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Further studies on the adrenergic neuron blocking activity of some β-adrenoceptor antagonists and guanethidine

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The effects of low (0·2) and high (20 μg ml^{-1}) concentrations of (±)- and (+)-propranolol, (±)-, (+)- and (−)-sotalol and guanethidine were tested for their ability to reduce responses to sympathetic stimulation in the isolated vas deferens preparation from the guinea-pig. At 0·2 μg ml^{-1} all drugs produced a slowly developing reduction in the responses to sympathetic stimulation while responses to noradrenaline were largely unchanged. The blockade, which was similar in extent in all six compounds, was reversed by (+)-amphetamine but not by washing. With high concentrations of (±)- and (+)-propranolol and guanethidine, the block was rapid in onset and rate and responses to noradrenaline were potentiated. The block was reversed by washing and unaffected by (+)-amphetamine. Sotalol and its isomers, which possess little non-specific depressant activity, had qualitatively similar actions at 0·2 and 20 μg ml^{-1}. At the latter concentration responses to noradrenaline were potentiated. The results suggest that low concentrations of the β-adrenoceptor antagonists produce a blockade which is typical of guanethidine-like drugs. At high concentrations non-specific depressant (local anaesthetic) actions of propranolol and its isomers are largely responsible for the blockade. A similar mechanism may also operate when high concentrations of guanethidine are used.

In a number of pharmacological preparations where adrenergic responses are mediated predominantly through α-adrenoceptors, β-adrenoceptor antagonists have been shown to depress responses elicited by sympathetic nerve stimulation while responses to noradrenaline are either unaffected or increased (Day, Owen & Warren, 1968; Barrett & Nunn, 1970; Ganguly & Bhattacharya, 1970; Mylecharane & Raper, 1970; Eliash & Weinstock, 1971). Both guanethidine-like adrenergic neuron blocking actions and non-specific depressant (local anaesthetic) actions have been proposed to account for the results. In the present experiments attempts have been made to distinguish the mechanisms involved by the use of high and low concentrations of (±)- and (+)-propranolol, (±)-, (+)-, and (−)-sotalol, and guanethidine, in isolated vas deferens preparations from the guinea-pig.

METHODS

Isolated vas deferens preparations from guinea-pigs were suspended in McEwen (1956) solution maintained at 37° and aerated with 5% CO_2 in oxygen. A bipolar platinum ring electrode was used to stimulate the intramural nerves. The preparation

was stimulated once every 5 min for 15 s using square wave pulses of 1 ms duration at a frequency of 10 Hz. Supramaximal voltages (50–60 V) were used. In some experiments, electrical stimulation was interrupted and responses to exogenous noradrenaline were obtained. Contractions were recorded on a smoked drum using an isotonic frontal writing lever.

The drugs used were (±)- and (+)-propranolol hydrochloride (ICI); (±)-, (+)- and (−)-sotalol hydrochloride (Mead Johnson); guanethidine sulphate (Ciba); (+)-amphetamine sulphate (Smith, Kline & French) and (−)-noradrenaline bitartrate (Sterling). Concentrations of noradrenaline refer to the base, and of the remaining compounds to their salts.

RESULTS

Effects of low concentrations of propranolol, sotalol and their isomers, and guanethidine

At concentrations of 0·2 μg ml^{-1}, (±)- and (+)-propranolol, (±)-, (+)- and (−)-sotalol, and guanethidine reduced contractions of the vas deferens in response to sympathetic stimulation by 25 to 50%. The onset of the blockade was slow, and for the β-adrenoceptor antagonists, was occasionally preceded by a small enhancement of the contractions. The time taken for maximal blockade was extremely variable with all compounds used and ranged from 30 to 235 min. In three experiments with each compound, contractions elicited by electrical stimulation were interrupted at the height of the block and responses elicited to exogenously administered noradrenaline (2 to 5 μg ml^{-1}). Responses to noradrenaline were little affected when compared with controls. After washout of noradrenaline and the various compounds used, there was no reversal of the blockade. The first response to electrical stimulation after washout of the noradrenaline was variable and frequently greater than responses produced by succeeding stimuli which remained at the blocked level. The results confirm and extend previous findings on the effects of (±)-propranolol and (±)-sotalol (Mylecharane & Raper, 1970). These effects for (+)-propranolol are shown in Fig. 1A, and the maximum blockade produced by all six compounds is indicated in Fig. 2.

