J. Physiol. (1980), **305**, *pp.* 59–71
With 8 text-figures
Printed in Great Britain

BARIUM AND STRONTIUM CAN SUBSTITUTE FOR CALCIUM IN NORADRENALINE OUTPUT INDUCED BY EXCESS POTASSIUM IN THE GUINEA-PIG

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(*Received 4 September 1979*)

SUMMARY

1. The ability of Ba^{2+} and Sr^{2+} to substitute for Ca^{2+} in the noradrenaline output induced by excess K^+ was examined using isolated guinea-pig vas deferens.

2. When the vas deferens was repeatedly exposed to excess K^+ (60 mM) at 40 min intervals, the noradrenaline output increased at least three-fold in incubation medium which contained either Ca^{2+}, Ba^{2+} or Sr^{2+}. The response decreased on repetition. The order of effectiveness was roughly $Ba^{2+} > Ca^{2+} > Sr^{2+}$.

3. In the absence of excess K^+, these cations had no significant stimulating effect on the noradrenaline output even when added after exposure to Ca^{2+}-free solution.

4. As the concentration of divalent cation was increased from 0·2 to 2·5 mM the noradrenaline output induced by excess K^+ increased. The maximum noradrenaline output was achieved at a divalent cation concentration of 2·5 mM and was 29·56 ± 3·52, 15·02 ± 1·12 and 7·45 ± 0·84 (mean ± S.E. of mean) n-mole/g per hr in the presence of either Ba^{2+}, Ca^{2+} or Sr^{2+}, respectively. Further increase in the concentration of the cations reduced the response.

5. The addition of either Sr^{2+} (2 mM) or Ca^{2+} (1 mM) to a solution containing various concentrations of Ba^{2+} facilitated the K^+-induced increase in the noradrenaline output when the Ba^{2+} concentration was low, but inhibited release of noradrenaline when higher concentrations of Ba^{2+} were used. The addition of Sr^{2+} (1 mM) to Ca^{2+}-containing solutions had a similar effect.

6. Mg^{2+} competitively inhibited the K^+-induced increase in the noradrenaline output in the presence of either Ba^{2+} or Sr^{2+} and blocked that in the presence of Ca^{2+}.

7. The results indicate that both Ba^{2+} and Sr^{2+} can substitute for Ca^{2+} in the release of noradrenaline at adrenergic nerve terminals. It seems likely that all these cations act through the same site at some stage in the process of K^+-induced transmitter release.

INTRODUCTION

Sr^{2+} is effective in substituting for Ca^{2+} in transmitter release from motor nerve terminals (Miledi, 1966; Dodge, Miledi & Rahamimoff, 1969) and according to Meiri & Rahamimoff (1971), Sr^{2+} acts as a partial agonist of the 'Ca' receptor at the

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0022-3751/80/1180-0486 $07.50

neuromuscular junction. In contrast, Ba^{2+} is not an effective substitute for Ca^{2+} in the transmitter release at the myoneural junction (Miledi, 1966; Blioch, Glagoleva, Liberman & Nenashev, 1968). However, acetylcholine output from the perfused superior cervical ganglion in response to stimulation of the preganglionic sympathetic nerve is maintained when Ca^{2+} is replaced by Ba^{2+} (Douglas, Lywood & Straub, 1961) and substitution of Ba^{2+} for Ca^{2+} causes an asynchronous transmitter release at the sympathetic ganglion (McLachlan, 1977). At motor nerve endings brief repetitive stimulation in Ba^{2+} or in Sr^{2+} solutions elicited a large increase in asynchronous release of ACh (Mellow, Phillips & Silinsky, 1978; Silinsky, Mellow & Phillips, 1977; Silinsky, 1977, 1978). These authors suggested that both divalent cations acted through the same conductance pathway normally traversed by Ca^{2+}, because the asynchronous release of transmitter seen in Ba^{2+} or Sr^{2+} solutions was competitively antagonized by Mg^{2+} or Co^{2+}. These latter two divalent cations have also been shown to competitively inhibit the synchronous release of transmitter in the presence of Ca^{2+} in an analogous manner.

Ba^{2+} and Sr^{2+} are also effective in increasing the catecholamine output from the adrenal medulla and from adrenergic nerve terminals in response to stimulation by secretagogues or stimulation of the sympathetic nerve (Douglas & Rubin, 1964*a*; Boullin, 1967; Kirpekar & Misu, 1967; Garcia & Kirpekar, 1973; Ito, Nakazato & Ohga, 1978). However, it is still not clear whether Ba^{2+} and Sr^{2+} act at the same site or through the same conductance pathway as Ca^{2+}, particularly at the adrenergic nerve terminals.

