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Inhibitory effect of high [Mg^{2+}] on the vasopressin-stimulated hydroosmotic permeability of the isolated perfused cortical collecting duct

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Abstract

High magnesium concentration inhibits the effect of arginine vasopressin (AVP) on smooth muscle contraction and platelet aggregation and also influences hepatocyte AVP receptor binding. The aim of this study was to determine the role of magnesium concentration [Mg^{2+}] in AVP-stimulated water transport in the kidney collecting duct. The effect of low and high peritubular [Mg^{2+}] on the AVP-stimulated osmotic water permeability coefficient (P_f) was evaluated in the isolated perfused rabbit cortical collecting duct (CCD). Control tubules bathed and perfused with standard Ringer bicarbonate solution containing 1 mM Mg^{2+} presented a P_f of 223.9 ± 27.2 µm/s. When Mg^{2+} was not added to the bathing solution, an increase in the AVP-stimulated P_f to 363.1 ± 57.2 µm/s ($P<0.05$) was observed. An elevation of Mg^{2+} to 5 mM resulted in a decrease in P_f to 202.9 ± 12.6 µm/s ($P<0.05$). This decrease in the AVP-stimulated P_f at 5 mM Mg^{2+} persisted when the CCDs were returned to 1 mM Mg^{2+}, P_f = 130.2 ± 20.3 µm/s, and was not normalized by the addition of 8-[4-chlorophenylthio]-adenosine 3',5'-cyclic monophosphate, a cAMP analogue, to the preparation. These data indicate that magnesium may play a modulatory role in the action of AVP on CCD osmotic water permeability, as observed in other tissues.

Key words
- Cortical collecting ducts
- Magnesium
- Arginine vasopressin
- Osmotic water permeability

The collecting duct, as the final portion of the nephron, plays an important role in the control of the extracellular water and salt balance. During water deprivation or extracellular volume contraction, increases in vasopressin release elicit a rise in osmotic water permeability in the kidney collecting ducts. The effect of vasopressin on water reabsorption in the collecting duct is mediated through the classic second messenger adenosine 3',5'-cyclic monophosphate (cAMP) via V_2 receptors. The increase in cyclic AMP levels in the cell leads to the activation of protein kinase A, which promotes the phosphorylation and trafficking of water channels, i.e., the aquaporin-2 protein, from the intracellular vesicles to the plasma membrane (1,2).

The hormone arginine vasopressin (AVP) has multiple functions in the organism, including the stimulation of kidney antidiure-

sis, the contraction of smooth muscle, platelet aggregation, stimulation of liver glycogenolysis, and the modulation of adrenocorticotropic hormone release from the pituitary (3).

The regulatory role of intracellular calcium concentration [Ca^{2+}] during AVP stimulation of water reabsorption in the collecting ducts is a well-known event. Calcium controls the activation or inhibition of the intracellular signaling pathways that are involved in the water channel insertion into the apical membrane (1,4). In the collecting ducts, high or low intracellular [Ca^{2+}]i has an inhibitory effect on the AVP-stimulated water hydroosmotic response (4,5). In contrast, the role of the cation magnesium (Mg^{2+}) in the action of AVP on renal epithelia is not clear. It is well established that extracellular Mg^{2+} has inhibitory effects on AVP-stimulated Ca^{2+} influx in smooth muscle cells and inhibits AVP receptor binding in platelets and hepatocyte membranes (6,7). Little is known about the effect of [Mg^{2+}] on AVP-stimulated water reabsorption in the collecting duct. Argy et al. (8) have already observed that the addition of 8 mM Mg^{2+} to the serosal surface of the toad bladder inhibits AVP stimulation of the osmotic water flow and urea permeability.

The use of magnesium supplements in several therapies is increasing. High doses of magnesium are used clinically for the prevention or improvement of several disorders and in the composition of organ preservation solutions. One of the main roles attributed to magnesium in these therapies is its action as a calcium blocker in several tissues (9). Therefore, it would be interesting to know the effect of high extracellular Mg^{2+} concentrations on the AVP-stimulated hydroosmotic response of renal epithelia.

