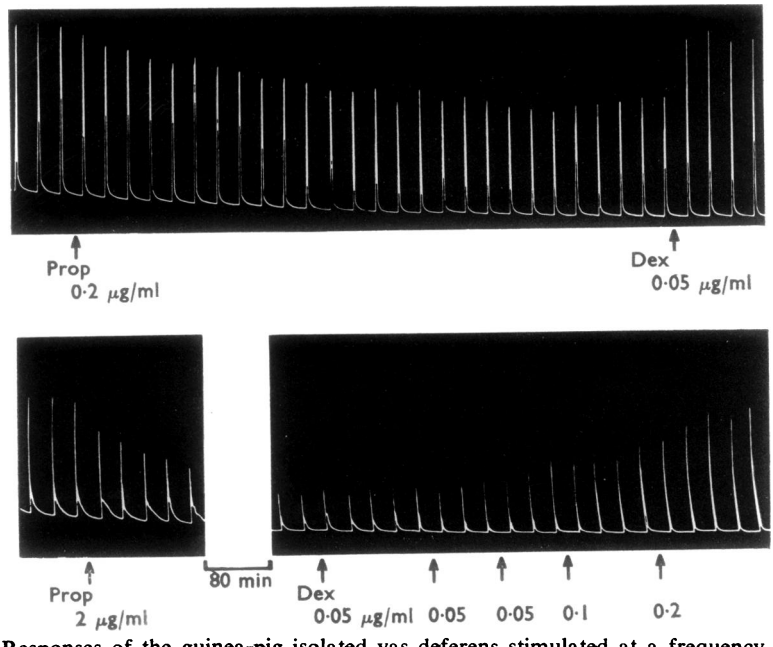

FIG. 4. Responses of the guinea-pig isolated vas deferens stimulated at a frequency of 10 Hz at 5 min intervals for 15 s. Upper panel, at Prop, propranolol (0·2 μg/ml) added. Reversal of the blockade occurred on addition of dexamphetamine (Dex, 0·05 μg/ml). Lower panels, at Prop, propranolol (2 μg/ml) added. Right-hand panel shows reversal of the blockade on the addition of dexamphetamine (Dex) at the concentrations indicated. The final cumulative concentration of dexamphetamine required for complete reversal was 0·45 μg/ml.

Obianwu, 1969). Similarly, dexamphetamine reversed the block produced by the four β-adrenoceptor antagonists used in the present experiments. However, the ability of dexamphetamine to cause reversal was dependent on the concentrations of the β-adrenoceptor antagonists used. In these experiments, single doses of the antagonists were given, and when the blockade of the responses was established, increasing doses of dexamphetamine were added and the effects on the block noted. The inhibition produced by low concentrations (0·05 to 0·5 μg/ml) of the four β-adrenoceptor antagonists was invariably reversed by low concentrations of dexamphetamine (0·05 to 0·1 μg/ml), while that produced by high concentrations of the β-adrenoceptor antagonists (1·0 to 4·0 μg/ml) was not regularly reversed by dexamphetamine. Experiments with pronethalol, Ciba 39089-Ba and MJ 1999 were all carried out using preparations stimulated at a frequency of 10 Hz. Experiments with propranolol were conducted at both 5 and 10 Hz. Similar results were obtained with both frequencies of stimulation in the latter experiments. In six experiments using pronethalol and Ciba 39089-Ba, the blockade produced by low concentrations (0·05 to 0·5 μg/ml) of the antagonists was completely reversed by dexamphetamine (0·05 μg/ml) while that produced by 2 μg/ml of the drugs was little affected. In twenty-seven experiments using propranolol, the blockade produced by 0·1 and 0·2 μg/ml was completely reversed by dexamphetamine (0·05 μg/ml) (Fig. 4). With a concentration of 2 μg/ml of propranolol, no significant reversal of the block occurred with dexamphetamine in concentrations of up to

