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CALCIUM AND MAGNESIUM IONS: INFLUENCE ON THE RESPONSE OF AN ISOLATED ARTERY TO SYMPATHETIC NERVE STIMULATION, NORADRENALINE AND TYRAMINE

BY

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Alterations in the external Ca^{2+} and/or Mg^{2+} concentrations of physiological salt solutions have been shown to modify the response of isolated tissues to sympathetic nerve stimulation. In the isolated vas deferens–hypogastric nerve preparation of the rat (Boeles, Irausquin-cath & Veldsema, 1963) and guinea-pig (Kuriyama, 1964) and in the isolated rabbit ileum-periarterial nerve preparation (Burn & Gibbons, 1964) an increase in the external Ca^{2+} concentration enhances the response to nerve stimulation. A decrease in the Ca^{2+} concentration or increase in the Mg^{2+} concentration reduces the response.

The aim of the present investigation was to examine the effect of altered external Ca^{2+} and/or Mg^{2+} concentrations on the response of the isolated central ear artery preparation of the rabbit to sympathetic periarterial nerve stimulation and to noradrenaline and tyramine. De la Lande & Rand (1965), who first described this preparation, showed that the artery constricted in response to periarterial nerve stimulation or noradrenaline. Tyramine (Farmer, 1966) was shown to produce a biphasic constriction which was partly due to a direct action and partly due to noradrenaline released from adrenergic nerves.

METHODS

Lop-eared or semi-lop-eared rabbits weighing 2–5 kg were anaesthetized with pentobarbitone 30 mg/kg injected intravenously. The central ear artery was cannulated *in situ* and removed according to the method described by de la Lande & Rand (1965). The arterial segment, usually 4–5 cm long, was immersed in McEwen solution, and the artery lumen was perfused with the same solution, delivered by means of a constant output pump (Watson–Marlow). Both the bathing and the perfusion solutions were maintained at 37° C and gassed with 95% O_2 and 5% CO_2. Perfusion pressure was measured with a Devices blood pressure transducer coupled to a Devices multi-channel recorder. Periarterial nerve stimulation was affected by means of bipolar platinum electrodes as described by Burn & Rand (1960). Trains of impulses of supramaximal strength and 1 msec pulse width were delivered from a Palmer electronic square-wave stimulator. Each train was of 5 sec duration and the artery was subjected to repeated trains of stimulation at 1, 2, 5, 10 and 20 c/s. Drugs, dissolved in McEwen solution, were injected into the solution perfusing the lumen,

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through a rubber connexion close to the artery. In all experiments the volume of each injection was 0.02 ml. Injections of tyramine (20 and 200 μg) and noradrenaline (2 and 20 ng) were given in addition to the trains of stimulation. Changes in Ca^{2+} and/or Mg^{2+} concentrations were made both in the solution perfusing the lumen and in the bathing solution. McEwen solution is Mg^{2+}-free, and when the effects of this ion were studied, hydrated magnesium chloride was added to the McEwen solution. The possibility was considered that pH changes might account for the effects observed on changing the Ca^{2+} and Mg^{2+} concentrations. The maximum pH change was 0.2 u. as measured by a Pye pH meter and this was considered insufficient to account for the results obtained.