The striking parallel between the properties of the late Ca^{2+} channel and those of the Ca^{2+}-dependent transmitter release mechanism (for example both mechanisms are competitively inhibited by Mg^{2+}, Mn^{2+}, Co^{2+}, La^{3+} and the organic Ca^{2+}-antagonist, D-600) have led Baker and his colleagues to suggest a possible function for the late Ca^{2+} channel in stimulus–secretion coupling (Baker, Hodgkin & Ridgway, 1971; Baker, 1972; Baker, Meves & Ridgway, 1973*a*). Noradrenaline output from adrenergic nerve terminals induced by the stimulation of the post-ganglionic sympathetic nerve (Boullin, 1967; Kirpekar & Misu, 1967) and by stimulation with K^+ (Kirpekar & Wakade, 1968) was inhibited by Mg^{2+}. The synaptic potentials due to noradrenaline release recorded from mouse vas deferens was suggested to be mediated by a Ca^{2+}-receptor complex which was dependent on the external concentration of Ca^{2+} and was competitively antagonized by Mg^{2+} (Bennett & Florin, 1975). These findings suggest that the late Ca^{2+} channel also involved in the release of noradrenaline at the adrenergic nerve terminals.

The purpose of the present experiments was to determine whether Ba^{2+} and Sr^{2+} can substitute for Ca^{2+} and, if so, whether the active site or the conductance pathway of these cations is the same as that for Ca^{2+} in noradrenaline release caused by depolarization of the adrenergic nerve terminal of guinea-pig vas deferens. We exposed the tissue to excess K^+ solutions, since the late Ca^{2+} channels first opened and more slowly closed in response to maintained depolarization produced either electrically or by exposure to K^+-rich solutions (Baker, Meves & Ridgway, 1973*b*).

METHODS

Male guinea-pigs weighing between 500–650 g were stunned and bled to death. Vasa deferentia (60–90 mg) were isolated and prepared for incubation. The procedure of incubation was the same as described before (Nakazato, Ohga & Onoda, 1978; Ito, Nakazato & Ohga, 1978).

The standard incubation medium was a Krebs solution of the following composition (mM): NaCl, 118; KCl, 4·8; $CaCl_2$, 2·5; $MgCl_2$, 1·2; $NaHCO_3$, 25; KH_2PO_4, 1·2; glucose 10, gassed with 5% CO_2 in O_2; pH 7·3. For Ba^{2+}- and Sr^{2+}-substituted Krebs solutions, $CaCl_2$ was replaced by $BaCl_2$ or $SrCl_2$, respectively. The concentrations of $CaCl_2$, $BaCl_2$ and $SrCl_2$ were varied from 0·2 to 5 mM and that of $MgCl_2$ from 0·4 to 10 mM without adjusting the tonicity. The excess K^+ solution was prepared by increasing the concentration of KCl to 60 mM with a corresponding reduction in the amount of NaCl. For the Ca^{2+}-free solution, $CaCl_2$ was omitted. In all incubation media, phenoxybenzamine (50 μM) and ascorbic acid (60 μM) were added to prevent the uptake and the oxidation of released noradrenaline, respectively.

The vasa deferentia were preincubated in either standard Krebs(Krebs), Ba^{2+}-, Sr^{2+}- substituted or Ca^{2+}-free Krebs solutions respectively for 40–50 min, during which time the solutions were changed at 10 min intervals. The organ was then transferred to the appropriate test solution and incubated. During the incubation, the solution was replaced at 5, 10, 20 or 60 min intervals. Following each incubation, the medium was acidified with concentrated perchloric acid (final concentration, 0·4 N) and stored on ice until centrifugation.

The acidified media were centrifuged at 25000 g at 5 °C for 10 min. The clear supernatants were then transferred to small test tube and stored on ice until assay. The assay was performed by the fluorimetric method of Anton & Sayre (1962).

RESULTS

Comparison of the K^+-induced noradrenaline output in the presence of Ca^{2+}, Ba^{2+} or Sr^{2+}

After preincubation in either Krebs, or Ba^{2+}- or Sr^{2+}-substituted Krebs solutions, vasa deferentia were exposed for 20 min to excess K^+ in the presence of 2·5 mM Ca^{2+}, Ba^{2+} or Sr^{2+} respectively. The exposure to excess K^+ was repeated twice more, with 40 min intervals between exposures, during which the organ was immersed in the appropriate fresh normal K^+ Krebs solutions. In all media tested, exposure to excess K^+ caused an increase in the noradrenaline output, though the effect decreased on repetition (Fig. 1). The order of effectiveness of the divalent cations was roughly $Ba^{2+} > Ca^{2+} > Sr^{2+}$.

