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The effects of calcium on adrenergic neuron blockade

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The effects of increasing extracellular calcium were investigated on the responses to sympathetic nerve stimulation of three isolated organs; rabbit ileum, guinea-pig vas deferens and rabbit ear artery. A rise in the calcium concentration increased the responses of the ileum to low frequency stimulation, the maximum increase being obtained at 8·8 mM calcium. After partial blockade by guanethidine of the responses of the ileum to high frequency stimulation, raised calcium concentrations again increased the responses. The increase was similar in guanethidine-treated and untreated preparations and the maximum increase in both was obtained using 8·8 mM calcium. In the vas deferens and rabbit ear artery preparations an increase in extracellular calcium did not antagonize the blocking action of guanethidine. These experiments do not therefore support the theory that guanethidine acts by preventing the entry of calcium into the sympathetic nerve endings.

The effects of calcium on the release of noradrenaline by sympathetic nerve endings have been studied by several workers using a variety of isolated organ preparations. Kuriyama (1964) provided strong evidence that calcium was necessary for the release of noradrenaline by the sympathetic nerves to the guinea-pig vas deferens. Other evidence to support this postulated role of calcium has been obtained using rabbit ileum (Burn & Gibbons, 1964), cat spleen (Kirpekar & Misu, 1967), rabbit colon (Boullin, 1967) and rabbit ear arteries (Farmer & Campbell, 1967). Burn & Welsh (1967) found that after the responses of the rabbit ileum to sympathetic nerve stimulation had been partially blocked by guanethidine, they could be restored by raising the extracellular calcium concentration. These observations were extended by Kirpekar, Wakade & others (1969) who suggested that adrenergic neuron blockade by guanethidine was due to prevention of the access of calcium to its site of action in the sympathetic nerve ending.

The object of the present experiments was to examine the effects of raising the calcium concentration on adrenergic neuron blockade due to guanethidine in three isolated organs: rabbit ileum, rabbit ear artery and guinea-pig vas deferens.

EXPERIMENTAL

Rabbit ileum

Segments of rabbit ileum with their sympathetic nerves were prepared by the method of Finkleman (1930). A length of ileum 2–3 cm long was set up in a 25 ml bath containing McEwen (1956) solution at 35° and gassed with 5% carbon dioxide in oxygen. Movements of the ileum were recorded with an isotonic frontal writing

lever exerting a tension of 2 g. The periarterial nerves were stimulated with bipolar platinum electrodes for 15 s every 2 min (or for 30 s every 4 min) at frequencies of 3–40 pulses/s using 2 ms pulses at supramaximal voltage.

Guinea-pig vas deferens

The preparation of Huković (1961) was used. The vas deferens was set up in a 25 ml organ bath containing McEwen (1956) solution at 35° gassed with 5% carbon dioxide in oxygen. The hypogastric nerve was stimulated with bipolar platinum electrodes for 5 s every 2 min using 2 ms pulses at 2–20 pulses/s and supramaximal voltage. Contractions of the vas deferens were recorded using an isotonic frontal-writing lever producing four times magnification and exerting a tension of 0·5 g.

Rabbit ear artery

The preparation was dissected and set up as described by de la Lande & Rand (1965). The artery was perfused with McEwen (1956) solution at 37° and gassed with 5% carbon dioxide in oxygen. The flow of perfusion fluid was maintained with a constant volume roller pump at a rate of 6 ml/min and the perfusion pressure was measured using a pressure transducer and a Devices recorder. The periarterial sympathetic nerves were stimulated by means of bipolar platinum ring electrodes placed on the upper end of the vessel. Stimulation was at a rate of 10–20 pulses/s for 5 s every 2 min using 2 ms pulses and supramaximal voltage.

RESULTS

Rabbit ileum

Fig. 1 shows the blocking action of guanethidine on the responses of the rabbit ileum to sympathetic nerve stimulation and reversal of the blockade when the concentration of calcium in the bath was doubled.