There was no significant difference in the maximum blockade produced by 0·2 μg ml^{-1} of propranolol, sotalol and their isomers (t-test, $P > 0{\cdot}05$). At this concentration the potency of guanethidine was not significantly different from that of any of the β-adrenoceptor antagonists (t-test, $P > 0{\cdot}05$) except (±)-propranolol (t-test, $P < 0{\cdot}05$).

In approximately half the experiments with each compound, (+)-amphetamine was added at the height of the blockade as shown in Fig. 1B for (+)-propranolol. The initial concentration of (+)-amphetamine (0·05 μg ml^{-1}) produced a significant reversal of the blockade (t-test, $P < 0{\cdot}05$) with all compounds except (±)-sotalol and guanethidine (Fig. 2). Significant reversal was obtained with the latter compounds when the (+)-amphetamine concentration was increased to 0·1 μg ml^{-1}.

In the remaining experiments, after maximum blockade was obtained, the tissue was washed at 15 min intervals over a period of at least 60 min. No significant change in the degree of blockade occurred with any of the compounds after washing (t-test, $P > 0{\cdot}05$) (Fig. 2). It was noted that after washout of the β-adrenoceptor antagonists or guanethidine, (+)-amphetamine, 0·05 to 0·5 μg ml^{-1}, produced some degree of reversal of the blockade. However with each compound the reversal by (+)-amphetamine *after* the washing procedure was much less than that produced in the previously described experiments where the (+)-amphetamine was added without washout.

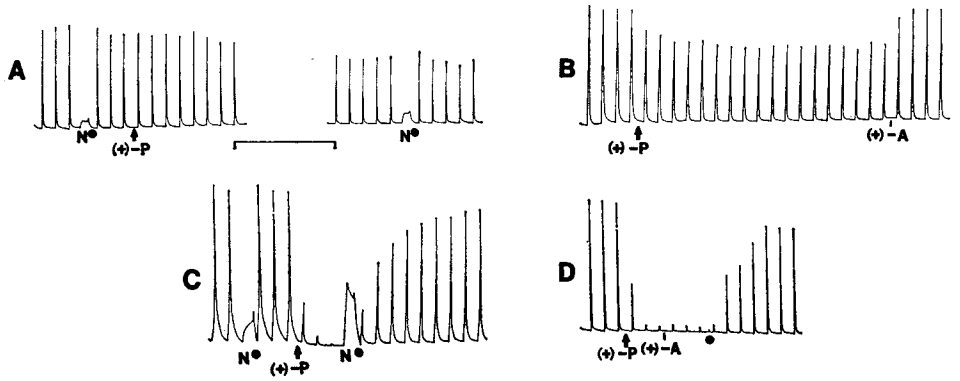

FIG. 1. Effects of (+)-propranolol ((+)-P) on responses to sympathetic stimulation in vas deferens preparations. In A and B, the concentration of (+)-propranolol was 0·2, and in C and D, 20 μg ml^{-1}. At N, noradrenaline (5 μg ml^{-1}) was added to the bath. Washing of the bath is indicated by ●. At (+)-A, (+)-amphetamine was added to the bath without washout of (+)-propranolol. The concentration of (+)-amphetamine was 0·05 μg ml^{-1} in B, and 0·5 μg ml^{-1} in D. The break in trace A represents a period of 125 min.

After the washout procedure, 0·05 μg ml^{-1} (+)-amphetamine produced a return of responses to 57 to 82% of control (*cf.* Fig. 2).