Lack of a direct effect of Ba^{2+}, Ca^{2+} and Sr^{2+}

Ba^{2+}, Ca^{2+} and Sr^{2+} are known to increase the catecholamine output from perfused adrenal glands when added after exposure of the glands to Ca^{2+}-free medium (Douglas & Rubin, 1961, 1964*a*). This occurs in the absence of any physiological stimulation such as ACh or excess K^+. Under these conditions, Ba^{2+}, at a high concentration such as 5 mM, was effective even in the presence of Ca^{2+} (Douglas & Rubin, 1964*b*). In the present experiments these divalent cations had no effect on noradrenaline output, when added after 2 hr exposure of the vas deferens to the Ca^{2+}-free medium. Even if the concentration of these cations was raised to 5 mM, no significant changes in the resting output of noradrenaline occurred. However exposure to excess K^+ after, but not before, the addition of divalent cations resulted in the release of noradrenaline. This suggested that there was little or no direct stimulating action of any of these divalent cations on the noradrenaline output. The results of Ca^{2+} and Ba^{2+} were shown in Fig. 2. Similar result was obtained by Sr^{2+}.

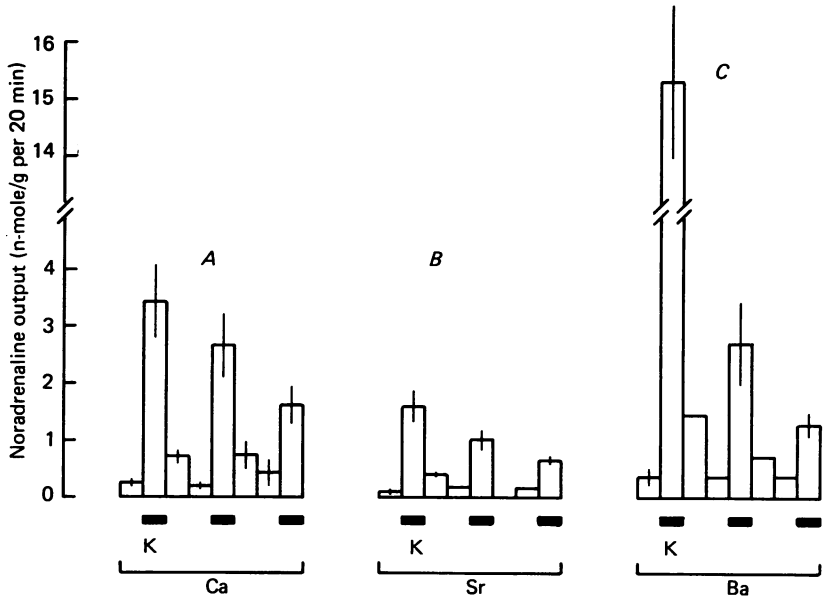

Fig. 1. Increase in noradrenaline output in response to exposure to excess K^+ in the presence of Ca^{2+} (*A*), Sr^{2+} (*B*) or Ba^{2+} (*C*). Columns represent the mean (±S.E.) of noradrenaline output obtained from eight (*A*) and four (*B* and *C*) experiments, except those without vertical bars ($n = 2$). Heavy horizontal bars indicate the period of exposure to excess K^+ (K, 60 mM). Ca, Sr and Ba indicate Krebs, Sr^{2+}-substituted or Ba^{2+}-substituted Krebs solutions, respectively.

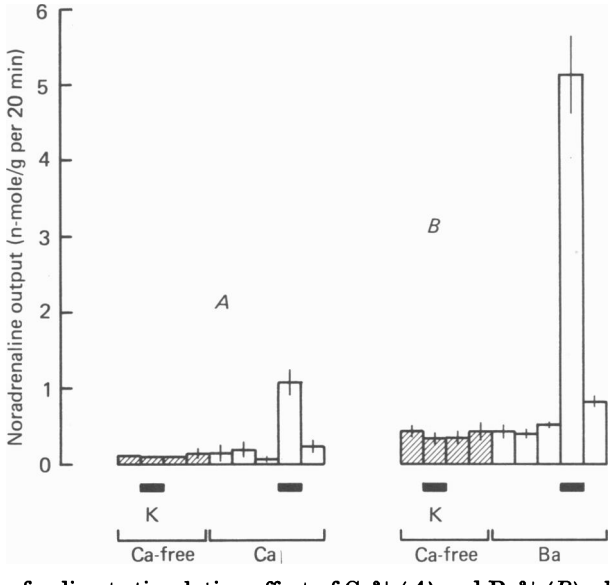

Fig. 2. Lack of a direct stimulating effect of Ca^{2+} (*A*) and Ba^{2+} (*B*) when these divalent cations are reintroduced after exposure of the vas deferens to Ca^{2+}-free media. Columns represent the mean (±S.E.) of noradrenaline output obtained from four (*A* and *B*) experiments. After exposure to Ca^{2+}-free Krebs solution for 2 hr (hatched columns) including a 40 min of preincubation period, the tissues were transferred to Krebs (Ca) or Ba^{2+}-substituted Krebs (*B*) solutions, respectively. Heavy horizontal bars indicate the period of exposure to excess K^+.

