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Magnesium Withdrawal and Contraction of Arterial Smooth Muscle: Effects of EDTA, EGTA, and Divalent Cations[1] (39300)

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Recently, we reported that withdrawal of external magnesium ($[Mg^{2+}]_o$) can induce contractions of rat arterial smooth muscle (1). Several different experiments suggested that the strength of these contractions may be dependent on not only the amount of $[Mg^{2+}]_o$ withdrawn from the medium but also on the amount of external Ca^{2+} present (1). Divalent cation chelating agents such as $CaNa_2EDTA$[2] and EGTA which can chelate Mg^{2+} and Ca^{2+}, respectively, should help to shed further light on these tenets. With this in mind, we undertook such experiments on isolated rat aortic strips. In addition, experiments were undertaken to determine whether other divalent cations could substitute for $[Mg^{2+}]_o$ in some of these responses.

Methods. Male rats (Wistar strain, 280-400 g) were sacrificed by decapitation and exsanguinated. The descending thoracic aortae were excised and placed in cold normal Krebs-Ringer bicarbonate solution, cleaned, freed of surrounding connective tissue, cut into helical strips, and set up isometrically under a resting tension of 1.5 g as described previously (1). All tissues were initially equilibrated for 2 hr in normal Krebs-Ringer bicarbonate. The composition of the normal Krebs-Ringer bicarbonate was (in millimoles): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 25.0; and glucose, 10.0. The loading tensions were maintained and periodically adjusted throughout the experiments. The Krebs-Ringer solution was oxygenated continuously with a 95% O_2-5% CO_2 mixture and kept at 37.5° (pH 7.4-7.5).

All of the vascular strips, after the equilibration period, were initially stimulated with a supramaximal dose of epinephrine (10 μg/ml; Adrenalin Chloride, Parke Davis and Co.,) in order to get an idea of each strips's maximal contractile response (1, 2). After this procedure the strips were washed and left in normal Krebs-Ringer bicarbonate solution 45-60 min for relaxation. Upon relaxation, the following different types of experiments were run on the aortic strips: (i) Certain vascular strips were exposed to Mg^{2+}-free Krebs-Ringer for periods of 5-60 min (1). (ii) In other experiments, $CaNa_2EDTA$ (1 or 5×10^{-3} *M*; J. T. Baker Co.) or EGTA (1 or 5×10^{-3} *M*; Sigma Chemical Co.) was added to an aortic strip after the contractile response produced by withdrawal of $[Mg^{2+}]_o$ had reached its plateau. The CaEDTA and EGTA solutions were adjusted to pH 7.4. (iii) Divalent cations (i.e., $SrCl_2$, $MgCl_2$, $NiCl_2$, $MnCl_2$, and $CdCl_2$) were added in various concentrations (i.e., from 10^{-6} to 10^{-3} *M*) to aortic strips after the contractile responses produced by withdrawal of $[Mg^{2+}]$ had plateaued. These latter experiments were carried out in order to determine whether divalent cations, other than Mg^{2+}, could induce relaxation of these arteries. Where appropriate, the means (± SEM) of the responses in control and experimental strips were compared for statistical significance by means of Student's *t* test, paired *t* test (3), or analysis of variance (4).

Results. Figure 1 shows recordings of typical changes in mechanical activity and resting tension, in two different isolated aortic strips, as the external Mg^{2+} concentration is reduced from a normal 1.2 m*M* to zero together with the effects of a subsequent addition of 5 m*M* CaEDTA and EGTA. This figure demonstrates that the contractile response elicited by withdrawal of $[Mg^{2+}]_o$ is enhanced approximately 200% by the Ca-

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[2] Abbreviations used: $CaNa_2EDTA$, calcium disodium ethylenediaminetetra-acetate; EGTA, ethyleneglycol-tetraacetic acid; EC50, concentration of divalent cation that is necessary to produce 50% relaxation.

EDTA and completely relaxed by the EGTA. Table I presents a summary of the data obtained in 63 different aortic strips using 1 and 5 m*M* CaEDTA and EGTA and indicates that 1 and 5 m*M* CaEDTA enhance the contractile responses approximately to the same extent. However, only 5 m*M* EGTA (not 1 m*M*) is capable of inducing complete relaxation of strips contracted by withdrawal of $[Mg^{2+}]_o$ (Table I). (Since 2.5 m*M* Ca^{2+} is present in the medium, one would not expect 1 m*M* EGTA to have any effect).

