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Neurohypophyseal Hormones and Analogues: Magnesium Dependence and Contraction of Arterial Smooth Muscle[1] (38683)

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It has been suggested that extracellular magnesium ions ($[Mg^{2+}]o$) selectively potentiate the contractile actions of neurohypophyseal hormones (NHPH) and analogues on vertebrate peripheral blood vessels by Somlyo *et al.* (1–3). Moreover, these investigators have postulated that $[Mg^{2+}]o$ potentiates neurohypophyseal peptides by enhancing the ability of these peptides to bind at their receptors (*i.e.* to enhance hormone-receptor affinity), similar to the mechanism of enhancement postulated by others for uterine smooth muscle (4, 5) rather than by altering events beyond the receptor sites, e.g., on excitation-contraction (E-C) coupling, metabolism or the contractile proteins.

In sharp contrast to these suggestions, it has recently been demonstrated that $[Mg^{2+}]o$ can potentiate vasoactive substances other than neurohypophyseal peptides on vascular smooth muscle, e.g., catecholamines and barium ion (6–8). These recent studies collectively suggest that $[Mg^{2+}]o$ may potentiate catecholamines and barium ion by acting at sites in vascular muscle other than the receptors (6–8). In addition, it has been demonstrated that different arterial vessels within a single mammalian species—the dog—show different contractile dependencies on $[Mg^{2+}]o$ for at least one neurohypophyseal hormone, [8-arginine]-vasopressin (9). These observations on different canine arteries, employing complete dose-response curves, indicate that $[Mg^{2+}]o$ may possibly potentiate the activities of neurohypophyseal peptides on certain peripheral vessels by acting at events beyond the peptide receptors. This would thus be quite different from the tenet promulgated by other workers (1–5).

Since the rat is the mammalian species primarily used to assay and standardize NHPH and synthetic analogues (10), it would be interesting to determine the exact, quantitative relationships that exist between $[Mg^{2+}]o$ and contraction of blood vessels in response to neurohypophyseal peptides in this vertebrate. But, to my knowledge, no such studies exist. Moreover, since different concentrations of $[Mg^{2+}]o$ have been shown to differentially affect contraction, ion content and tone of vascular muscle (6–8), it is important to examine the actions of a range of external magnesium concentrations on contraction of neurohypophyseal hormones and analogues. The present experiments utilizing four different NHPH and synthetic analogues, a range of $[Mg^{2+}]o$, and complete dose-response curves, were therefore undertaken with isolated rat aortas in order to acquire this information.

Methods. Thoracic aortas obtained from male rats (Wistar strain, 200–375g) were cut helically into vascular strips (1.3–1.5 mm in width by 25 mm in length) and set up isometrically *in vitro* under a resting tension of 1.5 g, in a manner essentially similar to that described previously for rabbit thoracic aorta (11). Only male rats were employed since estrogenic hormones are known to affect the reactivity of blood vessels to NHPH (12). All vascular strips were equilibrated for 2 hr in muscle chambers containing Krebs-Ringer bicarbonate solution with 1.2 m*M* Mg^{2+}, the composition of which has been given previously (13). The Krebs-

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Ringer bicarbonate solution was oxygenated continuously with a 95% O_2–5% CO_2 mixture and kept at 37° (pH 7.3–7.5). After the 2 hr incubation period, certain vascular strips were exposed either to Mg^{2+}-free Krebs-Ringer solutions or to Krebs-Ringer solutions containing various concentrations of Mg^{2+} (0.2, 1.2 or 6.0 m*M*) for a period of 60 min. These incubation media were routinely changed every 5–10 min as a precaution against the production of interfering metabolites (14). Complete, cumulative log dose-response curves, similar to those described previously (13, 15), were then obtained for one of four different synthetic NHPH and analogues (16). The results for these experiments are expressed as: (i) percentage of maximal contractile responses to [8-arginine]-vasopressin in 1.2 m*M* $[Mg^{2+}]o$ since (a) the latter is the native rat pituitary hormone and (b) 1.2 m*M* $[Mg^{2+}]o$ approximates the rat plasma concentration for Mg^{2+}; and (ii) the concentration of the NHPH required to elicit 50% of the maximal contractile response (*i.e.* ED_{50}) in different $[Mg^{2+}]o$. The latter is a measure of hormone-receptor affinity, while the former is a measure of the intrinsic activity or effectiveness of the drug-receptor complex (17).