Concentration–response relationship

To compare the effectiveness of Ba^{2+}, Ca^{2+} and Sr^{2+} in a quantitative way, the total output of noradrenaline released during a 1 hr exposure to the excess K^+ solution in the presence of various concentrations (0·2–5 mM) of each of the divalent cations was determined and plotted against the log concentration of the cations. As seen in Fig. 3, the pattern of each dose–response curve was quite similar, but the

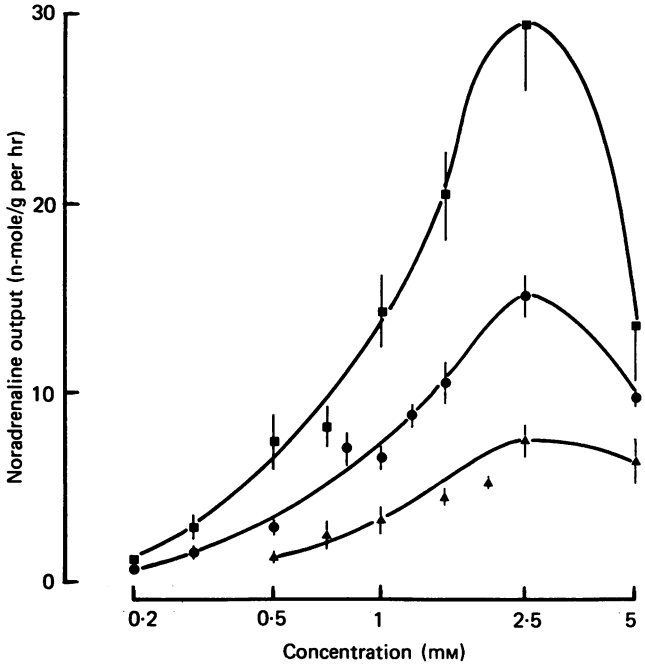

Fig. 3. Dose–response curves of noradrenaline output induced by excess K^+ in the presence of various concentrations of Ba^{2+}, Ca^{2+} or Sr^{2+}. The ordinate is the amount of noradrenaline released in n-mole/g per hr. The abscissa is the concentration of Ba^{2+}, Ca^{2+} or Sr^{2+} on a logarithmic scale. Symbols indicate the mean (± S.E.) of noradrenaline output obtained from four to nine experiments in the presence of Ba^{2+} (■), Ca^{2+} (●) or Sr^{2+} (▲). The lines are drawn by eye.

magnitude was different. The noradrenaline output increased with the increasing concentrations of divalent cation until it attained a maximum at a divalent cation concentration of 2·5 mM. Further increase in the divalent cation concentration decreased the noradrenaline output. The maximum value of noradrenaline released was $29·56 \pm 3·52$, $15·02 \pm 1·12$ and $7·45 \pm 0·84$ (mean ± S.E.) n-mole/g per hr in the medium containing Ba^{2+}, Ca^{2+} or Sr^{2+}, respectively.

Interaction between three cations

The site of action or the conductance pathway of these divalent cations in producing the K^+-induced noradrenaline output could be similar or different. This was tested by determining whether the cations interacted with each other in producing

the response and whether excess Mg^{2+} competitively inhibited the response in the presence of the respective cation.

Quantitative relationships between the concentration of divalent cation and noradrenaline output induced by excess K^+ can be expressed as a reaction between an ion A and a hypothetical active site, or carrier molecule X. Thus, $A + X \overset{K_A}{\rightleftharpoons} AX$ (del Castillo & Katz, 1954; Jenkinson, 1957; Dodge & Rahamimoff, 1967; Meiri & Rahamimoff, 1971), where K_A is the dissociation constant and AX is the complex necessary for inducing the noradrenaline output. Since the number of X molecules may be finite, the fraction of sites occupied by A will be given according to the Law of Mass Action and the concept of affinity and intrinsic activity or efficacy, introduced by Ariëns, Simonis & van Rossum (1964) and Stephenson (1956) for the analysis of drug–receptor interaction, by