In nine experiments, guanethidine (0·25 to 0·5 μg/ml), in contact with the preparation for 60 min, reduced the responses to stimulation of the sympathetic nerves at 20/s from complete inhibition of the pendular movements to 22·1% $\pm$ 1·9% (mean $\pm$ s.e.) inhibition. When the calcium content of the bath fluid was raised the responses of the preparation to sympathetic nerve stimulation were increased, a maximal

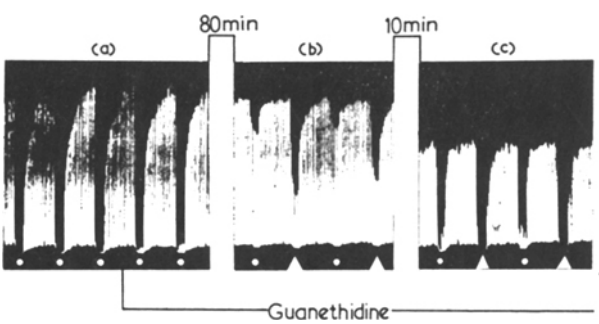

FIG. 1. Rabbit ileum. Effects of guanethidine on responses to stimulation of the sympathetic nerves for 30 s every 4 min using 2 ms pulses at frequencies of 20/s (●) and 40/s (△). In (*a*) and (b) McEwen solution containing 2·2 mM calcium was used. In (c) the calcium concentration was increased to 4·4 mM. Guanethidine dose: 0·4 μg/ml.

increase in the response being obtained in the presence of 8·8 mM calcium. The results of these experiments are shown in Fig. 2a.

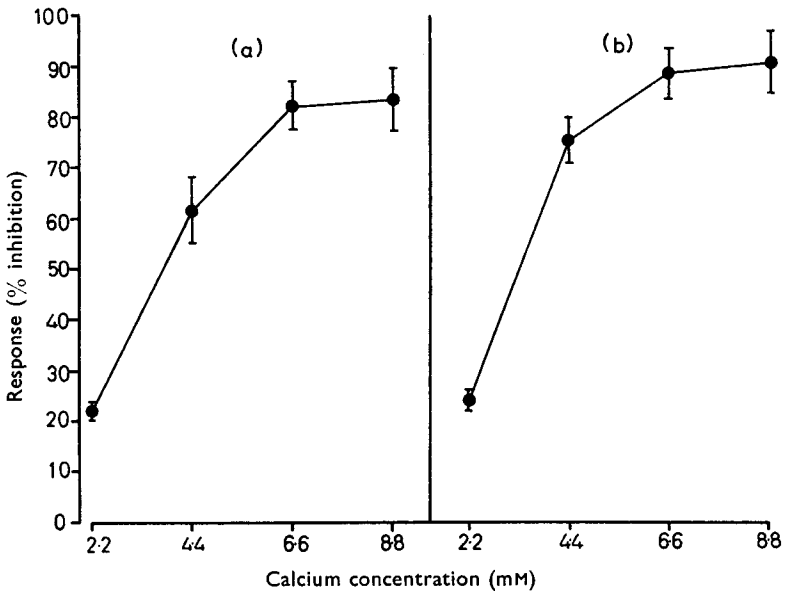

FIG. 2. Rabbit ileum. Responses (percentage inhibition of pendular movements) produced by stimulation of the sympathetic nerves using 2 ms pulses at frequencies stated and supramaximal voltage for 15 s every 2 min. (a) Stimulation at 20/s after exposure to guanethidine (0·25–0·5 μg/ml) for 60 min. (b) Stimulation at between 3/s and 6/s in the absence of guanethidine.