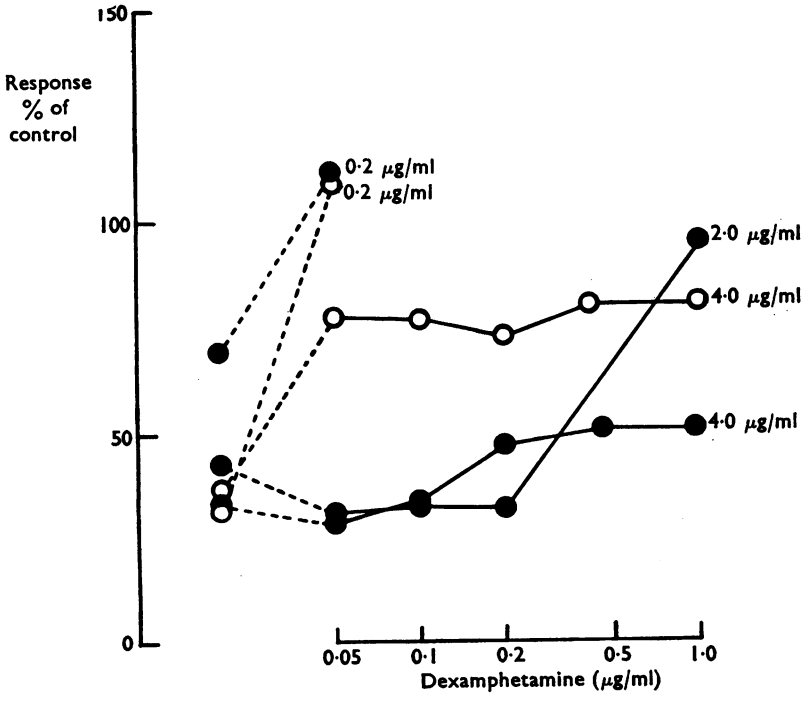

FIG. 5. Graphs showing the effects of dexamphetamine (cumulative concentration, μg/ml) in antagonizing the blockade produced by propranolol (●——●) and MJ 1999 (○——○) in the concentrations indicated. Each point shows the mean result from three or four experiments expressed as a percentage of control responses before the addition of the β-adrenoceptor antagonists. The first point in each graph shows the block produced by the β-adrenoceptor antagonists before addition of dexamphetamine.

0·2 μg/ml. Further increases in the concentration of dexamphetamine up to a total concentration of 0·45 to 1 μg/ml produced some reversal (Fig. 4). The block produced by 4 μg/ml of propranolol was unaffected by dexamphetamine in concentrations up to 1 μg/ml. MJ 1999 differed from the other β-adrenoceptor antagonists in that the blockade produced by both low and high concentrations (0·2 to 8·0 μg/ml, twelve experiments) was reversed by low concentrations of dexamphetamine (0·05 μg/ml).

Figure 5 shows graphically the effects of dexamphetamine on the blockade produced by low and high concentrations of propranolol and MJ 1999.

Previous workers have shown that the blockade produced by the adrenergic neurone blocking drug guanethidine may be antagonized by cocaine (Day, 1962; Gerkens *et al.*, 1969; Kirpekar, Wakade, Dixon & Prat, 1969). In a total of thirty-two experiments, the effects of cocaine on the blockade produced by propranolol, pronethalol, Ciba 39089-Ba and MJ 1999 was studied. The experimental procedure was similar to that described above for dexamphetamine reversal. In general, cocaine (0·05 to 0·1 μg/ml) reversed the blockade produced by low concentrations (0·1 to 0·5 μg/ml) of the four β-adrenoceptor antagonists, while the blockade produced by high concentrations (1 to 4 μg/ml) was variably affected. Twenty-two experiments were performed using various concentrations of propranolol. In eleven, 0·2 μg/ml of propranolol was used. The threshold concentration of cocaine required to initiate reversal of the blockade varied between 0·05 and 0·1 μg/ml, and complete reversal of the blockade was obtained with concentrations of 0·5 μg/ml or less. In five of eight experiments in which 2 μg/ml of propranolol was used, no reversal was apparent with concentrations of up to 1·0 μg/ml of cocaine. In the remaining three experiments cocaine (0·5 to 1·0 μg/ml) completely reversed the blockade. In three experiments in which 4 μg/ml of propranolol was used, no reversal was apparent with cocaine in concentrations of up to 1 μg/ml.