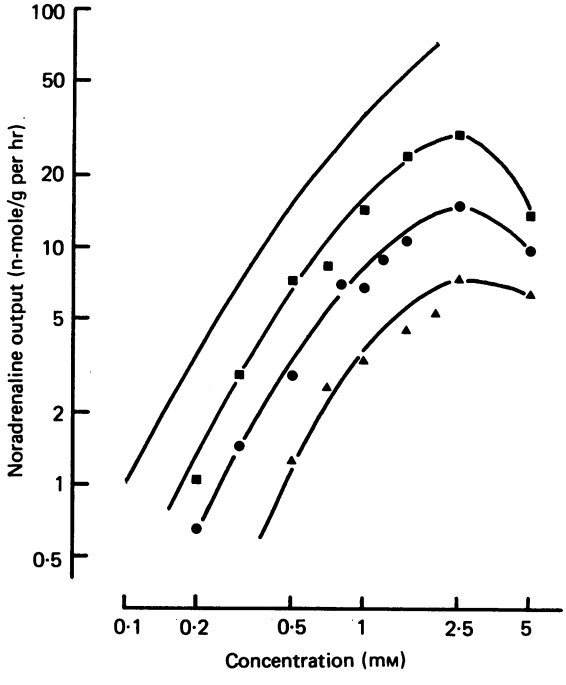

Fig. 4. Dose–response curves on double logarithmic scale for Ba^{2+}, Ca^{2+} and Sr^{2+}. The dose–response curves from Fig. 3 are replotted on a double logarithmic scale with similar symbols without vertical bars indicating S.E. to simplify the illustration. The lines are drawn by eye. The theoretical curve based on eqn. (3) is shown by continuous line without symbols. See the text and the legend of Fig. 3 for further details.

$$[AX] = \alpha \frac{[A]/K_A}{1+[A]/K_A}, \tag{1}$$

where α represents the (relative) intrinsic activity or efficacy of cation A. If the noradrenaline output is proportional to the concentration of AX, the amount of released noradrenaline R_A will be

$$R_A = K.[AX] = K.\left(\alpha \frac{[A]/K_A}{1+[A]/K_A}\right), \tag{2}$$

where K is a proportional constant. The initial slope of the plot of log R_A *vs.* log [A] gives the power of the relation between R_A and AX concentration (Dodge & Rahamimoff, 1967; Katz & Miledi, 1970).

To estimate the values of the power, the dose–response curves from Fig. 3 were replotted on a double logarithmic scale (Fig. 4). As indicated in this Figure, lines drawn by eye along each plot were approximately parallel and the initial slope was close to 2. This agrees well with the finding that the amplitude of the excitatory junction potential of the mouse vas deferens increased according to the second power

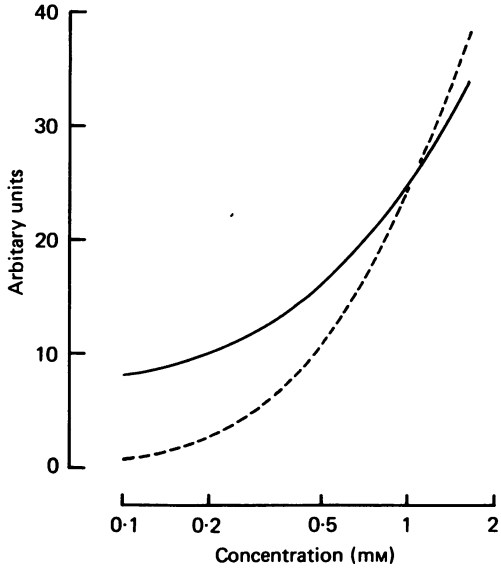

Fig. 5. Interaction between two agonists. The ordinate is the magnitude of the response in arbitrary unit. The abscissa is the concentration of agonist on a logarithmic scale. Dose–response curves for an agonist in the absence (dashed line) and presence (continuous line) of a fixed concentration (1 mM) of another, less potent agonist are constructed from theory. For further information, see text.

(2·3) of the concentration of Ca^{2+} (Bennett & Florin, 1975). Therefore, eqn. (2) can be rearranged as follows (Dodge & Rahamimoff, 1967):

$$R_A = K.[AX]^2 = K.\left(\frac{\alpha[A]/K_A}{1+[A]/K_A}\right)^2. \quad (3)$$

If two active ions, A and B act on the same site X, the interaction between them is given by

$$R_{A+B} = K.([AX]+[BX])^2 = K.\left(\frac{\alpha[A]/K_A+\beta[B]/K_B}{1+[A]/K_A+[B]/K_B}\right)^2 \quad (4)$$

(Meiri & Rahamimoff, 1971; Forman & Mongar, 1972), where β is again (relative) intrinsic activity or efficacy of cation B and K_B is the dissociation constant for the BX complex. If A is the agonist that gives the highest response, the intrinsic activity, α is taken as unity. Then a relative value for the intrinsic activity of B, a partial agonist, can be given ($0 < \beta < 1$). The curve showing the interaction between A and

B can be constructed from eqn. (4) by replacing, for example, K with 100, K_A, K_B and α with 1 and β with 0·5. If the concentration of A is varied from 0·2 to 5 mM in the presence of 1 mM of B, then the curve for A in the presence of B crosses that for A alone constructed from eqn. (3) as described by Forman & Mongar (1972) (Fig. 5). On this basis, we determined whether the dose–response curve for the more potent ion among Ba^{2+}, Ca^{2+} and Sr^{2+} was shifted by the addition of a given concentration of a less potent ion in a manner similar to the curves constructed from the theory (Fig. 5).