Results and Discussion. If the presence of $[Mg^{2+}]o$ potentiates NHPH and analogues solely by enhancing the binding of these peptides to their respective receptors (1–5), then one might expect to see a *parallel* displacement of the concentration-effect curves for the NHPH to the left of those obtained in Mg^{2+}-free solutions with no change in maximum contractile response. If, however, $[Mg^{2+}]o$ potentiates these NHPH and analogues on certain vascular muscle by acting at receptors and some other cellular site(s), then one should not see a left-ward parallel shift of the dose-response curves (6–9). The data shown in Figs. 1–4 suggest that although different concentrations of $[Mg^{2+}]o$ (0.2, 1.2 and 6.0 m*M*) strongly potentiate the contractile actions of NHPH and analogues on rat aorta, the concentration-effect curves *are not* shifted in a distinctly parallel manner to the left of those obtained in Mg^{2+}-free solutions. Instead, these findings indicate that the slopes of the dose-response curves are steepened and the maximum contractile responses are differentially affected and are dependent upon $[Mg^{2+}]o$, as well as on the type of NHPH. In addition, the data in Fig. 4 and Table I indicate that although the maximum contractile response to oxytocin is increased almost sixfold by 1.2 m*M* $[Mg^{2+}]o$, the ED_{50} is not significantly shifted to the left of that observed in Mg^{2+}-free solution as is the case with the other NHPH and analogues; in fact, $[Mg^{2+}]o$ shifts the ED_{50}'s rightward for this peptide (Fig. 4, Table I). It is thus

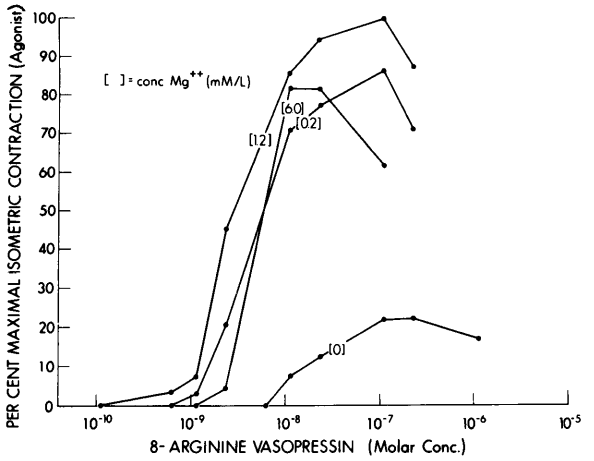

FIG. 1. Influence of $[Mg^{2+}]o([\ ])$ on [8-arginine]-vasopressin-induced contractions. Cumulative log dose-isometric response curves. 100% response = 945.5 ± 82 mg. *N* = 10–30 different strips for each dose-response curve.