Discussion

A common property of most β-adrenoceptor antagonists, including pronethalol, propranolol, Ciba 39089-Ba and MJ 1999, is their ability to cause specific β-adrenoceptor blockade at low dose levels, while at higher dose levels depressant activity, variously described as non-specific, quinidine-like or local anaesthetic, is observed (Sekiya & Vaughan Williams, 1963; Morales-Aguilerá & Vaughan Williams, 1965; Schmid & Hanna, 1967; Raper & Wale, 1968a, b). There is no significant correlation between the β-adrenoceptor blocking and the non-specific depressant actions of these drugs (Benfey & Varma, 1966; Raper & Jowett, 1967; Raper & Wale, 1968a, b).

In addition, the four β-adrenoceptor antagonists used in the present experiments have been shown to influence the uptake of noradrenaline (Iversen, 1967; von Euler & Lishajko, 1968; Foo, Jowett & Stafford, 1968), its release from isolated nerve granules (von Euler & Lishajko, 1966), and its release from sympathetically innervated tissues in response to tyramine (Aramendía & Kaumann, 1967; Foo *et al.*, 1968). These actions of the β-adrenoceptor antagonists did not correlate with their β-adrenoceptor blocking effects (Foo *et al.*, 1968).

In the present experiments, blockade of the responses to sympathetic stimulation occurred while responses to added noradrenaline were little affected. This suggests that a prejunctional action is responsible for the observed effects. The degree of

blockade of the response of the vas deferens to stimulation was almost maximal when low concentrations (0·05 to 0·5 μg/ml) of the β-adrenoceptor antagonists were used. Although there was little or no significant difference in the relative potencies of the drugs at these concentrations, MJ 1999 proved to be marginally the least effective inhibitor at higher concentrations. This result may be contrasted with the β-adrenoceptor blocking potency of the drugs established *in vitro* (Blinks, 1967; Raper & Wale, 1968a; Foo *et al.*, 1968); these authors found propranolol to be more than 100 times as potent as MJ 1999 in blocking β-adrenoceptors in cardiac muscle. These results suggest that blockade of β-adrenoceptors is not implicated in the effects seen in the present experiments.

Specific β-adrenoceptor blockade itself would be expected to result in increased contractions of the guinea-pig vas deferens preparation in response to sympathetic stimulation due to a further unmasking of α-adrenoceptor (excitatory) activity by antagonism of the weak β-adrenoceptor (inhibitory) actions of the released noradrenaline (Holman & Jowett, 1964; Large, 1965). This action may explain the short-lasting potentiation of the responses seen before the blockade in some experiments when low doses of the β-adrenoceptor antagonists were used.

Non-specific depressant actions of these β-adrenoceptor antagonists probably contribute little to the depression of contractions in response to stimulation produced by the lower concentrations of the drugs. The four drugs have been studied for local anaesthetic, quinidine-like and non-specific depressant actions in smooth muscle preparations (Morales-Aguilerá & Vaughan Williams, 1965; Raper & Jowett, 1967; Raper & Wale, 1968a, b) and MJ 1999 was some 10 to 80 times less depressant than propranolol—respectively the least active and the most active of the four drugs. The differences in the relative potencies of the drugs in producing blockade of responses to sympathetic stimulation in the vas deferens and in producing non-specific depressant actions in other tissues favour the concept that these actions are unrelated. Furthermore, blockade of responses occurred at concentrations below those required to produce evidence of quinidine-like actions in cardiac preparations; non-specific blockade of the actions of agonists in guinea-pig ileum preparations; and depression of the spontaneous pendular movements in the rabbit ileum (Raper & Mylecharane, unpublished observations).

