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Research Papers

Influence of the length of the stimulus period and frequency of sympathetic stimulation on the response of the guinea-pig isolated vas deferens to bretylium, guanethidine and amphetamine

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The height of contraction of guinea-pig isolated vas deferens preparations in response to pre- or postganglionic sympathetic nerve stimulation at various stimulus frequencies was shown to vary with varying length of stimulation period. Short stimulation periods, as used by some workers, produced suboptimal contractions especially at the lower frequencies. It is suggested that variations in the length of the stimulation period could account for some of the contradictory results reported by various workers using this preparation. Thus the effect of low concentrations of dexamphetamine was qualitatively changed as the length of the stimulation period was increased; with short stimulation periods dexamphetamine impaired the responses to sympathetic stimulation but enhanced them if stimulation was continued until contractions had reached the maximum height obtainable. Higher concentrations of dexamphetamine impaired the contractions, the effect being most marked against the higher stimulus frequencies. Using stimulation to maximal effect a qualitative difference in the blocking actions of bretylium and guanethidine was demonstrated. Bretylium reduced the responses to the higher stimulus frequencies to a greater extent than it reduced the responses to the lower frequencies. Guanethidine reduced the responses to all frequencies so that there was a parallel shift of the frequency/response curve to the right. The possible significance of these findings is discussed.

THE isolated sympathetically-innervated vas deferens preparation of the guinea-pig was first described by Huković (1961) and has since been widely used to elucidate sympathetic nervous mechanisms. The published pharmacological work on this preparation contains several conflicting reports concerning the action of various drugs. Some of these differences may be explained on the basis of the recent convincing evidence that most fibres in the hypogastric nerve supplying the vas deferens synapse at or near the organ (Bentley & Sabine, 1963; Birmingham & Wilson, 1963; Kuriyama, 1963; Ohlin & Stromblad, 1963).

Other contradictory results cannot be explained in this way. Edge (1964) showed that low concentrations of amphetamine potentiated the contractions of the vas deferens in response to hypogastric nerve stimulation, the effect being most marked at low frequencies of stimulation. Higher amphetamine concentrations inhibited the responses to sympathetic nerve stimulation with the higher frequencies being most affected. Using the same preparation and drug, Morrison & Parkes (1964) obtained the opposite effects. They found that low concentrations of amphetamine reduced responses to all frequencies of stimulation except the highest, which were potentiated; higher concentrations reduced responses to all frequencies of stimulation, the effect being most marked against the lower frequencies. Furthermore, Morrison & Parkes (1964) were unable

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to confirm the observations of Green and others, made on different preparations, that the sympathetic nerve blocking actions of bretylium and guanethidine could be differentiated by their blocking efficacy at different stimulation frequencies (Boura & Green, 1962; Green & Robson, 1964).

In view of the increasing use of the vas deferens preparation and of the drugs bretylium and guanethidine in the investigation of the sympathetic postganglionic mechanism, an attempt has been made to resolve these conflicting reports.

Experimental

Methods

Guinea-pigs weighing 400–500 g were killed by a blow on the head and vasa deferentia were removed and set up in 75 ml organ baths containing Tyrode solution gassed with air and maintained at 32°. Longitudinal contractions of the preparations were recorded kymographically by means of an isotonic frontal writing lever. The preparations were stimulated electrically either through the hypogastric nerve ("preganglionic") as described by Huković (1961) or by means of parallel electrodes ("postganglionic") as described by Birmingham & Wilson (1963).

Preganglionic stimulation was applied by threading the nerve through bipolar platinum electrodes of the type described by Burn & Rand (1960) and postganglionic stimulation by passing the whole preparation through an electrode consisting of two platinum rings embedded in epoxy resin. In some experiments both pre- and post-ganglionic stimulation were applied in the same preparation.

Electrical stimulation was supplied from Palmer electronic stimulators delivering rectangular pulses of 2 msec pulse width and supramaximal strength. Stimulation was applied for periods of 2 or 5 sec repeated at intervals of 1 to 3 min, or (as in most experiments) continuously until a maximal response was obtained. In the latter instance 5 min periods were allowed from the end of one stimulation period before the start of the next. This method of stimulation produced more consistent results than were obtained with short fixed periods of stimulation.