Figure 2 indicates that like Mg^{2+}, Mn^{2+}, Ni^{2+} as well as Cd^{2+} can effectively relax contractions of aortic strips produced by withdrawal of $[Mg^{2+}]_o$; Sr^{2+} although not capable of substituting for $[Mg^{2+}]_o$ induces spontaneous contractile activity in strips exposed to Mg^{2+}-free Krebs-Ringer. It is important to note that the contractions induced by exposing the arterial strips to zero $[Mg^{2+}]_o$ were maintained for the duration of exposure to Mg^{2+}-free Krebs-Ringer solution. Table II indicates that the half-time ($t_{1/2}$) of Mg^{2+} relaxation was not greater than Mn^{2+} relaxation. The $t_{1/2}$ for both Mg^{2+} and Mn^{2+} relaxation was, however, significantly less than that for Cd^{2+} or Ni^{2+} relaxation.

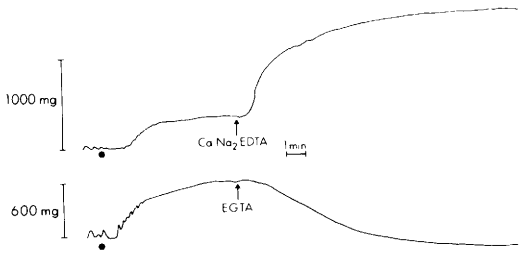

FIG. 1. Influence of 5 m*M* $CaNa_2$ EDTA and EGTA (added at arrows, respectively) on contractions of rat aortic strips produced by exposing the tissues to Mg^{2+}-free Krebs-Ringer solution (at dots).

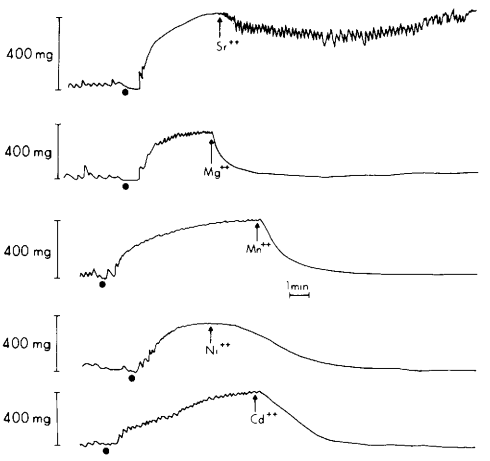

FIG. 2. Influence of 1.2 m*M* externally added divalent cations, Sr, Mg, Mn, Ni, and Cd, on contractile responses in rat aorta induced by complete withdrawal of $[Mg^{2+}]_o$ (at dot).

The same pattern was seen when equieffective concentrations of the divalent cations were used. Figure 3 shows the cumulative dose-response curves of these four divalent cations for relaxation of aortic strips precontracted by withdrawal of $[Mg^{2+}]_o$. For each ion, the degree of relaxation was proportional to the concentration of divalent cation added. In addition, the dose-response curves for Mn^{2+}, Mg^{2+}, and Ni^{2+} but not Cd^{2+} are parallel with one another ($P < 0.05$). The data in Fig. 3 when taken together with the EC50's presented in Table II indicate a relative descending order of potency for relaxation: Mn > Cd > Mg > Ni.

Discussion. The present experiments, employing two different divalent cation chelators, namely, CaEDTA and EGTA, lend support to the ideas that: (i) withdrawal of $[Mg^{2+}]_o$ produces contraction of rat aortic smooth muscle; and (ii) these latter re-

TABLE I. INFLUENCE OF CaEDTA AND EGTA ON CONTRACTIONS INDUCED BY WITHDRAWAL OF $[Mg^{2+}]_o$.