The objective of the present study was to examine the effects of extracellular magnesium depletion or increase on the AVP-stimulated osmotic water permeability coefficient in isolated perfused rabbit cortical collecting ducts (CCDs).

In vitro isolated rabbit CCDs were perfused by the method of Burg et al. (10) and as previously reported by our group (11). Briefly, New Zealand white rabbits weighing 1.0-2.0 kg were killed by cervical dislocation and the kidneys were immediately removed. Thin coronal slices of the kidney were placed in chilled (10°C) and oxygenated Ringer bicarbonate (Rb) solution for tubule dissection. Isolated CCDs (1-2.5 mm) were transferred to a controlled temperature Lucite chamber mounted on the stage of an inverted microscope (Reichert, Biostar, Vienna, Austria). The tubules were connected to a concentric glass pipette system. The CCDs were perfused at a flow rate of 15 to 20 nl/min obtained by hydrostatic pressure applied to the perfusion pipette. The bathing solution was heated and maintained at 37°C with a thermistor and exchanged continuously at a rate of 0.3 ml/min.

The tubules were dissected and bathed in modified Ringer solution containing 105 mM NaCl, 25 mM $NaHCO_3$, 10 mM Na acetate, 2.3 mM NaH_2PO_4, 5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgSO_4$, 8.3 mM glucose, 5 mM alanine. The osmolality of this solution was 286 ± 4 mOsm/kg H_2O and the pH was adjusted to 7.4 by bubbling with a 95% O_2 and 5% CO_2 gas mixture. The perfusion solution consisted of hypotonic Ringer bicarbonate of the same composition as the bathing solution, except for NaCl, which was reduced to 15 mM. The perfusate presented an osmolality of 126 ± 5 mOsm/kg H_2O. A tracer amount of [Carboxy-^{14}C] inulin (New England Nuclear, Boston, MA), 5 µCi/ml, was added to the perfusate as a volume marker.

The transepithelial osmotic water permeability coefficient P_f (µm/s) was computed from lumen to bath osmotic volume flow according to the equation (12):

$$P_f = (1/AV_w C_b^2)\{C_b(V_i - V_o) + C_i V_i [\ln (C_b - C_i)V_i - \ln (C_b V_o - C_i V_i)]\}$$

where V_i is the perfusion rate in cm/s, calculated from the collection rate (V_o) and the rate of ^{14}C-inulin in the perfused and collected fluid, according to $V_i = V_o$(^{14}C-inulin collected/^{14}C-inulin perfused); A is the surface area (cm^2), calculated from the length of the tubule, measured with an eyepiece reticule, and an assumed internal diameter of 20 µm; V_w is the partial molar volume of water (cm^3/mmol), and C_i and C_b are the osmotic concentrations of the perfusate and bathing medium, respectively.

Four groups of experiments were performed. Each experimental protocol started after a 60-min period of equilibration at 37ºC following the initiation of perfusion. A 30-min basal period was allowed to elapse, after which the tubules were bathed during the experimental period with Ringer bicarbonate solution containing different $MgSO_4$ concentrations and stimulated with 230 pM AVP or 10^{-4} M cAMP (8-[4-chlorophenylthio]-adenosine-3',5'-cyclic monophosphate, Sigma Chemical Co., St. Louis, MO) for P_f evaluation. Thereafter, the bathing solution was replaced with Rb solution containing 1 mM $MgSO_4$ for 30 min to remove AVP. During the recovery period, the tubules were bathed with 1 mM $MgSO_4$ Rb plus AVP or cAMP.

The data are reported as the mean ± SEM of individual values for each tubule, which represent the means of 3 to 4 collections per period. Statistical significance was evaluated at the 95% confidence level. Intergroup one-way analysis of variance (ANOVA) and the screening Bonferroni *t*-test were used. The paired Stundent *t*-test was employed for analysis of the differences between the experimental and recovery periods.