Drugs

The following drugs were used: (+)-amphetamine (dexamphetamine), (−)-amphetamine, (±)-amphetamine, as the sulphates; concentrations in the text are expressed in terms of the base. Concentrations of guanethidine sulphate and bretylium tosylate (*p*-toluene sulphonate) refer to the salts.

Results

Effect of duration and frequency of stimulation on responses of the isolated vas deferens

Postganglionic stimulation. In the initial experiments preparations were stimulated via parallel electrodes. This produced results corresponding closely to those expected after postganglionic stimulation

(Birmingham & Wilson, 1963), and largely precluded the possibility of ganglionic effects.

The lowest frequency of stimulation which regularly evoked a recordable contraction was 2 pulses/sec. The size of the contractions increased with increasing frequency in a linear fashion up to 20 pulses/sec; stimulation at 50 pulses/sec produced responses which were usually little different

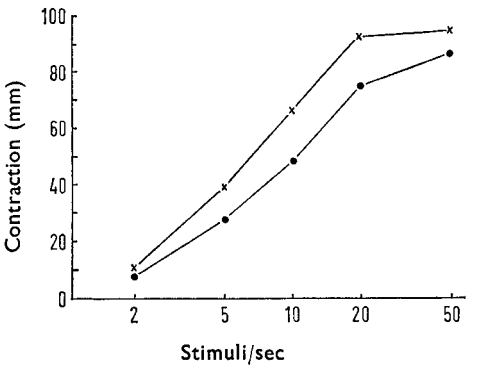

FIG. 1. Mean height of responses (in mm) from 17 preparations of guinea-pig isolated vas deferens stimulated postganglionically at various frequencies to maximal effect (—×—) and for 5 sec at each frequency (—●—).

from those obtained at 20 pulses/sec. The size of the contraction at any one frequency was to some extent dependent on the duration of the stimulation period. Thus, stimulation for periods of 5 sec produced responses that were consistently smaller than those obtained in the same preparations at the same stimulation frequencies in which stimulation was continued until a maximal effect was obtained. The frequency/response curves obtained were nevertheless roughly parallel (Fig. 1).

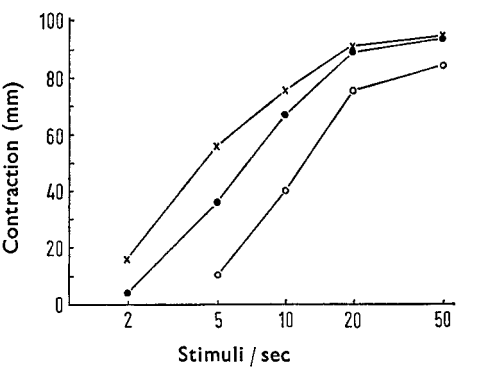

FIG. 2. Mean height of responses (in mm) from 6 vas deferens preparations stimulated preganglionically at various frequencies for 2 sec (—○—), 5 sec (—●—) and to maximal effect (—×—).

Preganglionic stimulation. In 6 preparations frequency/response data were obtained over the frequency range 2 to 50 pulses/sec by stimulating the hypogastric nerve in each preparation for periods of 2 and 5 sec, and

to a maximal effect, at each frequency. As with postganglionic stimulation a 5 sec stimulus period was insufficient to produce a maximal effect at any frequency of stimulation. The difference was more apparent with low rates of stimulation and was even more marked when 2 sec stimulation periods were used. The mean responses from these experiments are plotted in Fig. 2.