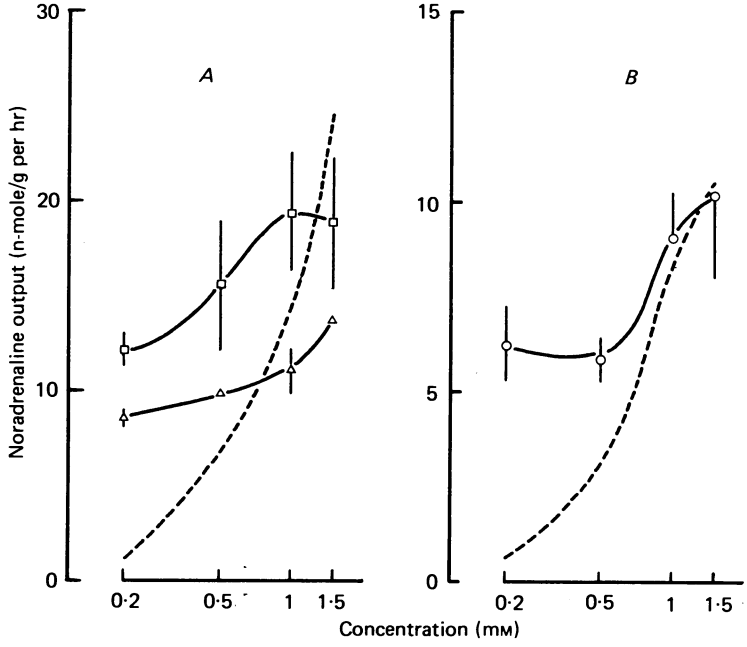

Fig. 6. Dose–response curves for Ba^{2+} and Ca^{2+} in the presence of less potent cations among Ba^{2+}, Ca^{2+} and Sr^{2+}. The ordinate is the amount of noradrenaline released in n-mole/g per hr. The abscissa is the concentration of Ba^{2+} (*A*) and Ca^{2+} (*B*) on a logarithmic scale. Symbols indicate the mean (± S.E.) of noradrenaline output obtained from four experiments, except those without vertical bar (n = 2) in the presence of Ca^{2+} 1 mM (□), Sr^{2+} 2 mM (△) and Sr^{2+} 1 mM (○). The dashed lines are part of the dose–response curves for Ba^{2+} (*A*) and Ca^{2+} (*B*) from Fig. 3.

Sr^{2+} (1 or 2 mM) or Ca^{2+} (1 mM) was added to solutions containing various concentrations (0·2–1·5 mM) of Ca^{2+} or Ba^{2+} and the noradrenaline output in response to excess K^+ was observed. As predicted from the theory, the K^+-induced increase in noradrenaline output was potentiated in a non-linear fashion at 0·2 mM of Ca^{2+} or Ba^{2+}, but inhibited at a concentration of 1·5 mM. As a result, the curves showing noradrenaline output versus various concentrations of Ca^{2+} in the presence of 1 mM-Sr^{2+} or various concentrations of Ba^{2+} in the presence of 1 mM-Ca^{2+} or 2 mM-Sr^{2+} crossed with the dose–response curves from Fig. 3 for Ca^{2+} and Ba^{2+} alone (Fig. 6).

Inhibitory effect of Mg^{2+}

At the adrenergic nerve–smooth muscle junction of the mouse vas deferens, an excess Mg^{2+} has been shown to inhibit the Ca^{2+}-dependent excitatory junction potentials in a competitive manner (Bennett & Florin, 1975). This suggests that Ca^{2+} and Mg^{2+} may compete for the same active site on or in the adrenergic nerve terminals. In the present experiments, the effect of various concentrations of Mg^{2+}