The prejunctional actions of the β-adrenoceptor antagonists described in this paper may be produced by a mechanism similar to that of adrenergic neurone blocking drugs such as guanethidine. The slow onset of the blockade, its persistence, the maintenance of responses to exogenously added noradrenaline, and its frequency-dependence, are reminiscent of the actions of guanethidine (Boura & Green, 1965). These results confirm and extend those of Day *et al.* (1968). In addition, the blockade produced by low doses of all four β-adrenoceptor antagonists was reversed by dexamphetamine and cocaine, as has been shown for guanethidine. However, Day *et al.* (1968) were unable to show a reversal by dexamphetamine of the block produced by propranolol (1 to 5 μg/ml) using other *in vitro* preparations. This inability of dexamphetamine to reverse the blockade produced by such high concentrations of propranolol was confirmed in our experiments and extended to Ciba 39089-Ba and pronethalol.

The mechanisms by which cocaine and dexamphetamine cause reversal of the adrenergic neurone blocking action of guanethidine are controversial (Day & Rand, 1963; Gerkens *et al.*, 1969; Kirkpekar *et al.*, 1969; Obianwu, 1969). It has been

suggested that guanethidine and noradrenaline are taken up in adrenergic neurones by a similar mechanism (Chang, Costa & Brodie, 1965 ; Obianwu, Stitzel & Lundborg, 1968) and that both these compounds are released during sympathetic nerve stimulation (Boullin, Costa & Brodie, 1966). Gerkens *et al.* (1969) have postulated that the ability of cocaine and dexamphetamine to reverse the adrenergic neurone blocking action of guanethidine is due to a blockade of its re-uptake following its release during sympathetic stimulation.

If the β-adrenoceptor antagonists are similar to guanethidine in that their uptake into sympathetic neurones is a necessary prerequisite for their prejunctional actions, their own ability to block the uptake process may lead to a self-antagonism of their uptake. This may explain the flattening of their concentration-response curves at higher concentrations where their ability to block the uptake process will be manifested to the greatest extent. Foo *et al.* (1968) have previously shown that in cardiac tissue the β-adrenoceptor blocking actions of these drugs occur with lower concentrations than those required to block the uptake of noradrenaline and that the threshold concentrations required for the latter effect are of the order of 0·6 μg/ml.

In the present experiments the blockade produced by low concentrations of the four β-adrenoceptor antagonists was reversible by dexamphetamine and cocaine, while, with the exception of MJ 1999, that produced by high concentrations was not reversible. This dose-dependent reversibility may also be due to the uptake blocking actions of the β-adrenoceptor antagonists at high concentrations. If, as with guanethidine, the ability of cocaine and dexamphetamine to reverse the prejunctional block produced by the β-adrenoceptor antagonists is in part dependent on a blockade of their re-uptake following release during sympathetic stimulation, high doses of the β-adrenoceptor antagonists will cause a blockade of uptake themselves, thus precluding the uptake blocking actions of dexamphetamine and cocaine. MJ 1999 itself has only weak actions as a blocker of uptake, a fact which may explain the ability of dexamphetamine and cocaine to reverse the blockade produced by high concentrations of this drug.

The degree of blockade produced by particular concentrations of the β-adrenoceptor antagonists given singly (Fig. 5) appeared to be less than that produced when the same concentrations were reached more slowly after cumulative administration of the drugs (Fig. 3). The possibility exists that, when the drugs are given in single doses, some self-inhibitory activity could occur because their own actions on the uptake mechanism might decrease the total amount of drug reaching an intracellular site of action. However, with cumulative administration, low concentrations of the drugs might reach the site of action unhindered by blockade of their own uptake, the latter action only being of consequence when higher concentrations had been given.

Further experiments will be required to determine the possible significance of an "adrenergic neurone blocking" action of β-adrenoceptor antagonists in producing antihypertensive effects.

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