Chelator	*N*	Contractile tension before adding chelator (mg)	Contractile tension after adding chelator (mg)	Change in tension (%)
CaEDTA				
1 m*M*	9	379.6 ± 38.6	795.6 ± 48.6[a]	+111.3
5 m*M*	25	435.8 ± 32.2	993.8 ± 76.2[a]	+128.0
EGTA				
1 m*M*	7	428 ± 29.2	445.1 ± 34.5	+4.0
5 m*M*	22	499.7 ± 36.7	0[a]	−100.0

[a] Significantly different from paired tension before chelator ($P < 0.01$).

TABLE II. INFLUENCE OF VARIOUS DIVALENT CATIONS ON CONTRACTIONS INDUCED BY WITHDRAWAL OF $[Mg^{2+}]_o$.

Cation	N	Relaxation after 1.2 mM (%)	$t_{\frac{1}{2}}$ after 1.2 mM (min ± SEM)	EC 50[a] (10^{-5} ± SEM)
Sr	12	0	–	–
Mg	65	100	1.50 ± 0.10	3.9 ± 0.6
Mn	9	100	1.77 ± 0.23	0.39 ± 0.07[b]
Cd	10	100	3.02 ± 0.32[b]	1.5 ± 0.4[b]
Ni	10	100	3.04 ± 0.28[b]	15.2 ± 2.8[b]

[a] These values are taken from the complete dose response curves shown in Fig. 3. N = 6–9 each.
[b] Significantly different from values for Mg ($P < 0.01$).

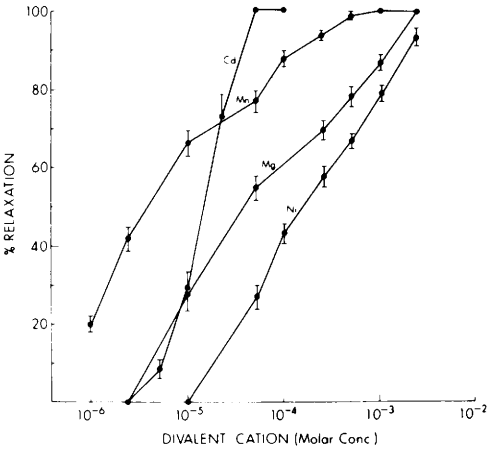

FIG. 3. Divalent cation-induced relaxation of rat aorta contracted by withdrawal of $[Mg^{2+}]_o$. N = 6–9 for each cumulative dose-response curve. Using analysis of variance and regression line analysis, the lines for Mn^{2+}, Mg^{2+}, and Ni are parallel ($P < 0.05$).

sponses are due to an inward movement of $[Ca^{2+}]_o$ (1). CaEDTA which has affinity for Mg^{2+} (5) is probably chelating and removing surface membrane Mg from vascular smooth muscle cells. Such an effect could be expected to result in a greater influx of $[Ca^{2+}]_o$ (1), thereby potentiating the contraction produced by withdrawal of $[Mg^{2+}]_o$. EGTA which is known to selectively chelate Ca^{2+}, in preference to Mg^{2+} (5), is probably binding with the external Ca^{2+}, thereby preventing its continued influx. The net result of the latter could be expected to result in rapid relaxation of the contractions induced by withdrawal of $[Mg^{2+}]_o$. Since there is no evidence to indicate that CaEDTA or EGTA can bind to substances like proteins, carbohydrates or lipids (6), it is likely they are binding Mg^{2+} and Ca^{2+}, respectively. The rapidity of the potentiation or reversal of the contraction, evoked by EDTA and EGTA, respectively, suggests actions on the cell membrane since these divalent cation chelating agents do not readily cross cell membranes (6). Overall, these findings lend support to the ideas that $[Mg^{2+}]_o$ either directly regulates membrane permeability to $[Ca^{2+}]_o$ or occupies membrane sites which are exchangeable with membrane-bound Ca in vascular smooth muscle (1, 7, 8). The present experiments with Mn^{2+}, Ni^{2+}, and Cd^{2+} could be used as further support for this tenet.