The responses to sympathetic nerve stimulation in the presence of various concentrations of calcium were also investigated in preparations which had not been treated with guanethidine. In twelve experiments the frequency of stimulation was adjusted to produce approximately 25% inhibition of the pendular movements. These responses were therefore similar in size to those obtained previously at a frequency of 20/s after exposure to guanethidine. When the calcium concentration was raised the responses were increased by an amount which was of the same order as in the guanethidine-treated preparations (Fig. 2b). The maximum responses in these preparations also were obtained at a calcium concentration of 8·8 mM. Four experiments were made in which guanethidine was added in the presence of 8·8 mM calcium. The concentration of guanethidine required to block the responses to sympathetic nerve stimulation in these experiments was between 0·25 and 0·5 μg/ml, i.e. the same as the concentration which blocked responses in the presence of 2·2 mM calcium. The blockade produced by guanethidine in the presence of 8·8 mM calcium could not be reversed by raising the calcium concentration still further. As shown in Fig. 3 calcium concentrations of up to 22 mM had no effect on the blocking action of guanethidine. Calcium concentrations higher than 22 mM were not used because of precipitation of calcium above this level.

In four experiments an increase in the calcium concentration from 2·2 to 4·4 mM also produced a small increase in the response of the ileum to added noradrenaline. However, this was insignificant compared with the increase in the response to

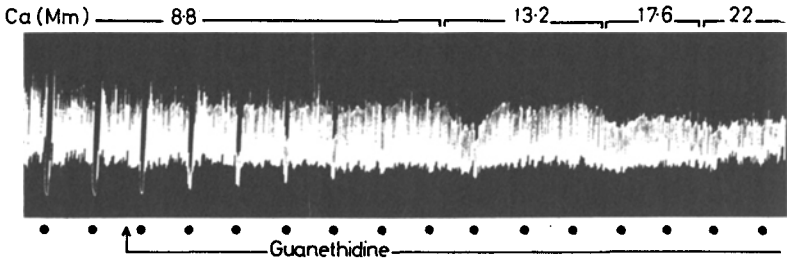

FIG. 3. Rabbit ileum. Effect of guanethidine (0·5 μg/ml) on responses of the rabbit ileum to stimulation with 2 ms pulses at a rate of 4/s for 15 s every 2 min.

sympathetic nerve stimulation. This confirmed the finding of Burn & Gibbons (1964) that the enhancement of the responses to sympathetic nerve stimulation could not be explained by supersensitivity to noradrenaline.

Guinea-pig vas deferens

Responses of the vas deferens to stimulation of the hypogastric nerve were recorded in the presence of calcium concentrations from 1·1 to 13·2 mM. In five preparations out of six the optimal calcium concentration was found to be 2·2 mM (Fig. 4) and in

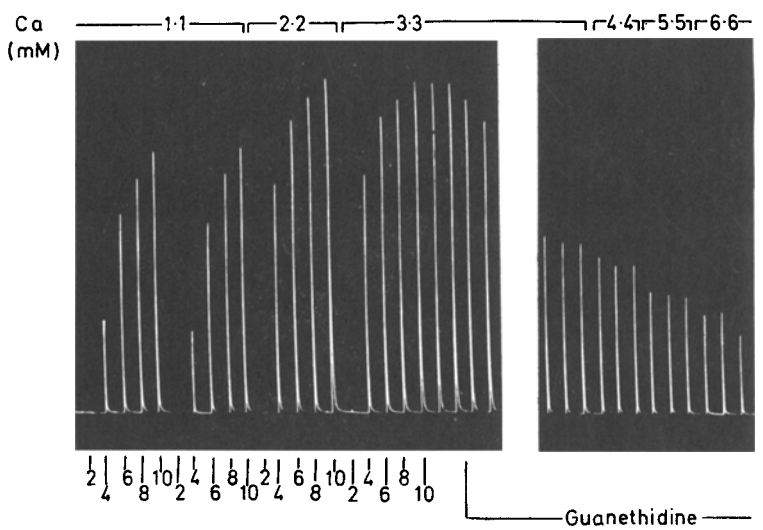

FIG. 4. Guinea-pig vas deferens. Responses to stimulation of the hypogastric nerve at rates of 2–10/s (responses where no frequency is indicated are to 10/s) for 5 s every 2 min using 2 ms pulses at maximal voltage. The record shows the effect of guanethidine (1 μg/ml) at various calcium concentrations. The right and left hand records are separated by an interval of 30 min.

one experiment it was 4·4 mM. In the experiment shown in Fig. 4 guanethidine (1 μg/ml) was added in the presence of a supramaximal concentration of calcium (3·3 mM). The responses were reduced by 50% in about 30 min, and the calcium concentration was then raised by 1·1 mM steps to 6·6 mM. This increase in calcium concentration did not diminish the blocking action of guanethidine. Similar results were obtained in four other experiments carried out in this way. When the calcium

concentration was increased above 6·6 mM, the blocking action of guanethidine appeared to be intensified (Fig. 5). However, the responses to hypogastric nerve stimulation were also reduced by calcium concentrations of 8·8 to 13·2 mM in preparations not treated with guanethidine.