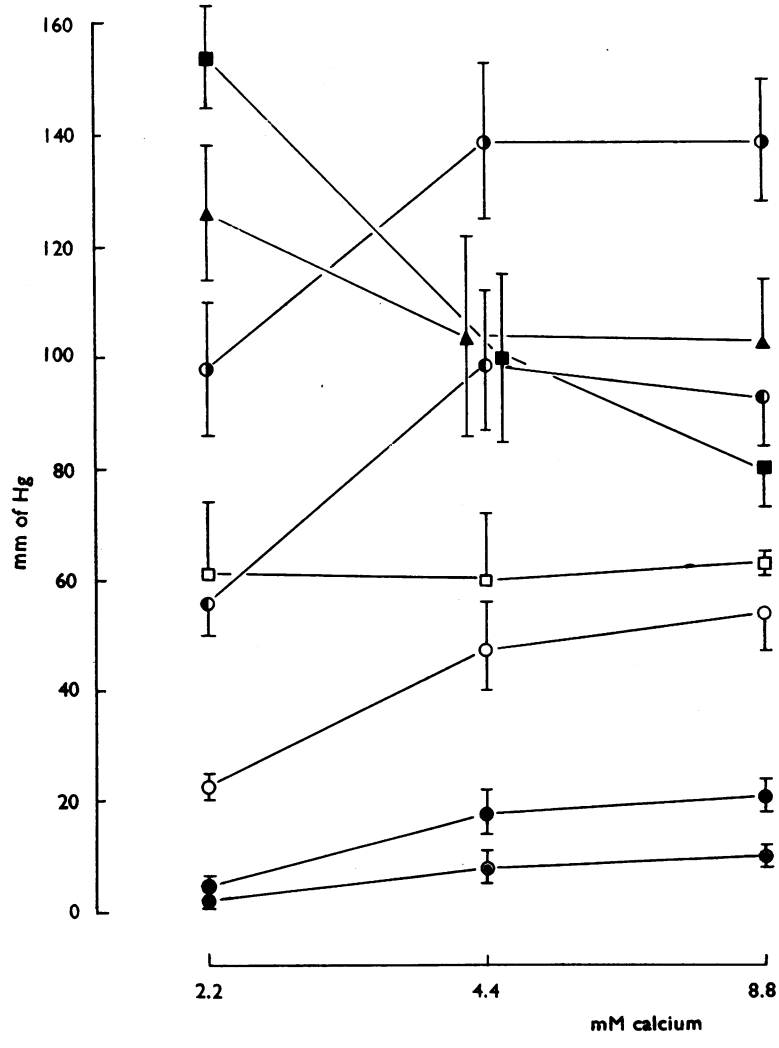

Fig. 1. The effects of increased Ca^{2+} concentrations on responses of the isolated central ear artery preparations of the rabbit to periarterial sympathetic nerve stimulation, noradrenaline and tyramine. The points plotted are the mean of eight experiments, and standard errors are shown. ⊙ 1 c/s, ● 2 c/s, ○ 5 c/s, ◐ 10 c/s, ◑ 20 c/s, ▲ 20 ng noradrenaline, □ 200 μg tyramine, direct phase, ■ 200 μg tyramine, indirect phase.