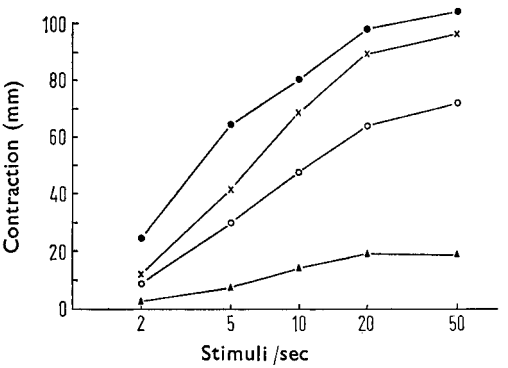

FIG. 3. Effect of dexamphetamine on the mean contraction heights (in mm) from 6 vas deferens preparations stimulated postganglionically to maximal effect at various frequencies; control observations (—×—), and in the presence of 1 μg/ml (—●—), 30 μg/ml (—○—) and 100 μg/ml (—▲—) of dexamphetamine.

Previous workers using this preparation have stimulated the nerve for periods of 2 to 5 sec (Huković, 1961; Morrison & Parkes, 1964; Large, 1965) or for a fixed number of pulses at each frequency (Burn & Weetman, 1963; Edge, 1964). The results plotted in Fig. 2 suggest the possibility that qualitative differences in drug actions obtained by various workers in this preparation may be a consequence of variations in methods of nervous stimulation.

ACTION OF DEXAMPHETAMINE ON THE VAS DEFERENS

Postganglionic stimulation. Dexamphetamine (1–10 μg/ml) caused a potentiation of the responses to all frequencies of sympathetic stimulation whether this was applied for 5 sec periods or continued until a maximal response was obtained. In both instances the enhancement was most marked at low frequencies of stimulation and was greater in the concentration range 1–3 than at 10 μg/ml. In concentrations above 10 μg/ml, dexamphetamine impaired sympathetic responses. Fig. 3 shows graphically the potentiation produced by a low concentration of dexamphetamine (1 μg/ml) and the impairment produced by higher concentrations (30 and 100 μg/ml) on supramaximally stimulated preparations.

The potentiation of responses with the low concentration of dexamphetamine are in accord with the results of Edge (1964) but differ from those obtained by Morrison & Parkes (1964) who reported that this concentration of amphetamine reduced low frequency stimulation (6 to 16 pulses/sec) but potentiated high (20 and 24 pulses/sec).

Preganglionic stimulation. Both Edge (1964) and Morrison & Parkes (1964) used preganglionic stimulation in most of their experiments so it is unlikely that a ganglionic action of amphetamine could account for the disparity in their results. However, Morrison & Parkes (1964) stimulated

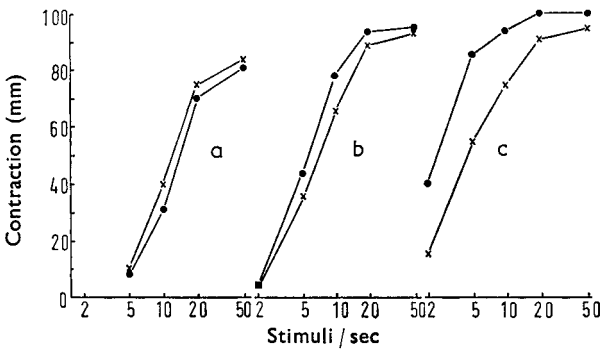

FIG. 4. Modification of the action of dexamphetamine on mean responses from 6 vas deferens preparations caused by changing the duration of the stimulation period. Preganglionic stimulation at various frequencies; control observations (—×—), and in the presence of 1 μg/ml of dexamphetamine (—●—). In 'a' with 2 sec stimulation periods the responses are slightly reduced after dexamphetamine whilst in 'b' with 5 sec periods the responses are slightly enhanced. In 'c' the stimulation was continued until a maximal response was obtained at each frequency, and dexamphetamine caused a potentiation of the responses which was most marked at the lower frequencies.

for fixed periods of 2 to 5 sec at each frequency and thus gave increasing numbers of stimuli with increased frequency whilst Edge (1964) stimulated with 100 pulses at each frequency. To determine whether the divergent results obtained by these workers were explicable on the basis of the differing duration of their stimulation periods, 6 preparations were stimulated over the frequency range 2 to 50 pulses/sec for periods of 2 and 5 sec, and to a maximal effect, both before and in the presence of dexamphetamine (1 μg/ml). The mean results from these experiments are plotted in Fig. 4 and show that both a qualitative and a quantitative change in the action of dexamphetamine occurs with increase in stimulation period.