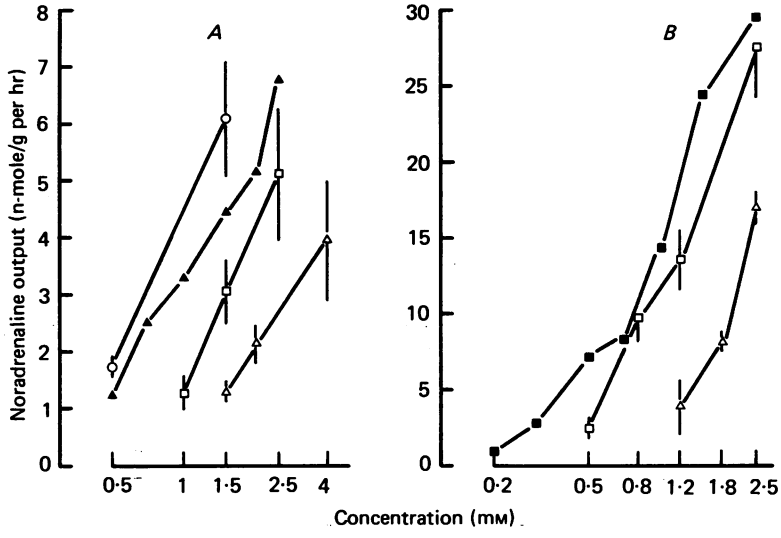

Fig. 7. Effect of Mg^{2+} on the dose–response curves for Sr^{2+} and Ba^{2+}. The ordinate is the amount of noradrenaline released in n-mole/g per hr. The abscissa is the concentration of Sr^{2+} (*A*) and Ba^{2+} (*B*) on a logarithmic scale. Symbols indicate the mean (± S.E.) of noradrenaline output obtained from four to seven experiments in the presence of Mg^{2+}, 0·4 mM (○), 5 mM (□) and 10 mM (△). The dose–response curves of filled symbols (▲, ■) are transferred from Fig. 3 (Mg^{2+}, 1·2 mM).

from 0·4 to 10 mM on the K^+-induced noradrenaline output in Ba^{2+}- or Sr^{2+}-substituted Krebs solutions were determined. As shown in Fig. 7, the dose–response curves in Ba^{2+} and in Sr^{2+} solutions were shifted in a parallel fashion to the right as the concentration of Mg^{2+} was increased. Since the inhibitory effect of Mg^{2+} was so variable from preparation to preparation an estimation of the dissociation constant of Mg^{2+} was not determined. However, these data at least suggest that the response induced by excess K^+ in the presence of either Ba^{2+} or Sr^{2+} may be competitively inhibited by Mg^{2+}. As well indicated previously (Boullin, 1967; Kirpekar & Misu, 1967; Kirpekar & Wakade, 1968; Bennett & Florin, 1975), excess Mg^{2+} (10 or 20 mM) completely blocked the K^+-induced noradrenaline output in the presence of Ca^{2+} 2·5 mM. However, we failed to observe the competitive interaction between Ca^{2+} and Mg^{2+} in a wider range of their concentrations, because the presence of high concentration of both the ions increased light scattering considerably and made the fluorimetric assay for noradrenaline impossible.

Dissociation constant

The interaction between the three cations and the competitive antagonism by Mg^{2+} suggest that these divalent cations act through the same site at some stage in the process of depolarization-induced increase in the noradrenaline output. Based on this assumption the difference in potency between Ba^{2+}, Ca^{2+} and Sr^{2+} could result from a difference in affinity of these cations for an active site or from a difference in

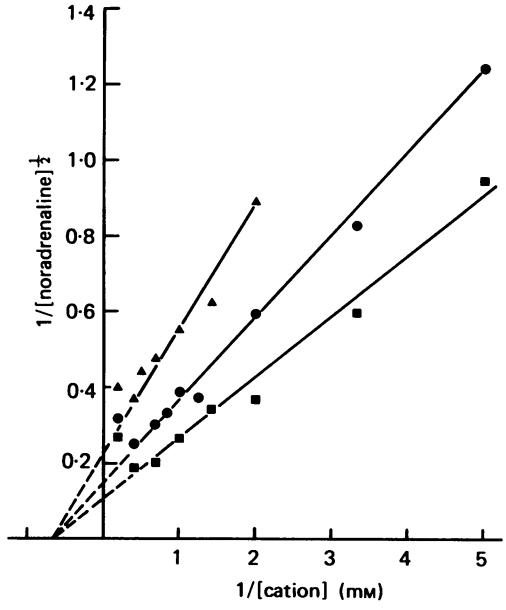

Fig. 8. Double reciprocal plot of [noradrenaline]$^{\frac{1}{2}}$ against [cation]. The ordinate is reciprocal of square root of the amount of noradrenaline released in n-mole/g per hr. The abscissa is the reciprocal of the concentration of Ba^{2+} (■), Ca^{2+} (●) and Sr^{2+} (▲). The lines are fitted by eye.

the effectiveness of each cation or cation-active site complex as suggested by Meiri & Rahamimoff (1971) and Forman & Mongar (1972). To distinguish between these possibilities, the dissociation constant (reciprocal of affinity constant) was estimated from a double reciprocal plot of [noradrenaline]$^{\frac{1}{2}}$ against [Ba^{2+}], [Ca^{2+}] and [Sr^{2+}] according to the following rearrangement of eqn. (3)

$$1/R_{Me}^{\frac{1}{2}} = 1/K^{\frac{1}{2}}\,(1/[Me].K_{Me}+1), \qquad (5)$$

where Me represents the divalent cation and K_{Me} is the dissociation constant. As shown in Fig. 8, eqn. (5) gave straight lines for Ba^{2+}, Ca^{2+} and Sr^{2+}. In each case, the extrapolated line intercepted the abscissa at approximately the same point, 1/1·3 mM, indicating that the affinity of each cation for the hypothetical active site may be the same. The curve constructed from eqn. (3) by applying 1·3 mM for K_A and 200 and 1 for K and α respectively on a double logarithmic scale is confirmed to be almost in parallel with those of experimental results (see Fig. 4).