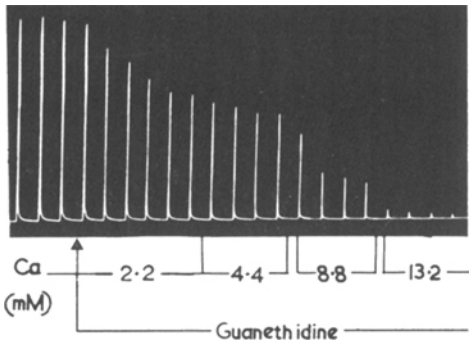

FIG. 5. Guinea-pig vas deferens. Responses to stimulation of the hypogastric nerve for 5 s every 2 min using 2 ms pulses at a frequency of 16/s. The record shows the effect of varying the calcium concentration on the action of guanethidine (0·5 μg/ml).

Rabbit ear artery

In experiments using the rabbit isolated ear artery a partial blockade of the responses to sympathetic nerve stimulation was produced by perfusing with 0·5–1 μg/ml guanethidine. An increase in the calcium concentration of the perfusion fluid to 4·4 or 8·8 mM had no effect on the blockade produced by guanethidine. A further increase in calcium concentration to 13·2 mM reduced the responses (Fig. 6).

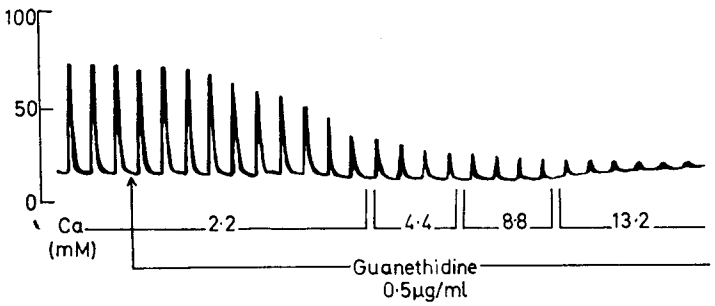

FIG. 6. Rabbit ear artery. Responses to stimulation of the periarterial sympathetic nerves for 5 s every 2 min using 2 ms pulses at a frequency of 14/s. The scale indicates the perfusion pressure in mm Hg. The record shows the effect of increasing the calcium concentration on the adrenergic neuron blockade produced by guanethidine (0·5 μg/ml).

DISCUSSION

The experiments reported here, using the rabbit ileum, confirm the findings of Burn & Welsh (1967). Thus, after a partial blockade of the responses of the ileum to sympathetic nerve stimulation by guanethidine, the responses could be restored to their original size by a 2- to 4-fold increase in the concentration of calcium in the organ bath. This led Burn & Welsh (1967) to suggest that guanethidine acted by preventing the entry of calcium into the sympathetic nerve ending, a process which is believed to be necessary for the release of noradrenaline from sympathetic nerves

(Kirpekar & Misu, 1967; Boullin, 1967). However, doubling the calcium concentration of the bath fluid (from 2·2 to 4·4 mM) increases responses of the rabbit ileum to sympathetic nerve stimulation even in the absence of guanethidine by providing an optimal calcium concentration. It therefore seems possible that the apparent reversal of the blocking action of guanethidine is due simply to physiological antagonism. The enhancement of sympathetic inhibition by providing optimal calcium concentration merely opposes the effect of guanethidine.