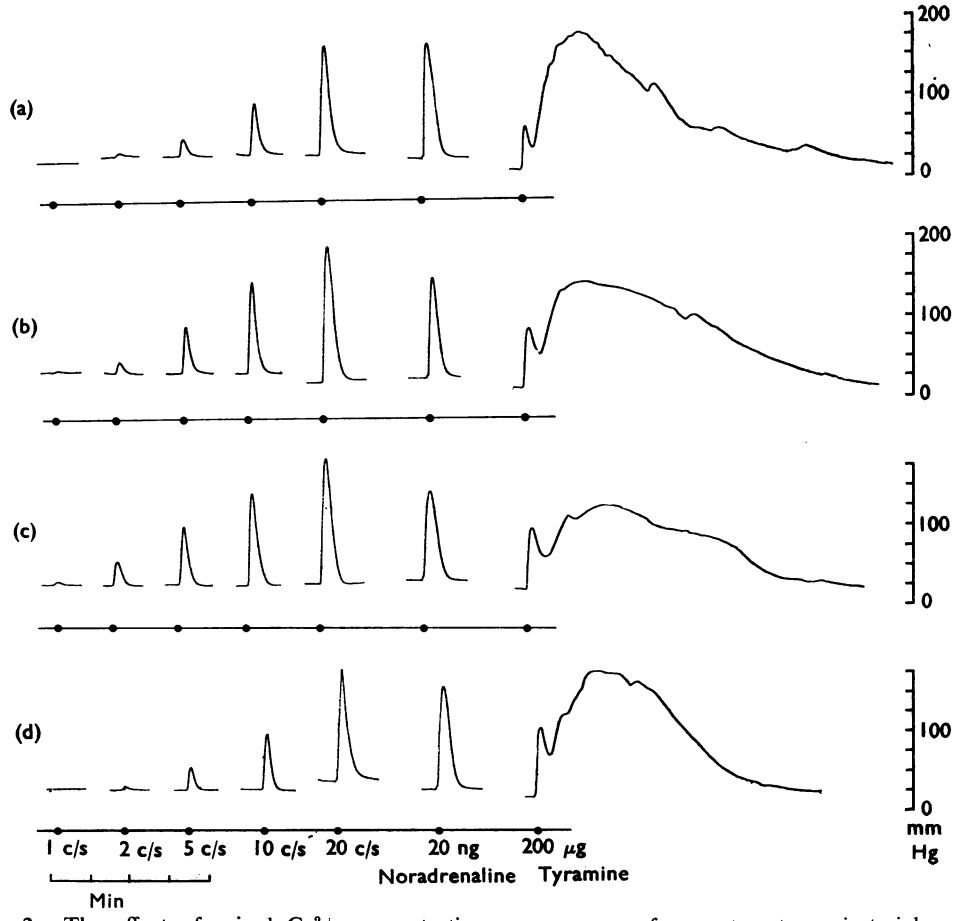

Fig. 2. The effect of raised Ca^{2+} concentration on response of an artery to periarterial nerve stimulation at 1, 2, 5, 10 and 20 c/s, 20 ng noradrenaline and 200 μg tyramine. (a) Control responses obtained at a Ca^{2+} concentration of 2.2 mM. (b) Responses obtained 30 min after changing to a Ca^{2+} concentration of 4.4 mM. (c) Responses obtained 30 min after changing to a Ca^{2+} concentration of 8.8 mM. (d) Responses obtained 30 min after return of tissue to normal McEwen solution (2.2 mM calcium).

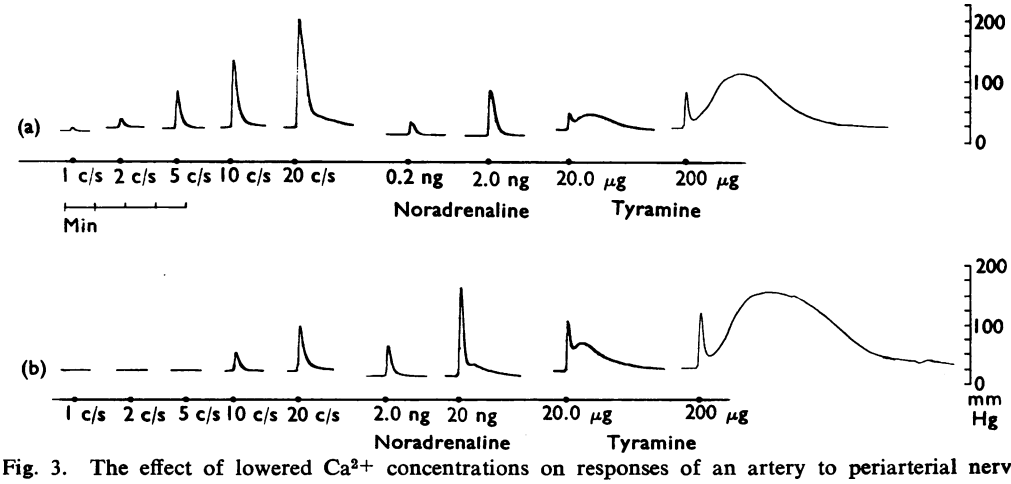

Fig. 3. The effect of lowered Ca^{2+} concentrations on responses of an artery to periarterial nerve stimulation at 1, 2, 5, 10 and 20 c/s, 2.0 and 20 ng noradrenaline and 20 and 200 μg tyramine. (a) Control responses obtained at a Ca^{2+} concentration of 2.2 mM. (b) Responses obtained 30 min after changing to a Ca^{2+} concentration of 0.55 mM.

RESULTS

Changes in Ca^{2+} concentration

In eight preparations the responses to periarterial nerve stimulation at all frequencies used were enhanced by an increase in the external Ca^{2+} concentration from 2.2 to 4.4 mM (Figs. 1 and 2). Further increase in the Ca^{2+} concentration from 4.4 to 8.8 mM did not further enhance the response to nerve stimulation (Figs. 1 and 2c). These changes in Ca^{2+} concentration were without consistent effect on the response of the artery to noradrenaline and on the direct phase of the response to tyramine (Fig. 1). However, the indirect phase of the response to tyramine was approximately halved by increasing the Ca^{2+} concentration to 4.4 mM and 8.8 mM (Fig. 2b and c). The effects of raised