In 8 additional experiments using guanethidine and (±)-propranolol, after maximum blockade was produced the drugs were left in contact with the tissue for a further 60 min (equivalent to the washing time) before (+)-amphetamine was added. In these experiments (+)-amphetamine produced a reversal of the blockade that was of the same order as that obtained in experiments where it was added immediately after maximum blockade had occurred.

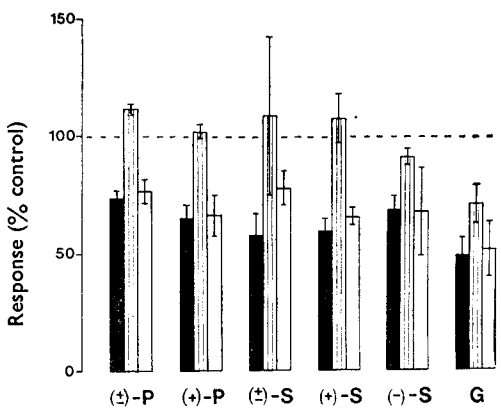

FIG. 2. Histograms showing results obtained in experiments using low concentrations (0·2 μg ml^{-1}) of (±)-propranolol ((±)-P); (+)-propranolol ((+)-P); (±)-sotalol ((±)-S); (+)-sotalol ((+)-S); (−)-sotalol ((−)-S); and guanethidine (G). Solid columns, maximum blockade of responses to sympathetic stimulation produced by the compounds; lined columns, effects of (+)-amphetamine (0·05 μg ml^{-1}) added to the bath after maximum blockade; open columns, effects of washout of the compounds after maximum blockade. Results are expressed as means (±s.e.) of responses expressed as percentages of control before addition of the compounds. Results for blockade were obtained from 8 to 14 experiments with each compound. In approximately half these experiments the effect of washout was tested, and in the remainder the effect of (+)-amphetamine was investigated.

Effects of high concentrations of (±)- *and* (+)-*propranolol and guanethidine*

High concentrations (20 μg ml^{-1}) of (±)- and (+)-propranolol and guanethidine produced a rapid and almost complete blockade of responses to sympathetic stimulation. There was no significant difference in the degree of blockade produced by any of the compounds 15 min after their addition to the bath (*t*-test, $P > 0{\cdot}05$) (Fig. 3).

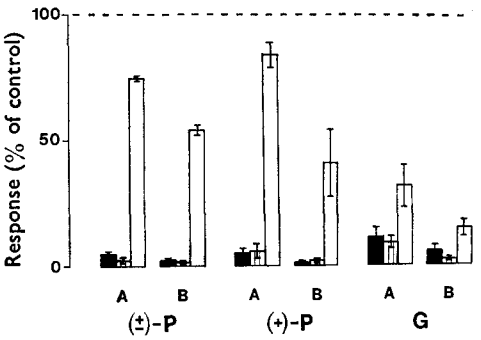

FIG. 3. As in Fig. 2, using high concentrations (20 μg ml^{-1}) of (±)- and (+)-propranolol and guanethidine. In these experiments (+)-amphetamine was used at 0·5 μg ml^{-1}. Effects are shown after a 15 min (A) and a 60 min (B) contact time of the tissues with the compounds. Eight experiments were performed with each compound.

In 4 of the experiments with each compound the effects of exogenously administered noradrenaline (5 μg ml^{-1}) were tested before and approximately 15 min after addition of the drug. Mean responses to noradrenaline were increased 6- and 3-fold after (±)- and (+)-propranolol respectively, and 17-fold after guanethidine. After washout of noradrenaline and the blocking compound, responses to sympathetic stimulation rapidly returned towards control levels (Fig. 3). Fig. 1C shows (+)-propranolol-induced blockade, noradrenaline potentiation, and the recovery of responses after washout of noradrenaline and (+)-propranolol.

In the remaining 4 experiments with each compound, (+)-amphetamine (0·5 μg ml^{-1}) was added to the bath and failed to reverse the blockade produced after 15 min contact with the compounds (*t*-test, $P > 0{\cdot}05$). Fig. 3 shows this lack of effect. After the (+)-amphetamine had been in contact with the tissue for 15 min, the bath was washed, and responses rapidly returned towards control level. This recovery level was slightly greater than that obtained by washout in tissues which had not been exposed to (+)-amphetamine. Fig. 1D shows these effects with (+)-propranolol.