With 2 sec stimulation periods the responses were slightly but uniformly depressed after dexamphetamine (Fig. 4a), whilst they were slightly enhanced if the stimulation period was extended to 5 sec (Fig. 4b). When the nerve trunk was stimulated until a maximal response was obtained the action of dexamphetamine was to produce a much greater potentiation of responses which was particularly marked at the lower frequencies (Fig. 4c). This latter effect was like that seen with this concentration of dexamphetamine on preparations subjected to supramaximal postganglionic stimulation (Fig. 3).

ANTISYMPATHETIC ACTION OF AMPHETAMINE ON THE VAS DEFERENS

As shown in Fig. 3 dexamphetamine in a concentration of 30 μg/ml or above caused a considerable impairment of the responses to sympathetic

nerve stimulation. Morrison & Parkes (1964) obtained a similar result with this concentration although they did not state which optical isomer of amphetamine they used in their experiments. Using (±)-amphetamine, Edge (1964) reported that concentrations up to 100 μg/ml potentiated the responses whilst 500 μg/ml were necessary to inhibit sympathetic responses. These divergent results suggest the possibility that there might be a marked difference between the blocking efficacy of the (+)- and (−)- form of amphetamine. Accordingly, experiments were made in which the effect of (+)-, (−)- and (±)-amphetamine was compared on the maximal responses to sympathetic nerve stimulation over the frequency range 2 to 50 pulses/sec, using both pre- and post-ganglionic stimulation. In these experiments it was found that both optical isomers and the racemic mixture produced identical results. Thus, each substance potentiated the responses to sympathetic stimulation in concentrations up to 10 μg/ml the effect being most marked at the low frequencies, whilst higher concentrations (30–100 μg/ml) caused impairment which was most marked at the higher stimulation frequencies.

COMPARISON OF THE BLOCKING ACTIONS OF BRETYLIUM AND GUANETHIDINE ON THE VAS DEFERENS

Boura & Green (1962) showed in the cat nictitating membrane preparation that the blocking actions of bretylium and guanethidine could be distinguished because guanethidine caused a preferential block of low frequency stimulation while bretylium preferentially blocked the high frequency. Morrison & Parkes (1964) were unable to confirm this observation in the vas deferens preparation; in their experiments both drugs produced a uniform degree of block at all stimulus frequencies. Since a qualitative difference between the blocking actions of bretylium and guanethidine may provide a clue to their precise mode of action at the sympathetic nerve ending, it was decided to re-examine this phenomenon on this preparation.

Postganglionic stimulation. In these experiments stimulation was applied until a maximal contraction was attained at each frequency over the range 2 to 50 pulses/sec. The effects of bretylium (3 μg/ml) and guanethidine (1 μg/ml) were compared in 12 preparations taken from 6 guinea-pigs; the two drugs being compared on preparations from the same animal in each experiment. Control frequency/response data were obtained initially and again after each drug had been left in the bath for periods of 30–45 min or until a virtually steady state of blockade had been attained. The mean results from these experiments are shown in Fig. 5; a clear qualitative difference was observed between the blocking actions of bretylium and guanethidine over the frequency range 2 to 20 pulses/sec. Bretylium had a relatively greater effect against the higher frequencies and thus flattened the frequency-response curve whilst guanethidine decreased the response at all frequencies causing a parallel shift of the frequency/response curve to the right. At 50 pulses/sec this difference was not so apparent, both drugs causing a greater degree of block than at 20 pulses/sec.

Preganglionic stimulation. Morrison & Parkes (1964) used preganglionic stimulation applied for short periods in their experiments and were unable to show a qualitative difference between the blocking actions of bretylium and guanethidine. In the present investigation their experiments were repeated using preganglionic stimulation but applying stimulation at each frequency until a maximal response was obtained. The results obtained were virtually the same as those shown in Fig. 5 for postganglionic stimulation.