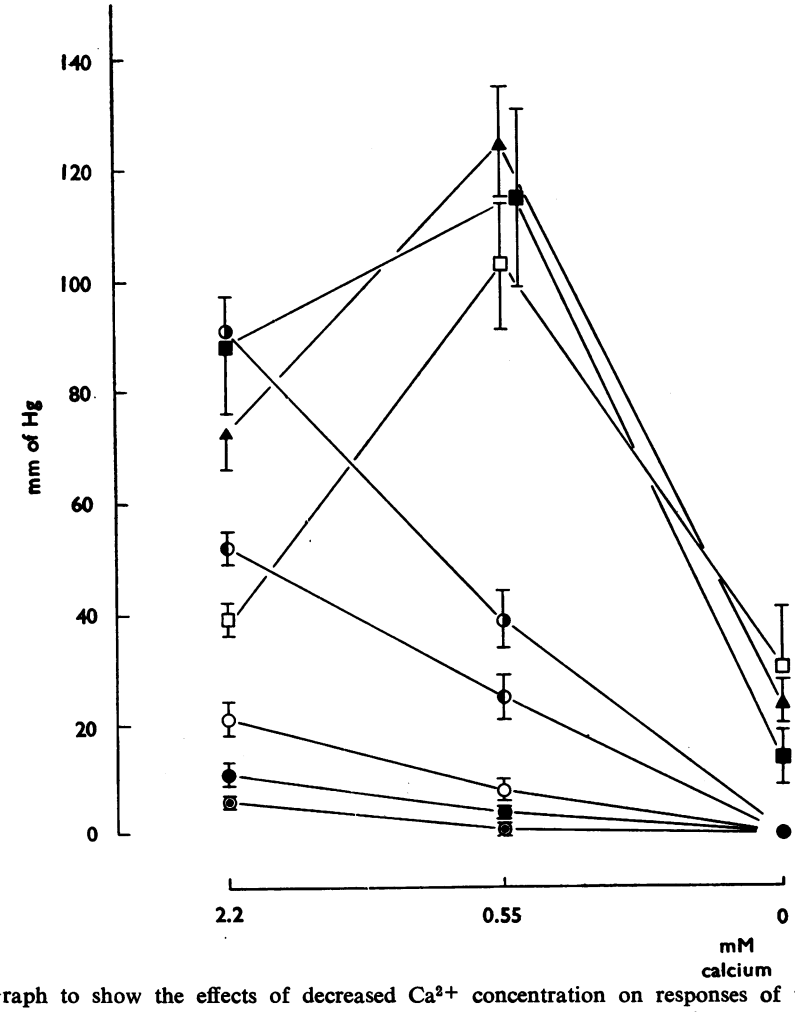

Fig. 4. Graph to show the effects of decreased Ca^{2+} concentration on responses of the isolated central ear artery preparation of the rabbit to periarterial sympathetic nerve stimulation, noradrenaline and tyramine. The points plotted are the mean of 12 experiments, and standard errors are shown. ⊙ 1 c/s, ● 2 c/s, ○ 5 c/s, ◐ 10 c/s, ◑ 20 c/s, ▲ 20 ng noradrenaline, □ 200 μg tyramine, direct phase, ■ 200 μg tyramine, indirect phase.

Ca^{2+} concentration on responses to nerve stimulation, noradrenaline and tyramine were reversible when the preparations were returned to normal McEwen solution (Fig. 2d). In one experiment the Ca^{2+} concentration was increased to 17.6 mM ; at this concentration a white precipitate formed and all responses to drugs and nerve stimulation were impaired.

In 12 preparations the responses to periarterial nerve stimulation at all frequencies used were decreased when the external Ca^{2+} was lowered to 0.55 mM (Figs. 3b and 4). No response to periarterial nerve stimulation was obtained in the absence of Ca^{2+} (Fig. 5b). In contrast to the effect on periarterial nerve stimulation the responses of the artery to noradrenaline and tyramine (direct and indirect phases) were enhanced when the Ca^{2+} concentration was reduced to 0.55 mM (Figs. 3b and 4). In the absence of Ca^{2+} the responses to noradrenaline and tyramine were considerably less than those obtained in normal calcium concentration (Figs. 4 and 5b). Arteries perfused with calcium-free solution for 3 hr still gave a small response to noradrenaline and tyramine (Fig. 5b). The effect of decreased calcium concentrations could be reversed by returning the preparation to a solution which contained 2.2 mM Ca^{2+} (Fig. 5c).