Preliminary experiments showed that the blockade of responses produced by high doses of the compounds left in contact with the tissues for 15 min as described above were reproducible at hourly intervals.

In view of the long time required for a maximal blockade of responses to sympathetic stimulation in experiments where 0·2 μg ml^{-1} of the drugs were used, the effects of (±)- and (+)-propranolol and guanethidine were tested over a longer time. After the initial 15 min contact and washout of the drugs, the administration of the compounds was repeated and they were left in contact with the tissue for 60 min.

The degree of blockade produced, the potentiation of noradrenaline responses, and the lack of effect of (+)-amphetamine in reversing the block, were similar after the 15 and 60 min contact periods. However, after the 60 min treatment with (±)- and (+)-propranolol, washing of the tissue was less effective in reversing the blockade. After a 60 min contact with guanethidine there was no significant recovery of responses with washing (*t*-test, $P > 0{\cdot}05$). These results are summarized in Fig. 3.

Effects of high concentrations of (±)-, (+)- *and* (−)-*sotalol*

In similar experiments to those where 20 μg ml^{-1} of (±)- and (+)-propranolol and guanethidine were studied, (±)-, (+)- and (−)-sotalol (20 μg ml^{-1}) produced qualitatively different effects. The degree of blockade produced by sotalol and its isomers was time-dependent. Responses were reduced by approximately 10 and 30% after 15 and 60 min contact times respectively (Fig. 4). At each individual contact time there was no significant difference in the potency of sotalol and its isomers (*t*-test, $P > 0{\cdot}05$). Concentrations of 0·05 to 0·1 μg ml^{-1} of (+)-amphetamine produced a reversal of the blockade produced by the three compounds after both 15 and 60 min

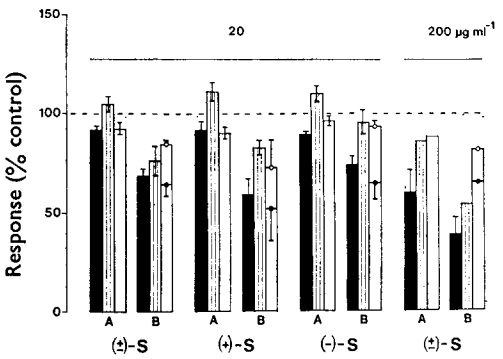

FIG. 4. As in Fig. 2, showing effects obtained with high concentrations (20 and 200 μg ml^{-1} as indicated) of sotalol and its isomers. In these experiments (+)-amphetamine was used at 0·05 μg ml^{-1}. After experiments where a 60 min contact period was allowed (B) the effects of washout were biphasic. Initially the responses were reversed (mean level shown by ○) and thereafter responses declined and stabilized at a lower level (mean level shown by ●). Eight experiments were performed with each compound at 20 μg ml^{-1} and four at 200 μg ml^{-1}.

contact times (Fig. 4). The reversal was significant (*t*-test, $P < 0{\cdot}05$) in all cases except with (±)-sotalol after a 60 min contact time.

Responses to noradrenaline were increased (approximately 2-fold) in the presence of sotalol and its isomers (20 μg ml^{-1}). This potentiation was less than that seen with (±)- and (+)-propranolol and guanethidine at this dose. After washout of the noradrenaline and (±)-, (+)-, or (−)-sotalol there was little or no reversal of the blockade produced following a 15 min contact with the compounds (Fig. 4). However, after 60 min contact, washout of noradrenaline and the compounds resulted in a biphasic effect. Initially there was a slight reversal of the blockade which lasted some 10 to 30 min. This reversal was followed by a decrease in the responses to the level obtained before washout (Fig. 4). Fig. 5 shows results obtained in tissues where a 60 min contact time with (+)-sotalol was used.