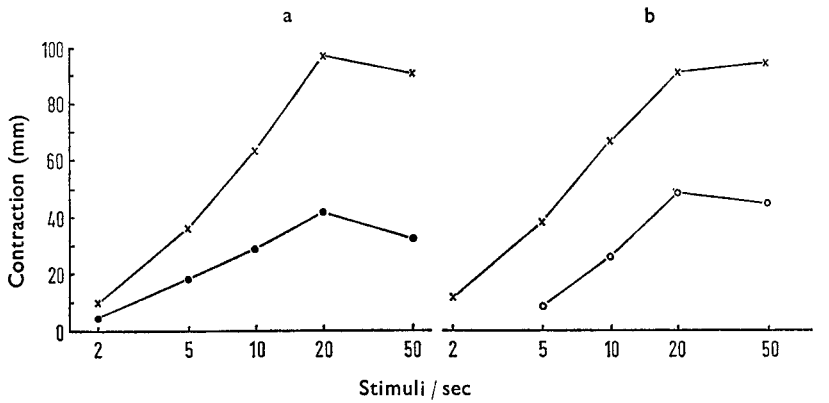

FIG. 5. Differential blocking action of bretylium and guanethidine on mean responses (in mm) from 12 vas deferens preparations taken from 6 guinea-pigs. Contractions in response to postganglionic sympathetic nerve stimulation at various frequencies and continued to maximal effect. In 'a', control responses from 6 preparations (—×—) were impaired most at the higher frequencies after 3 μg/ml of bretylium (—●—). In 'b' the contralateral preparations from 'a' were used and after 1 μg/ml guanethidine (—○—) the control responses (—×—) were preferentially blocked at the lower frequencies.

Reversal of guanethidine and bretylium blockade by dexamphetamine. As shown by previous workers (Day & Rand, 1963; Morrison & Parkes, 1964) dexamphetamine in low concentrations reversed the blocking action of bretylium and guanethidine on the vas. In the present experiments this restoration was found to occur over the full frequency range (2 to 50 pulses/sec) with both bretylium and guanethidine. This is similar to the behaviour of the cat nictitating membrane preparation (Day & Rand, 1963).

Discussion

The results described in this paper indicate that in the isolated vas deferens preparation both qualitative and quantitative differences in the action of a single drug can be obtained by varying the length of the period of electrical stimulation to the sympathetic nerves. Thus, using periods of stimulation of up to 5 sec as described by some workers (Hukovič, 1961; Morrison & Parkes, 1964) the contractions of the preparation are incomplete at all stimulus frequencies. Moreover, if frequency response data are obtained in this way the total numbers of stimuli applied at each frequency differ and consequently the lower frequencies will be more completely submaximal than the higher ones,

causing a steepening of the frequency/response curve. In the present experiments (Fig. 2), with 2 sec stimulus periods, the actual number of stimuli delivered varied between 4 (at 2 pulses/sec) and 100 (at 50 pulses/sec). It would be expected that 100 pulses at any frequency might produce a more nearly maximal response than would 4 pulses. That this was so is shown in Fig. 1; with stimulation periods of 2 or 5 sec the responses to the lower frequencies are smaller than those to the higher frequencies, when compared with the maximal responses obtainable at each frequency with prolonged stimulation.

Another disadvantage of stimulating this preparation for a fixed arbitrary time, irrespective of whether contraction is complete or not, is that drugs which affect the speed of contraction of the preparation may produce qualitatively different effects according to the length of the stimulation period. This is apparently the case with dexamphetamine; Morrison & Parkes (1964) stimulated for 2 to 5 sec and reported that amphetamine depressed the responses to all frequencies of sympathetic stimulation except the highest. In the present experiments a blocking action of low concentrations of dexamphetamine was similarly demonstrated (Fig. 4a) with 2 sec stimulus periods, but this was converted to a slight enhancement if stimulation was extended to 5 sec, and to a marked enhancement if stimulation was applied to maximal effect. The most likely explanation of this phenomenon is that amphetamine slows the rate of contraction of the vas in response to sympathetic stimulation whilst potentiating the actual extent of the contraction. Thus, it seems likely that differing durations of stimulation period could be an important factor in explaining the divergent results obtained by other workers using this preparation.