The P_f data for the CCDs during the basal, experimental and recovery periods are presented in Table 1. When the magnesium was omitted from the Rb solution during the experimental period, a significant elevation ($P<0.05$) of the AVP-elicited P_f to 363.1 ± 57.2 µm/s was observed, in relation to the control group. This increase in osmotic water permeability persisted after the addition of 1 mM Mg^{2+} to the Rb solution (P_f of 371.0 ± 67.9 µm/s), during the recovery period. The control group bathed with 1 mM Rb Mg^{2+} showed a P_f of 223.9 ± 27.2 µm/s. When the CCDs were incubated in 5 mM Rb Mg^{2+} a slight decrease in P_f to 202.9 ± 12.6 µm/s was noted. During the recovery period, these tubules presented a greater decrease of the AVP-stimulated P_f to 130.2 ± 20.3 µm/s ($P<0.05$), resulting in an inhibition of 38 ± 10% of the P_f response (Figure 1). This decrease in the stimulated hydroosmotic response was not reversed by the replacement

Table 1 - Effect of increasing [Mg^{2+}] on the AVP- or cAMP-stimulated osmotic water permeability response.

The number of experiments is given in parentheses and the results are reported as the means ± SEM for the basal, experimental (Exp) and recovery (Rec) periods. *$P<0.05$ for experimental x recovery period. $^a P<0.05$ experimental 0 mM ≠ 1 and 5 mM/AVP; $^b P<0.05$ recovery 0 mM ≠ 1 and 5 mM/AVP and cAMP (ANOVA followed by the Bonferroni t-test). V_o: Perfusion flow rate; P_f: osmotic water permeability coefficient; AVP: arginine vasopressin; cAMP: adenosine 3',5'-cyclic monophosphate; [Mg^{2+}]: magnesium concentration.

Group/[Mg^{2+}](mM)		V_o (nl/min)			P_f (µm/s)		
		Basal	Exp	Rec	Basal	Exp	Rec
AVP/0	(5)	18.3 ± 2.6	22.1 ± 0.9	22.1 ± 1.8	71.7 ± 39.2	363.1 ± 57.2	371.0 ± 67.9
AVP/1	(8)	19.1 ± 1.1	20.7 ± 0.7	20.3 ± 0.9	48.7 ± 17.2	223.9 ± 27.2^a	198.6 ± 42.5^b
AVP/5	(5)	15.9 ± 0.6	18.1 ± 1.5	17.4 ± 0.2	35.1 ± 9.2	202.9 ± 12.6^a	130.2 ± 20.3^{*b}
cAMP/5	(4)	14.0 ± 0.6	18.4 ± 0.3	16.9 ± 1.4	29.4 ± 19.3	224.7 ± 33.9	105.4 ± 11.4^{*b}

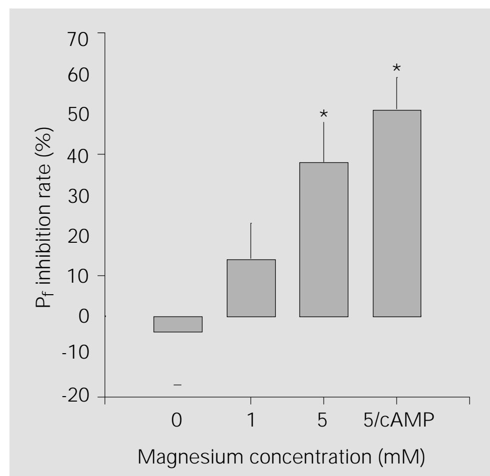

Figure 1 - Inhibitory effect of high magnesium concentrations on AVP or adenosine 3',5'-cyclic monophosphate (cAMP)-stimulated osmotic water permeability coefficient (P_f), in the recovery period. The P_f inhibition rate was calculated as: [($P_f$experimental - $P_f$recovery)/$P_f$experimental] x 100. Data are reported as mean ± SEM. *P<0.05, 0 mM ≠ 5 mM and 5 mM Mg^{2+} plus cAMP stimulation (Bonferroni t-test).

of AVP with the cAMP analogue and resulted in an inhibition of P_f of 51 ± 8% (P<0.05) between the recovery and the experimental periods (Figure 1). In the control group, in which no variation in [Mg^{2+}] occurred between the experimental and the recovery period, only a 14 ± 9% inhibition of P_f was noted after two consecutive AVP doses.