All three divalent cations, namely, Mn, Cd, and Ni but not Sr, have been demonstrated to reduce the uptake of Ca in several different types of muscle, including smooth muscle (9–14). Such an effect of these cations on the membranes of aortic strips, precontracted by exposure to Mg^{2+}-free Krebs-Ringer, could thus be expected to result in relaxation of these responses. The ability of Mn, Cd, and Ni but not Sr to substitute for Mg in relaxation of aortic smooth muscle probably resides in the physical properties of the various cations. The ionic radii may be quite important. In this context, it is of interest to note that Ni has an ionic radius of 0.69 Å while that for Mg is 0.65 Å (15–17). Although both Mn (0.80 Å) and Cd (0.97 Å) also have ionic radii below 1.00 Å, that of Sr is 1.13 Å (15–17). The hydration energies (i.e., ease with which the cations can be dehydrated) are also similar for Mg, Mn, Cd, and Ni, while that of Sr is different (15, 17). Thus, cations that possess properties similar to Mg, like Mn, Ni, and Cd, but not Sr could occupy membrane binding sites for Mg and Mg channels, thereby preventing entry of $[Ca^{2+}]_o$. The fact that the Cd dose-response curves do not parallel those of Mg,

Ni, and Mn suggests that Cd may act at cellular sites other than those affiliated with Mg. For example, although Cd is known to bind strongly at SH groups, the other divalents do not (12, 16, 17). Irrespective of the exact mechanism of action, the present findings demonstrate that some divalent cations can substitute for Mg in certain responses of vascular smooth muscle.

Summary. The divalent cation chelators, CaEDTA and EGTA, were demonstrated to exert opposite effects on contractions of rat aortic smooth muscle induced by withdrawal of external magnesium ($[Mg^{2+}]_o$). Addition of CaEDTA potentiated such contractions more than 100%, while EGTA promoted rapid relaxation. Rapid relaxation of contractions induced by withdrawal of $[Mg^{2+}]_o$ could also be induced by Mn, Ni, and Cd but not Sr. Using EC50's, a relative descending order of contractile inhibition was noted for the divalent cations: Mn > Cd > Mg > Ni. The ability of CaEDTA to potentiate contractions produced by withdrawal of $[Mg^{2+}]_o$, as well as the ability of divalent cations to relax contractions of aortic smooth muscle, appears to be related to actions on the transmembrane flux of Ca^{2+}. These findings thus lend support to the view that Mg ions either play an important role in regulating membrane permeability to $[Ca^{2+}]_o$ or occupy membrane sites which are exchangeable with membrane-bound Ca in certain types of arterial smooth muscle.

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1. Altura, B. M., and Altura, B. T., Microvas. Res. **7,** 145 (1974).
2. Altura, B. M., and Altura, B. T., J. Pharmacol. Exp. Ther. **190,** 300 (1974).
3. Bancroft, H., "Introduction to Biostatistics," p. 172, Hoeber-Harper, New York (1962).
4. Finney, D. J. "Statistical Method in Biological Assay," p. 99, Charles Griffin, (1964).
5. Sillén, L., and Martell, A., "Stability Constants of Metal-ion Complexes, Supplement 1," The Chemical Society, London (1971).
6. Baker, P. F., Prog. Biophysic **24,** 177 (1972).
7. Altura, B. M., and Altura, B. T., Amer. J. Physiol. **220,** 938 (1971).
8. Turlapaty, P. D. M. V., and Carrier, O., Jr., J. Pharmacol. Exp. Ther. **187,** 86 (1973).
9. Sullivan, L. J., and Briggs, A. H., J. Pharmacol. Exp. Ther. **161,** 205 (1968).
10. Bulbring, E., and Tomita, T., J. Physiol. (London) **196,** 137 (1968).
11. Collins, G. A., Sutter, M. C., and Teiser, J. C., Can. J. Physiol. Pharmacol. **50,** 300 (1972).
12. Toda, N., Amer. J. Physiol. **225,** 350 (1973).
13. Osa, T., Jap. J. Physiol. **24,** 101 (1974).
14. Washizu, Y., Comp. Biochem. Physiol. **24,** 301 (1968).
15. Pauling, L., "Nature of the Chemical Bond," 3rd ed., Cornell Univ. Press, Ithaca (1960).
16. Albert, A., "Selective Toxicity," 5th ed., Wiley, New York (1973).
17. Triggle, C. R., Grant, W. F., and Triggle, D. J., J. Pharmacol. Exp. Ther. **194,** 182 (1975).

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