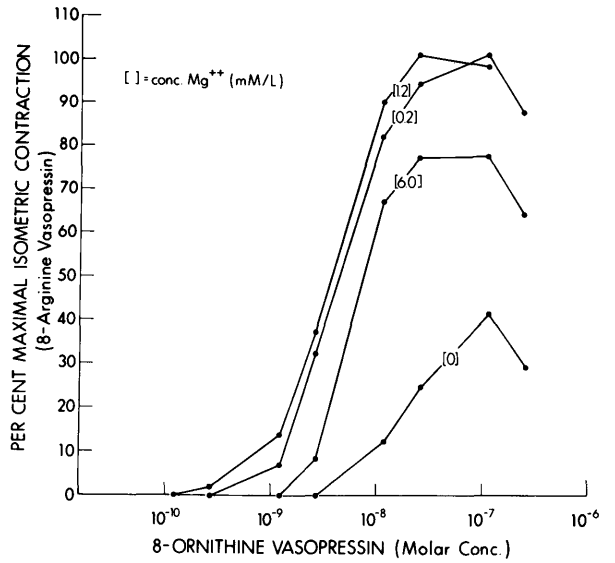

FIG. 2. Influence of $[Mg^{2+}]o$ ([ ]) on [8-ornithine]-vasopressin-induced contractions. 100% response = 938.2 ± 76 mg. N = 10–30 different strips for each dose-response curve.

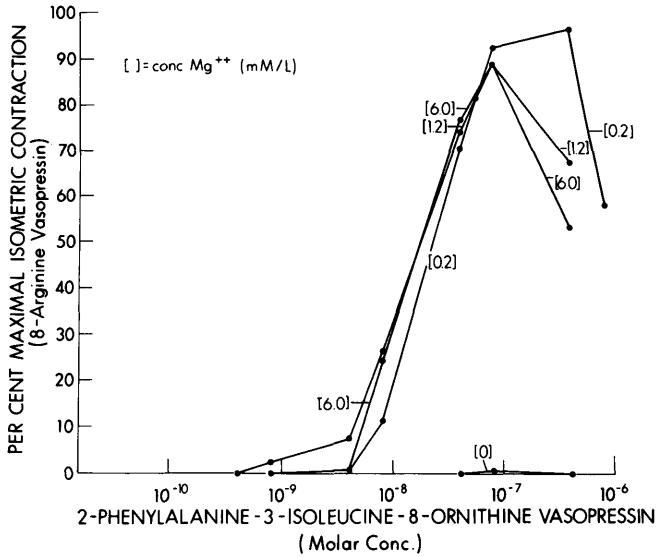

FIG. 3. Influence of $[Mg^{2+}]o$ ([ ]) on [2-phenylalanine, 3-isoleucine, 8-ornithine]-vasopressin-induced contractions. 100% response = 955.5 ± 86.5 mg. N = 10–25 different strips for each dose-response curve.

exceedingly difficult, if not impossible, to interpret such data as supporting the concept that Mg^{2+} solely potentiates activities of NHPH analogues on vascular muscle by enhancing hormone-receptor affinity (1–3). The complex concentration-effect curves for the NHPH, observed in the present experiments, in the presence of $[Mg^{2+}]o$, rather strongly support, at least for rat aorta, the idea that Mg^{2+} potentiates NHPH by acting either at cellular sites other than the receptors for these peptides (E-C coupling, metabolism, contractile proteins, etc.) or at several sites, e.g., receptors, membranes and intracellularly (6–8).

One of these sites for Mg^{2+} action may be intracellular in nature. The increased maximum responses observed in the presence of

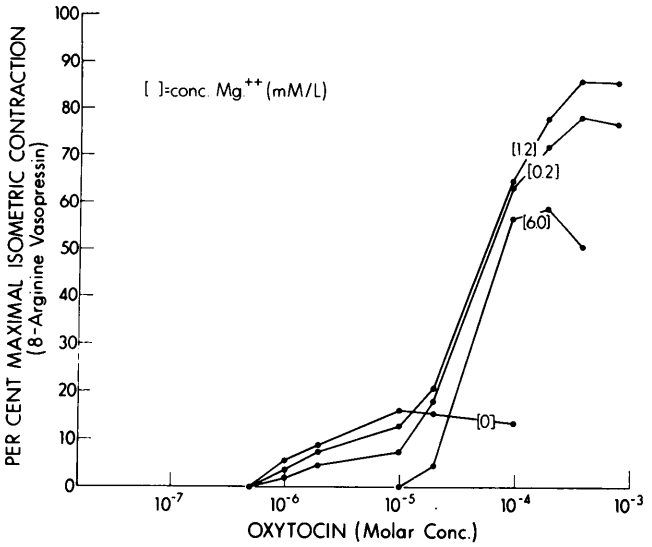

FIG. 4. Influence of $[Mg^{2+}]o$ ([ ]) on oxytocin-induced contractions. 100% response = 942.4 ± 69.5 mg. N = 12–20 different strips for each dose-response curve.