In the present experiments an approximately 25% inhibition of the ileum was obtained either by low frequency stimulation of the sympathetic nerves in the absence of guanethidine or by high frequency stimulation after 60 min exposure to guanethidine. The amounts by which these responses were increased when the calcium concentration was raised from 2·2 to 4·4, 6·6 and 8·8 mM were similar whether or not guanethidine was present. In some experiments, pieces of ileum were set up in McEwen solution containing 8·8 mM calcium, the optimal concentration for responses to sympathetic nerve stimulation in this preparation. In spite of the high calcium content of the bath fluid, guanethidine blocked the responses in the same concentration (0·25 to 0·5 μg/ml) as in 2·2 mM calcium, and a further increase in calcium concentration to 22 mM produced no reversal of the blockade.

Experiments using the isolated hypogastric nerve—vas deferens of the guinea-pig also provided no evidence for a specific antagonism between calcium and guanethidine. This preparation differed from the rabbit ileum in having a lower calcium requirement for the production of maximal responses to sympathetic nerve stimulation. In the vas deferens, maximal responses were obtained in McEwen solution containing the usual calcium concentration of 2·2 mM. When experiments were made using a supramaximal calcium concentration (3·3 mM) no reversal of the blocking action of guanethidine could be produced by further increases in calcium concentration. When the calcium concentration was raised to 8·8 mM or above, the contractions of the vas deferens were depressed whether or not guanethidine was present. This may be due to the stabilizing effect of calcium on the smooth muscle membrane. These experiments with the vas deferens are more difficult to interpret because the hypogastric nerve is preganglionic and the effect of calcium on ganglionic transmission must be taken into account.

A similar lack of antagonism between calcium and guanethidine was seen using the isolated artery preparation. This preparation has been shown by Farmer & Campbell (1967) to respond maximally to sympathetic nerve stimulation when perfused with a solution containing 4·4 mM calcium. Thus, if the antagonism between calcium and guanethidine is "physiological" (i.e. they are producing opposing effects) a two-fold increase in the calcium content of the McEwen solution might be expected to oppose the blocking action of guanethidine, whereas higher calcium concentrations would have no further effect. In the above experiments, however, increases in calcium concentration up to 13·2 mM did not antagonize the action of guanethidine.

The experiments reported here show that the optimal calcium concentration for responses of isolated organs to sympathetic nerve stimulation varies from one preparation to another. Thus the responses of the vas deferens to sympathetic stimulation were not enhanced by increased calcium, since McEwen solution in which the preparation is set up, already contains the optimal calcium concentration.

However, this solution contains only 25% of the optimal concentration for the responses of the rabbit ileum to sympathetic nerve stimulation, so that a rise in calcium concentration increases these responses. These observations suggest that the reversal of the blocking action of guanethidine by calcium is due to physiological antagonism and do not support the theory that guanethidine acts by limiting the access of calcium to its site of action in the sympathetic nerve endings.

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REFERENCES

BOULLIN, D. J. (1967). *J. Physiol., Lond.*, **189**, 85–99.
BURN, J. H. & GIBBONS, W. R. (1964). *Br. J. Pharmac. Chemother.*, **22**, 540–548.
BURN, J. H. & WELSH, F. (1967). *Ibid.*, **31**, 74–81.
DE LA LANDE, I. S. & RAND, M. J. (1965). *Aust. J. exp. Biol. med. Sci.*, **43**, 639–656.
FARMER, J. B. & CAMPBELL, I. K. (1967). *Br. J. Pharmac. Chemother.*, **29**, 319–328.
FINKLEMAN, B. (1930). *J. Physiol., Lond.*, **70**, 145–157.
HUKOVIĆ, S. (1961). *Br. J. Pharmac. Chemother.*, **16**, 188–194.
KIRPEKAR, S. M. & MISU, Y. (1967). *J. Physiol., Lond.*, **188**, 219–234.
KIRPEKAR, S. M., WAKADE, A. R., DIXON, W. & PRAT, J. C. (1969). *J. Pharmac. exp. Ther.*, **165**, 166–175.
KURIYAMA, H. (1964). *J. Physiol., Lond.*, **175**, 211–230.
MCEWEN, L. M. (1956). *Ibid.*, **131**, 678–689.