Interaction of Ca^{2+} and Mg^{2+}

In 10 preparations responses to all frequencies of periarterial nerve stimulation investigated were reduced by a concentration of 10 mM Mg^{2+} (Figs. 6 and 7b). Similarly, the responses to noradrenaline, and both phases of the responses to tyramine, were also reduced (Figs. 6 and 7b). An increase in the Ca^{2+} concentration from 2.2 to 4.4 mM in

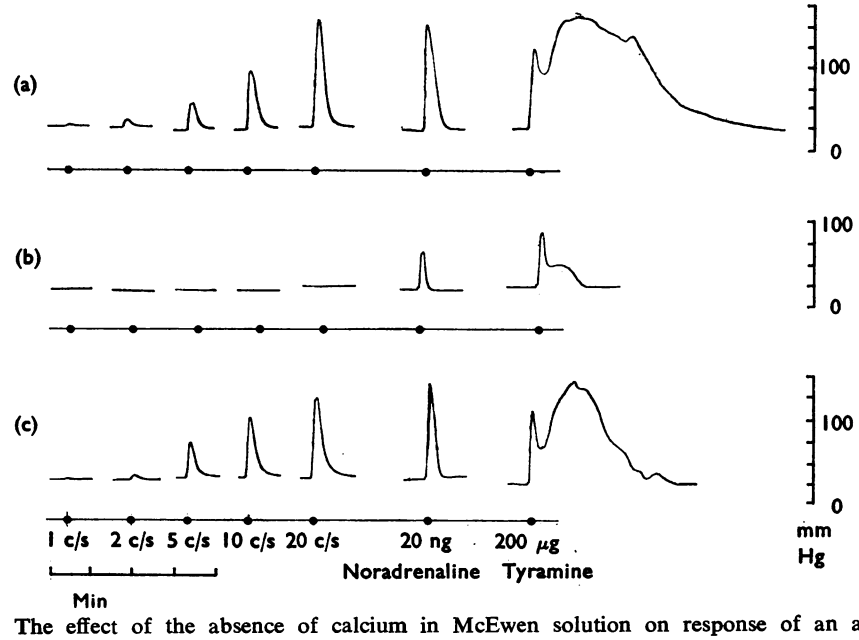

Fig. 5. The effect of the absence of calcium in McEwen solution on response of an artery to periarterial nerve stimulation at 1, 2, 5, 10 and 20 c/s, 20 ng noradrenaline, and 200 μg tyramine. (a) Control responses obtained at a Ca^{2+} concentration of 2.2 mM. (b) Responses obtained 3 hr after changing to calcium-free McEwen solution. (c) Responses obtained 30 min after the return of the tissue to normal McEwen solution (2.2 mM calcium).

the presence of 10 mM Mg^{2+} produced a partial restoration of the responses to periarterial nerve stimulation, to noradrenaline and to the direct phase of the response to tyramine (Figs. 6 and 7c). The indirect phase of the response to tyramine, depressed by 10 mM Mg^{2+}, could not be reversed by an increase in the external Ca^{2+} concentration (Figs. 6 and 7c). In fact an increased depression of responses was observed. However, all the effects of increased Mg^{2+} concentration were reversed when the tissue was returned to Mg^{2+} free solutions (Fig. 7d).