In 4 further experiments the effects of 200 μg ml^{-1} of (±)-sotalol were investigated. In comparison with effects obtained with 20 μg ml^{-1}, the blockade of responses was

greater; noradrenaline responses were potentiated to a similar extent; washout produced a greater degree of recovery; and (+)-amphetamine was less effective in reversing the blockade (Fig. 4).

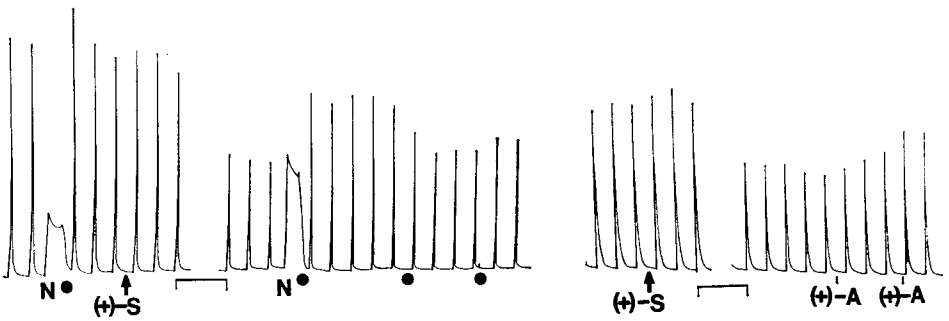

FIG. 5. Effects of (+)-sotalol ((+)-S, 20 μg ml^{-1}) on responses to sympathetic stimulation in vas deferens preparations. Left trace, at N, noradrenaline (5 μg ml^{-1}) was added to the bath. At ●, the bath was washed. The break in the trace represents a period of 35 min. Right trace, reversal of the block by (+)-amphetamine (+)-A, 0·05 μg ml^{-1}). The break in the trace represents 25 min.

DISCUSSION

The results obtained in the present experiments using low concentrations of (±)-propranolol and (±)-sotalol confirm the findings of Mylecharane & Raper (1970). In addition, it would appear that (+)-propranolol and the (+)- and (−)-isomers of sotalol possess quantitatively equivalent actions at low concentration, thus confirming and extending previous suggestions (Mylecharane & Raper, 1970) that the blockade is unrelated to β-adrenoceptor antagonism as in this respect the (+)-isomers are less potent than the (−)- or (±)-compounds (Levy & Richards, 1966; Ariëns, 1967; Barrett & Cullum, 1968). The actions produced by all the β-adrenoceptor antagonists are typical of adrenergic-neuron blocking compounds in that the rate of onset of blockade of responses to sympathetic stimulation is slow, responses to exogenous noradrenaline are not antagonized, and the blockade of neurally elicited responses is resistant to washing but can be reversed in the presence of (+)-amphetamine (Boura & Green, 1965). Furthermore, the adrenergic neuron blocking drug guanethidine at the same concentration (0·2 μg ml^{-1}) produced qualitatively and quantitatively similar effects to those observed with the β-adrenoceptor antagonists used.

The observation that when (+)-amphetamine was given after a 60 min washing period the blockade produced by a low concentration of the six compounds was not reversed is somewhat surprising and no explanation is apparent.

The effects of high concentrations of (±)- and (+)-propranolol and guanethidine differed markedly from those produced by the low concentrations. Thus, the blockade was rapid in onset and rate, was reversed readily by washing, and was unchanged in the presence of (+)-amphetamine. These results are similar to those described by Rand & Wilson (1967) and Day & others (1968) when the local anaesthetics procaine and lignocaine were tested for their effects on responses to sympathetic stimulation in guinea-pig and rat vasa deferentia. These authors found that the blockade of sympathetic responses produced by these compounds was quick in onset and rate and was rapidly reversed by washing. Rand & Wilson (1967) also showed that the blockade was unaffected by (+)-amphetamine. Guanethidine and (±)- and (+)-propranolol

possess potent non-specific depressant (local anaesthetic) actions (Boura & Green, 1965; Levy & Richards, 1966; Rand & Wilson, 1967; Barrett & Cullum, 1968).