different $[Mg^{2+}]o$ could be used to suggest that this divalent cation may enhance maximal contractile responses by displacing calcium ions from intracellular sites (6–8). For example, although different vasotropic agents, including NHPH, are known to induce contraction of vascular smooth muscle by different receptor systems (3), it is believed (i) that all stimulants require calcium ions (Ca^{2+}) for E-C coupling, and (ii) the source of activator Ca^{2+} may be extracellular or intracellular in nature, depending upon stimulant (18). The fact that $[Mg^{2+}]o$ has been shown to (i) alter the binding of Ca in vascular muscle (6, 7), including rat aorta (8), and (ii) enhance the maximal contractile response of depolarized rat aorta to $[Ca^{2+}]o$ (8), supports the idea that Mg ions can compete with Ca^{2+} for certain membrane and intracellular sites. A freeing-up of intracellular ionized Ca for interaction with actomyosin could account for the increased maximal contractile responses seen in the present study. An alternative likely contributing mechanism is that more actomyosin is activated by the presence of Mg^{2+} (19). Russell (19) has shown that Mg^{2+} has a direct effect on the physical state of arterial actomyosin which is correlated with its activation. Although the present data do not rule in or out the possibility that $[Mg^{2+}]o$ enhances NHPH induced contractions by acting on vascular smooth muscle cell metabolism, previous studies would tend to discount this possibility, at least with respect to aortic smooth muscle (6).

The data in Figs. 1–4 and Table I suggest that although $[Mg^{2+}]o$ does indeed enhance NHPH-induced contractions in arterial smooth muscle, there appears to be an optimal $[Mg^{2+}]o$, approximately 1.2 mM Mg^{2+}. Concentrations lower or higher than 1.2 mM not only result in development of lower maximal contractions but in less of a left-ward shift in the concentration-effect curves; [2-phenylalanine, 3-isoleucine, 8-ornithine]—vasopressin may, however, be an exception. Hypermagnesemic levels (e.g. 6.0 mM), although resulting in potentiation when compared to Mg^{2+}-free conditions, usually result in the lowest hormone-receptor affinities (high ED_{50}'s) and smallest maximal tensions when compared to 1.2 mM $[Mg^{2+}]o$. In this context, it is of interest to note that hypermagnesemic levels have previously been shown not only to depress maximal contractile responses of other vasoactive hormones and amines on vascular muscle but to shift the dose-responses to higher concentration levels as well (6). Such

TABLE I. RELATIVE HORMONE-RECEPTOR AFFINITIES AND INTRINSIC ACTIVITIES OF NEUROHYPOPHYSEAL HORMONES AND SYNTHETIC ANALOGUES ON RAT AORTIC SMOOTH MUSCLE IN DIFFERENT CONCENTRATIONS OF $[Mg^{2+}]o$.

NHPH or Analogue	Peptide (−vasopressin)		Affinity ($\times 10^{-9} M$)[a]				Intrinsic Activity[c]			
			0[b]	0.2	1.2	6.0	0[b]	0.2	1.2	6.0
Arginine-vasopressin	[Arg^8]	—	19.2±0.7	4.7±0.3[d]	2.5±0.2[d]	4.9±0.3[d]	0.24±0.02	0.86±0.05[d]	1.00±0.00[d]	0.83±0.05[d]
8-ornithine-vasopressin	[Orn^8]	—	17.5±0.6	4.4±0.3[d]	3.4±0.2[d]	5.6±0.4[d]	0.43±0.06	1.03±0.03[d]	1.03±0.03[d]	0.77±0.06[d]
2-phenylalanine-8-ornithine-oxytocin	[Phe^2, Ile^3, Orn^8]	—	68.6±3.5	21.3±0.7[d]	15.5±0.6[d]	15.5±0.7[d]	0.02±0.01	0.96±0.03[d]	0.88±0.05[d]	0.88±0.04[d]
Oxytocin	[Ile^3, Leu^8]	—	1755.8±75	44,000±575[d]	36,500±542[d]	44,600±590[d]	0.16±0.02	0.78±0.07[d]	0.85±0.05[d]	0.58±0.07[d]