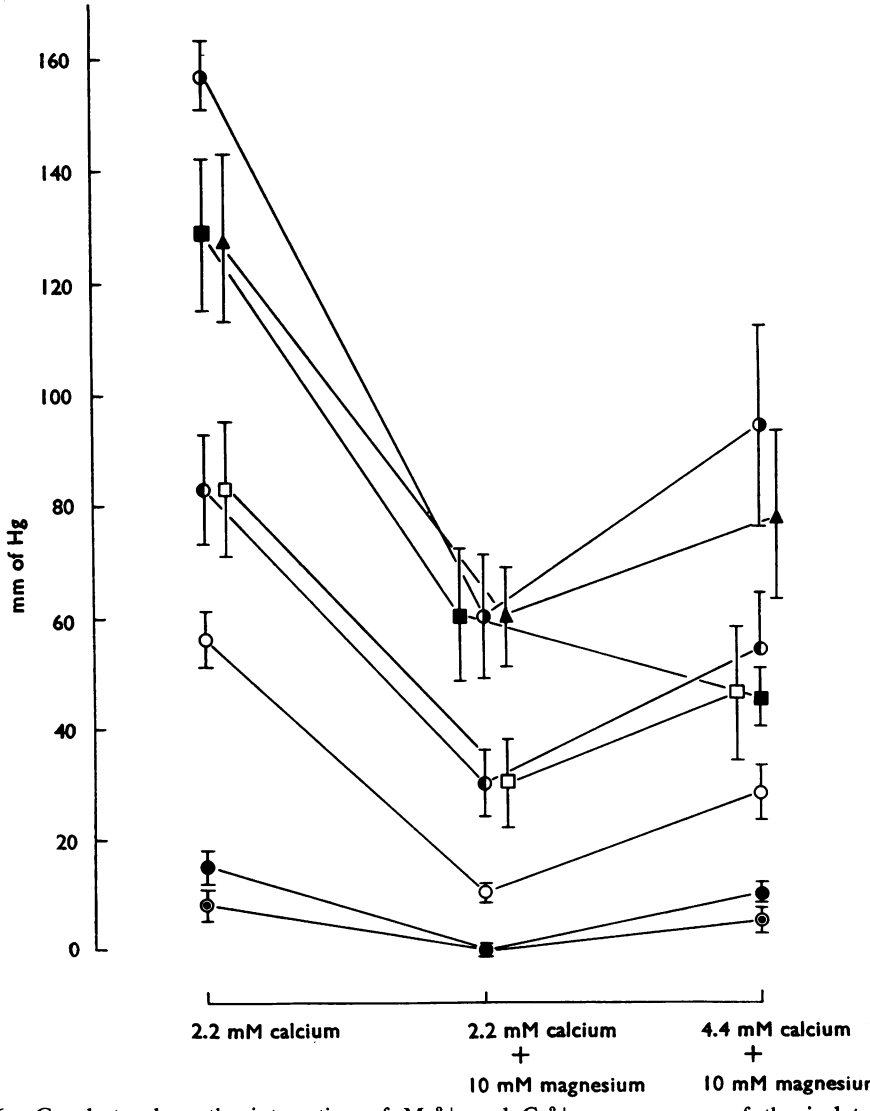

Fig. 6. Graph to show the interaction of Mg^{2+} and Ca^{2+} on responses of the isolated central ear artery preparation of the rabbit to periarterial sympathetic nerve stimulation, noradrenaline and tyramine. The points plotted are the mean of 12 experiments, and standard errors are shown. ⊙ 1 c/s, ● 2 c/s, ○ 5 c/s, ◐ 10 c/s, ◑ 20 c/s, ▲ 20 ng noradrenaline, □ 200 μg tyramine, direct phase, ■ 200 μg tyramine, indirect phase.