Both high and low concentrations of (±)-, (+)- and (−)-sotalol produced similar qualitative effects. The absence of effects similar to those produced by 20 μg ml^{-1} concentrations of propranolol and guanethidine might be explained by the relative lack of depressant and local anaesthetic actions of sotalol and its isomers (Lish, Weikel & Dungan, 1965; Levy & Richards, 1966; Raper & Wale, 1968). The initial short-lasting reversal of the blockade following 60 min contact of the tissue with sotalol and its isomers (20 μg ml^{-1}) may reflect the removal of a weak non-specific depressant component apparent at this dose level. With a higher concentration of (±)-sotalol (200 μg ml^{-1}) a non-specific depressant component in its action is more in evidence.

With high concentrations of all five β-adrenoceptor antagonists and guanethidine, responses to exogenous noradrenaline were potentiated. This might be explained by blockade of the noradrenaline uptake mechanism reported with guanethidine, (+)- and (+)-propranolol, and (±)-sotalol (Iversen, 1967; Foo, Jowett & Stafford, 1968). The comparatively smaller potentiation of the responses produced with (±)-sotalol may be due to its weaker uptake blocking potency (Foo & others, 1968).

The ability of a number of β-adrenoceptor antagonists to reduce responses to sympathetic nerve stimulation while responses to exogenous noradrenaline are unaffected or potentiated has been ascribed to non-specific depressant (local anaesthetic) activity and to a guanethidine-like adrenergic neuron blocking action. Barrett & Nunn (1970) and Ganguly & Bhattacharya (1970) favour the former mechanism, whereas Mylecharane & Raper (1970) and Eliash & Weinstock (1971) favour the latter. Day & others (1968), while suggesting that local anaesthetic actions provided the most likely explanation for their results, pointed out that the effects of propranolol and pronethalol could not be mimicked by lignocaine. On this basis they suggested that a specific local anaesthetic action of the β-adrenoceptor antagonists on sympathetic nerve endings could not be precluded.

The results of the present experiments where high and low concentrations of β-adrenoceptor antagonists and guanethidine were used throws some light on the differing mechanistic interpretations advanced. With high concentrations of (±)- and (+)-propranolol (20 μg ml^{-1}) the effects obtained were similar to those obtained by Barrett & Nunn (1970) with 4·6 to 20 μg ml^{-1} of the compounds. The apparent lack of blocking effects with lower concentrations as reported by the above authors could well be due to the relatively brief period they allowed for the development of blockade. Day & others (1968) and Ganguly & Bhattacharya (1970) used an intermediate concentration range of propranolol and pronethalol (1 to 5 μg ml^{-1}) and obtained results similar to those of Mylecharane & Raper (1970) with 4 μg ml^{-1} of propranolol, in that the blockade was unaffected by washing or the administration of (+)-amphetamine. In the present experiments using lower concentrations of the β-adrenoceptor antagonists (0·2 μg ml^{-1}) the effects obtained were similar to those produced by the same concentration of guanethidine. The blockade was unaffected by washing, and reversed by (+)-amphetamine. Similar results were obtained by Eliash & Weinstock (1971) when low doses of (±)- and (+)-propranolol were used *in vivo*, and when low concentrations of (±)-propranolol, (±)-sotalol, (±)-pronethalol and (±)-oxprenolol were used *in vitro* (Mylecharane & Raper, 1970).

Thus, it would appear that with low concentrations of the β-adrenoceptor antagonists typical guanethidine-like adrenergic neuron blocking effects are obtained, while

with progressively higher concentrations a non-specific depressant (local anaesthetic) effect becomes increasingly important in producing blockade of responses to sympathetic nerve stimulation. The similar qualitative effects found with guanethidine and propranolol at both low and high concentrations suggest that when large concentrations of the former compound are used, blockade of responses to sympathetic stimulation may result from depressant rather than specific adrenergic neuron blocking actions of the accepted type.

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