DISCUSSION

The present experiments show that Ba^{2+} and Sr^{2+} can substitute for Ca^{2+} in the noradrenaline output induced by exposure of guinea-pig vas deferens to excess K^+. Excess K^+ is thought to open the late Ca channel (Baker, Meves & Ridgway, 1973*b*) at the adrenergic nerve terminals. The interaction between Ca^{2+}, Ba^{2+} and Sr^{2+} and/or the competitive antagonism by Mg^{2+} have been demonstrated for transmitter release from motor nerve endings (Meiri & Rahamimoff, 1971; Silinsky *et al.* 1977; Mellow *et al.* 1978; Silinsky, 1978), sympathetic ganglion (McLachlan, 1977) and adrenergic nerve terminals (Bennett & Florin, 1975) and in the anaphylactic histamine release from mast cells (Forman & Mongar, 1972), suggesting a similar active site for these cations. Consistent with these findings is our observation that Ca^{2+}, Ba^{2+} and Sr^{2+} interacted with each other during the K^+-induced noradrenaline output, and Mg^{2+} competitively antagonized the response in the presence of Ba^{2+} and Sr^{2+}. Bennett & Florin (1975) have shown that Mg^{2+} competitively antagonizes the Ca^{2+}-dependent excitatory junction potentials in the mouse vas deferens. In the present experiments, 10 or 20 mM-Mg^{2+} blocked the K^+-induced noradrenaline output from vas deferens bathed in Krebs solution, though antagonism between Ca^{2+} and Mg^{2+} in a wide range of concentrations could not be demonstrated for the reason described in the Results. Therefore, the site of action of Ca^{2+}, Ba^{2+} and Sr^{2+} during the process of depolarization induced noradrenaline output may be the same.

It has been reported that the amplitude of the excitatory junction potentials in the mouse vas deferens (Bennett & Florin, 1975) evoked by the nerve stimulation increases with the second power of the concentration of Ca^{2+} in the medium. However, according to Stjärne (1973), the release of [^{3}H]noradrenaline from guinea-pig vas deferens evoked by stimulation of the hypogastric nerve is a simple function of the Ca^{2+} concentration and obeys Michaelis–Menten kinetics. We believe the present results support those of Bennett & Florin (1975) for the following reasons. First, the dose–response curves for either Ba^{2+}, Ca^{2+} or Sr^{2+} on a double logarithmic scale had an initial slope of 2, suggesting the noradrenaline output caused by excess K^+ increases with the second power of the concentration of any of these cations in the medium. Thus, the curve plotted from eqn. (3) was parallel with the curves obtained from the experimental results using the lower range of concentrations, and the curve of interaction constructed from eqn. (4) was also consistent with those obtained from the experimental data.

In agreement with the report by Meiri & Rahamimoff (1971) for Ca^{2+} and Sr^{2+} at the motor nerve endings, Ba^{2+}, Ca^{2+} and Sr^{2+} were suggested to have a similar affinity for the active site, because a double-reciprocal plot of $[\text{noradrenaline}]^{\frac{1}{2}}$ against [cation] ranging from 0·2 to 2·5 mM gave straight lines, the extrapolation of which intercepted the abscissa scale at the same point. If this suggestion is true, then the differences in effectiveness of these cations in producing the K^+-induced increase in the noradrenaline output may reflect a difference in intrinsic activity of each cation or a difference in efficacy of each cation–active site complex.

We have not defined the term 'active site' for the divalent cations in the strict sense. Miledi (1973) showed that Ca^{2+} ions, injected into the presynaptic nerve terminals in the giant synapse of the squid, evoked transmitter release while similar

doses of Mg^{2+} and Mn^{2+} were ineffective. Similar results were also obtained on the histamine release from rat peritoneal mast cells (Kanno, Cochrane & Douglas, 1973). If this is the case in the adrenergic nerve terminals, it is possible that at least two sets of active sites for the divalent cations participated in the K^+-induced noradrenaline output, one extracellular which may play a role in determining the ease with which the divalent cations cross the membrane, and another intracellular set which may be involved in the activation of the release process. If so, the situation should be much more complicated. However, we do not know how to approach such intracellular active sites in the adrenergic nerve terminals 'in situ' at present. The problem is unsettled and left for the future.

We thank Professor A. Ohga for his advice, continual encouragement and reading the manuscript and Dr D. E. Cochrane, Department of Biology, Tufts University for help in correcting the English of this manuscript and helpful discussion. We also thank Dr S. Ito for valuable discussion during the preparation of the manuscript.

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