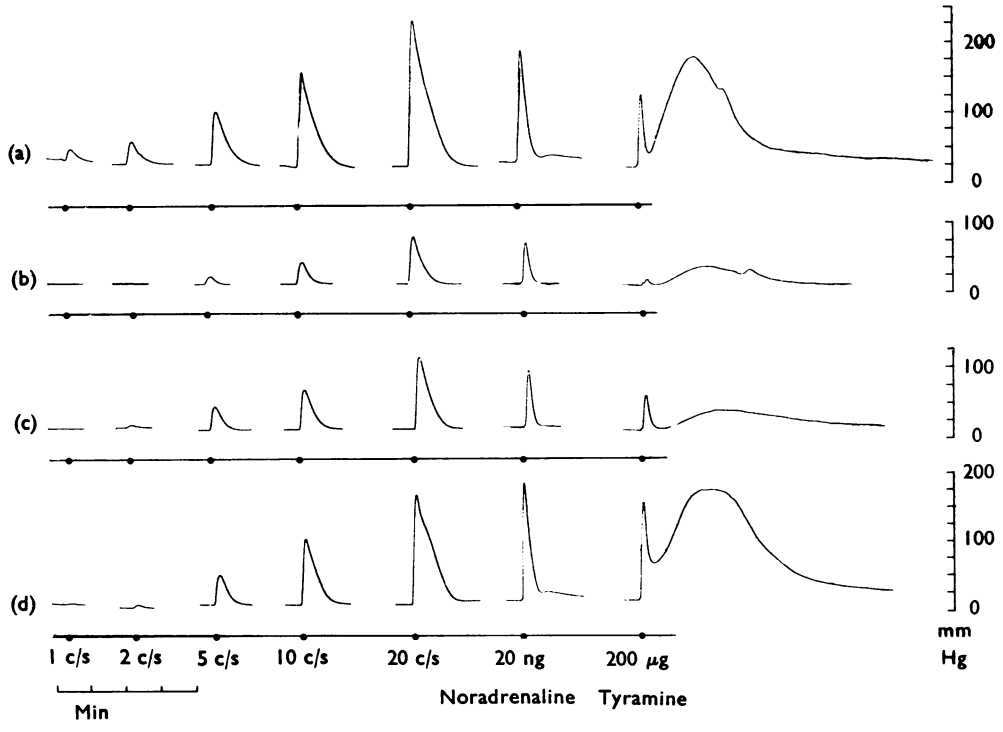

Fig. 7. The effect of the addition of Mg^{2+} and Ca^{2+} on responses of an artery to periarterial nerve stimulation at 1, 2, 5, 10 and 20 c/s, 20 ng noradrenaline and 200 μg tyramine. (a) Control responses obtained at a Ca^{2+} concentration of 2.2 mM. (b) Responses obtained 30 min after changing to McEwen solution with 10 mM Mg^{2+} added. (c) Responses obtained 30 min after changing to McEwen solution containing 10 mM Mg^{2+} and 4.4 mM Ca^{2+}. (d) Responses obtained 30 min after washing bath with normal McEwen solution (2.2 mM calcium).

DISCUSSION

Burn & Gibbons (1964) observed that the inhibitory response of the rabbit ileum to periarterial nerve stimulation was progressively enhanced by concentrations of Ca^{2+} up to 17.6 mM. The response of the ear artery of the rabbit to periarterial nerve stimulation was enhanced when the Ca^{2+} concentration was raised from 2.2 to 4.4 mM but further increases in the Ca^{2+} concentration produced no further increase in the response to nerve stimulation. Also changes in the external Ca^{2+} concentration were shown to alter the sensitivity of the artery to injected noradrenaline. Since the changes in the responses of the artery to periarterial nerve stimulation were not affected in parallel with the response of the artery to injected noradrenaline it may be assumed that Ca^{2+} ions exert a specific action on adrenergic neurones. The likely explanation of this action is that there is an increase in the amount of noradrenaline liberated from the nerve when the concentration of Ca^{2+} is raised and a decrease when the Ca^{2+} concentration is reduced. The effect of increased Ca^{2+} concentration could also be seen in experiments in which the responses of the artery to sympathetic stimulation were depressed by raised

Mg^{2+} concentration. An increase in the Ca^{2+} concentration from 2.2 to 4.4 mM partly overcame the effect of added Mg^{2+}. Katz (1962) has reviewed the effect of Ca^{2+} and Mg^{2+} on neuromuscular transmission in mammalian and amphibian skeletal muscle. The release of acetylcholine from motor nerves is proportional to the Ca^{2+} concentration. Toda, Fujiwara & Shimamoto (1964) observed that Ca^{2+} augmented the inhibitory actions of vagal stimulation in isolated rabbit and guinea-pig atria. Attention has also been drawn to the similar effects that Ca^{2+} ions have on the release of noradrenaline (by acetylcholine) from the perfused adrenal gland and their effect on post-ganglionic sympathetic transmission to the ileum (Burn & Gibbons, 1964). All these observations are compatible with the proposal that Ca^{2+} availability is essential for both acetylcholine and noradrenaline release from nerve endings. The present results confirm that Ca^{2+} availability is a controlling factor in the release of noradrenaline from sympathetic nerves.