[a] Mean concentration of agonist (± SEM) required to produce 50% of its maximal contractile response. N = 10–30 different vessels for each peptide and each $[Mg^{2+}]o$. No more than *one* peptide was tested on any one aortic strip.

[b] $[Mg^{2+}]o$.

[c] Where maximal contractile response to [Arg^8]-vasopressin in 1.2 mM $[Mg^{2+}]o$ is taken as 1.00.

[d] Significantly different from value in Mg^{2+}-free solution ($P < 0.01$).

phenomena may also be due to effects on translocation of Ca^{2+} (8).

It has been suggested by several workers that potentiation of structurally different NHPH and analogues by Mg^{2+} on different effector systems, e.g., uterus, mammary gland, blood vessels, is inversely related to potency of the molecules and not to intrinsic differences between uterine smooth muscle, myoepithelial or vascular smooth muscle receptors, per se (3–5, 20). The ED_{50}'s presented in Table I (compare values in 1.2 m*M* Mg^{2+} with Mg^{2+}-free), however, suggest that the greater the rat pressor potency of the NHPH or analogue, the greater is the shift of the concentration-effect curve to the left in the presence of $[Mg^{2+}]o$. These data tend to suggest that the degree of potentiation of a NHPH or analogue on rat aortic smooth muscle by $[Mg^{2+}]o$ may be *directly*, rather than inversely, proportional to the rat pressor potency of the NHPH molecules. In other terms, the more potent the NHPH or analogue in raising rat blood pressure, the more dependent it is on $[Mg^{2+}]o$ for causing contraction of the rat aorta.

At the very least, the present observations indicate that the receptor which subserves contraction for NHPH on mammalian blood vessels is probably different from the one in uterine smooth muscle for vasopressin and oxytocin. $[Mg^{2+}]o$ could thus prove useful in the characterization of the receptors for these posterior pituitary hormones in blood vessels.

Summary. The present quantitative results, using isolated rat aorta, demonstrate that different $[Mg^{2+}]o$ (*i.e.* 0.2, 1.2 and 6.0 m*M*) potentiate the contractile actions of a variety of neurohypophyseal hormones and synthetic analogues on vascular smooth muscle. $[Mg^{2+}]o$ can alter both the hormone-receptor affinities (H-RA) and intrinsic (contractile) activities (i.a.) of these peptides on vascular muscle; 1.2 m*M* $[Mg^{2+}]o$ (approximately that found in rat plasma) appears to optimize H-RA and i.a. on rat aortic smooth muscle. The presence of $[Mg^{2+}]o$ not only steepens the concentration-effect curves to the neurohypophyseal peptides but increases the maximum contractile responses as well. The present findings question that $[Mg^{2+}]o$ potentiates responses to neurohypophyseal peptides by vascular muscle solely by affecting H-RA. The present study supports the notion that Mg^{2+} potentiates responses to these peptides by acting at sites other than the receptor in mammalian vascular muscle. In addition, the present experiments suggest that the $[Mg^{2+}]o$ dependence of neurohypophyseal peptides on at least one mammalian vascular muscle-rat aorta- is *directly rather than inversely* proportional to the rat pressor potency of the molecules. Further, the vasopressin receptor which subserves contraction in mammalian blood vessels may differ in this respect from that in uterine smooth muscle.

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IU/mg), [8-ornithine]-vasopressin (360 IU/mg), [2-phenylalanine, 3-isoleucine, 8-ornithine]-vasopressin (120 IU/mg), and [3-isoleucine, 8-leucine]—vasopressin (oxytocin, 5 IU/mg).
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