In the present experiments using dexamphetamine, bretylium or guanethidine the effects of these drugs were unchanged whether pre- or post-ganglionic stimulation was used. However, the presence of ganglionic synapses along the hypogastric nerve trunk is another factor which may account for disparity between the results of different workers using other drugs, as shown by Birmingham & Wilson (1963).

Using maximal stimulation it was shown that dexamphetamine in concentrations of 1 to 10 μg/ml potentiates the effects of sympathetic nerve stimulation the effect being most marked on the lower frequencies, confirming the observation of Edge (1964). However, doses of 30 μg/ml and above depressed the responses to sympathetic nerve stimulation the effect being most marked at the higher frequencies. This latter observation is not in complete agreement with the findings of Edge (1964) who reported that concentrations up to 100 μg/ml of amphetamine potentiated responses whilst 500 μg/ml were necessary to cause a blocking action. The reason for the difference between the blocking potency of amphetamine in the present experiments and those of Edge (1964) is not apparent. It is not due to differing potencies of the optical isomers of amphetamine since in these experiments both optical isomers and the racemic mixture produced indistinguishable effects.

Edge showed that high concentrations of amphetamine produced an

anti-adrenaline effect in addition to blocking the contractions to sympathetic nerve stimulation. However, the fact that high frequencies of stimulation are more impaired than low frequencies suggests some other mechanism perhaps in addition to an anti-adrenaline effect. Day & Rand (1963) postulated that the blocking action of dexamphetamine might be due to a weak adrenergic neurone blocking effect and this is compatible with the recent report that dexamphetamine has a very marked affinity for the noradrenaline uptake site (Iverson, 1964). Concentrations of dexamphetamine below those necessary to impair responses to sympathetic nerve stimulation oppose the blocking action of both bretylium and guanethidine.

The observations of Green and his colleagues (Boura & Green, 1962; Green & Robson, 1964) on the relative blocking efficiency of bretylium and guanethidine against high and low frequencies of sympathetic nerve stimulation have provided what may be an important clue to the precise mode of action of these drugs and perhaps to the phenomenon of tolerance to their effects sometimes met in clinical practice. By using prolonged stimulation to produce a maximal contraction at each stimulation frequency this differential blocking effect has been demonstrated in the vas deferens preparation which may therefore provide a convenient tool for the further examination of this interesting phenomenon.

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References

Bentley, G. A. & Sabine, J. R. (1963). *Brit. J. Pharmacol.*, **21**, 190–201.
Birmingham, A. T. & Wilson, A. B. (1963). *Ibid.*, **21**, 569–580.
Boura, A. L. A. & Green, A. F. (1962). *Ibid.*, **19**, 13–41.
Burn, J. H. Rand, M. J. (1960). *Ibid.*, **15**, 56–66.
Burn, J. H. & Weetman, D. F. (1963). *Ibid.*, **20**, 74–82.
Day, M. D. & Rand, M. J. (1963). *Ibid.*, **20**, 17–28.
Edge, N. D. (1964). *Ibid.*, **22**, 408–414.
Green, A. F. & Robson, R. D. (1964). *Ibid.*, **22**, 349–355.
Huković, S. (1961). *Ibid.*, **16**, 188–194.
Iverson, L. L. (1964). *J. Pharm. Pharmacol.*, **16**, 435–436.
Kuriyama, H. (1963). *J. Physiol. (Lond.)*, **169**, 213–228.
Large, B. J. (1965). *Brit. J. Pharmacol.*, **24**, 194–204.
Morrison, A. B. & Parkes, M. W. (1964). *J. Pharm. Pharmacol.*, **16**, 647–654.
Ohlin, P. & Stromblad, B. C. R. (1963). *Brit. J. Pharmacol.*, **20**, 299–306.