The mobilization of noradrenaline from tissue stores by tyramine was also affected when the external Ca^{2+} and Mg^{2+} concentrations were altered. The changes in the direct and indirect responses of the artery to tyramine could not be explained entirely on the basis of altered sensitivity of the artery to injected noradrenaline. When the external Ca^{2+} concentration was raised to 4.4 mM and then to 8.8 mM, the indirect phase of the response to tyramine was progressively reduced at a time when the direct phase of the response to tyramine was unaffected and that to noradrenaline was only marginally reduced. This specific action of increased Ca^{2+} concentrations in reducing the indirect sympathomimetic action of tyramine may indicate impeded uptake of tyramine into tissue stores within the adrenergic neurones. In addition, it may be implied that the release of noradrenaline by tyramine does not involve a cholinergic mechanism as has been proposed for the release of noradrenaline from sympathetic nerves (Burn & Rand, 1959). At 10 mM Mg^{2+} concentration both the direct and indirect phases of the response to tyramine were reduced; however elevation of Ca^{2+} concentration reversed the direct phase but further decreased the indirect phase of the response to tyramine. Thus, Ca^{2+} ions were found to antagonize the effect of Mg^{2+} ions on responses of the artery to nerve stimulation or to noradrenaline but not the effect of Mg^{2+} on release of endogenous noradrenaline by tyramine.

The effect of lowered Ca^{2+} concentrations on the response of the artery to both the direct and indirect phases of tyramine could be explained in terms of a change in sensitivity of the effector cells to noradrenaline since both phases of the response to tyramine and the response to injected noradrenaline were greatly enhanced. In Ca^{2+}-free solutions the responses to noradrenaline and tyramine (both phases) were reduced but not abolished even after perfusion for 3 hr. Whilst these conditions are unphysiological it is of interest that many workers have observed complete loss of the contractile response of isolated muscle in the absence of Ca^{2+} (Edman & Schild, 1961, 1962; Bohr, 1963). It is well established that Ca^{2+} ions are essential for the contraction of smooth muscle and it may be that the persistence of the response to noradrenaline in this preparation is due to Ca^{2+} ions being bound to " critical sites " within the muscle (Bohr, 1964) and these sites may not easily be exhausted.

The experiments described in this paper point to an important role for Ca^{2+} in the maintenance of normal levels of excitability of adrenergic neurones and arterial smooth muscle cells. The results are interpreted as meaning that alteration in the external

Ca^{2+} concentration produces specific action on the mobilization of noradrenaline by sympathetic nerve stimulation and tyramine, and on the responsiveness of the arterial muscle to α-adrenergic stimulation.

SUMMARY

1. The effects of altered Ca^{2+} and Mg^{+2} concentrations on responses to periarterial nerve stimulation, noradrenaline and tyramine have been investigated in the isolated central ear artery of the rabbit.

2. Reducing the Ca^{2+} concentration of the McEwen solution to 0.55 mM (25% normal) produced a reduction in responses to nerve stimulation at 1, 2, 5, 10 and 20 c/s. The responses to tyramine and noradrenaline were potentiated.

3. Complete removal of Ca^{2+} caused complete inhibition of the responses to nerve stimulation at all frequencies used. The responses to noradrenaline and tyramine were greatly reduced but were never completely abolished.

4. An increase in the Ca^{2+} concentrations to 4.4 mM produced a potentiation of responses to sympathetic stimulation. The response to noradrenaline and the direct phase of the response to tyramine were not significantly altered. The indirect phase of the response to tyramine was reduced by increasing Ca^{2+} concentration.

5. Adding 10 mM Mg^{2+} to the McEwen solution greatly reduced the responses to sympathetic stimulation, noradrenaline and tyramine. Doubling the Ca^{2+} concentration to 4.4 mM partially reversed the blockade of responses to sympathetic stimulation, to noradrenaline and the direct phase of the response to tyramine, but further increased the blockade of the indirect phase of tyramine.

6. It is concluded that the actions of Ca^{2+} and Mg^{2+} on sympathetic transmission are basically similar to the actions of these ions on skeletal neuro-muscular transmission. It is also concluded that these ions play an important role in determining the responsiveness of arterial smooth muscle to directly and indirectly acting sympathomimetic amines.

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