These data demonstrate that the AVP-stimulated osmotic water permeability of cortical collecting ducts could be modulated by extracellular magnesium concentration, as is the case for the action of AVP on the smooth muscle contraction response.

The present study demonstrates the importance of extracellular magnesium concentration in kidney epithelial ADH-dependent water transport. Under our experimental conditions, cortical collecting ducts submitted to reduced peritubular [Mg^{2+}] displayed an increase in the AVP-stimulated osmotic water permeability coefficient.

In the rat vascular smooth muscle cells (VSMC), AVP stimulation through V_1 receptors induces the mobilization of intracellular [Mg^{2+}]i and cytosolic free [Ca^{2+}]i in a dose-dependent manner (6,7). In these cells, it was also observed that the removal of extracellular Mg^{2+} enhanced the AVP-mobilized [Ca^{2+}]i but not [Mg^{2+}]i (6). On the other hand, studies with VSMC have demonstrated that high extracellular [Mg^{2+}] suppressed the activation of a non-selective cation current of Ca^{2+} induced by AVP-stimulation (13). Similarly, in human myometrial cells a high extracellular magnesium concentration reduces the intracellular free [Ca^{2+}] induced by oxytocin (14). If these mechanisms are also present in the CCD epithelial cells, it would be expected that elevated extracellular [Mg^{+}] would decrease cytosolic free Ca^{2+} concentration, which would have an inhibitory effect on the AVP-stimulated hydroosmotic response (5). In agreement with this hypothesis, under our experimental conditions CCDs incubated in 5 mM Mg^{2+} solution showed a fall in AVP- and cAMP-stimulated water osmotic permeability in comparison to tubules incubated in the absence of Mg^{2+}.

The effects of magnesium concentration on the action of other hormones and AVP-related systems have been reported in the literature. AVP release from nerve endings in the neurohypophysis is controlled by the [Mg^{2+}] (15); platelet formation of inositol triphosphate depends on [Mg^{2+}] (16). Increases in [Mg^{2+}] also inhibit the PTH stimulation of cAMP production in renal cortical slices (17).

Our data suggest that a high magnesium concentration inhibits AVP-stimulated hydroosmotic permeability. This decrease in AVP response persisted after the tubules were returned to normal Ringer bicarbonate solution containing 1 mM Mg^{2+}, showing a continued inhibitory response. This inhibition was not reversed by the addition of the cAMP-analogue to the preparation, indicating that extracellular magnesium probably acts on a level beyond the second messenger for the insertion of the aquaporin-2 protein in the apical membrane.

It is also possible that there is an increased binding affinity of AVP for its receptor, contributing to an enhanced response and/or receptor and cAMP desensitization (18,19). In agreement with this view are

studies on other tissues reporting that extracellular Mg^{2+} enhanced vasopressin binding to its receptors in the pig kidney epithelial cell line LLC-PK1, in intact platelets and in rat liver membranes (16,18,20). Several studies have demonstrated that high doses of AVP decrease water osmotic permeability and cAMP production by activating inhibitory G proteins, prostaglandin production, the rise in intracellular Ca^{2+}, the increase in cGMP and/or the stimulation of the protein kinase C (3,19-21).

Finally, the conclusion that a high extracellular magnesium concentration inhibits the AVP-stimulated osmotic water permeability in the cortical collecting ducts needs further study to determine the pathway that is implicated in this mechanism.

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