ELSEVIER

European Journal of Pharmacology 320 (1997) 81–86

ejp

Extracellular Mg^{2+} inhibits receptor-mediated Ca^{2+}-permeable non-selective cation currents in aortic smooth muscle cells

Toshiaki Nakajima [a,*], Kuniaki Iwasawa [a], Hisanori Hazama [a], Michiko Asano [a], Yukichi Okuda [b], Masao Omata [a]

[a] *Second Department of Internal Medicine, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan*
[b] *Department of Internal Medicine, University of Tsukuba, Tsukuba, Ibaraki 305, Japan*

Received 21 October 1996; accepted 1 November 1996

Abstract

The effects of extracellular Mg^{2+} on receptor-mediated Ca^{2+}-permeable non-selective cation currents were investigated in a cultured aortic smooth muscle cell line (A7r5) from rat thoracic aorta, using the whole-cell voltage-clamp technique. Under the Cs^{+}-containing internal solution, both vasopressin and endothelin-1 (100 nM) activated a long-lasting inward current with a high noise level. The reversal potential of these agonists-induced current was approximately +0 mV, and was not significantly altered by the replacement of $[Cl^{-}]_i$ or $[Cl^{-}]_o$, suggesting that the inward current was a cation-selective channel. La^{3+} and Cd^{2+} (1 mM) almost completely abolished the vasopressin or endothelin-induced non-selective cation current; however, nifedipine (10 μM) failed to inhibit it significantly. Extracellular Mg^{2+} (3–20 mM) also markedly inhibited the vasopressin- or endothelin-induced non-selective cation current in a concentration-dependent manner. When a non-hydrolysable GTP-analogue, GTPγS (1 mM), was applied from the patch pipette, the non-selective cation current was gradually activated even in the absence of agonist (vasopressin or endothelin-1), probably due to the direct activation of GTP-binding proteins coupled to the receptors. Extracellular Mg^{2+} (3–20 mM) also suppressed the activation of non-selective cation current induced by GTPγS, suggesting that the inhibitory sites of Mg^{2+} are not located on the receptors. These results suggest that extracellular Mg^{2+} inhibits receptor-mediated non-selective cation current, which may contribute to the relaxation effects of Mg^{2+} in vascular smooth muscle cells.

Keywords: Smooth muscle cell, vascular; Ca^{2+}-permeable non-selective cation channel; Vasopressin; Endothelin-1; Mg^{2+}, extracellular

1. Introduction

Extracellular Mg^{2+} not only induces relaxation per se but also affects the vasomotor reactivity to a number of contractile agonists such as noradrenaline, prostaglandin $F_{2\alpha}$ and histamine (Altura and Altura, 1981; Howell and Carrier, 1986; Karaki, 1989; D'Angelo et al., 1992). Several mechanisms for the relaxant actions of Mg^{2+} have been proposed (Karaki, 1989). Mg^{2+} decreases intracellular Ca^{2+} (D'Angelo et al., 1992) due to the alteration of membrane permeability, disruption of agonist-receptor interaction, and/or blockade of Ca^{2+} channels, i.e. the voltage-dependent L- or T-type Ca^{2+} channels. The inhibitory effects of Mg^{2+} on receptor-mediated Ca^{2+} influx have also been reported (Ruegg et al., 1989; Wallnofer et al., 1989; Karaki, 1989). In various kinds of cells including vascular smooth muscle, the contractile agonists such as vasopressin and endothelin induce Ca^{2+} release from the internal stores and Ca^{2+} influx through receptor-operated Ca^{2+} channels or Ca^{2+} influx through the voltage-dependent Ca^{2+} channels. The receptor-activated Ca^{2+} entry is mediated in part by Ca^{2+}-permeable non-selective cation channels, which is resistant to organic Ca^{2+} antagonist, such as nifedipine (Benham and Tsien, 1987; Van Renterghem et al., 1988; Matsunaga et al., 1994; Nakajima et al., 1996). Furthermore, the activation of this channel depolarizes the membrane, which may indirectly activate the voltage-dependent L-type Ca^{2+} channel, and then increase Ca^{2+} influx. Thus, Ca^{2+}-permeable non-selective cation channel is thought to play an important role in regulating vascular tone. However, the effects of extracellular Mg^{2+} on the channels have not been directly investi-

* Corresponding author. Tel.: (81-3) 3815-5411; Fax: (81-3) 3814-0021.

PII S0014-2999(96)00873-4

gated. Therefore, the purpose of the present study is to clarify the effects of extracellular Mg^{2+} on receptor (vasopressin or endothelin)-mediated Ca^{2+} permeable non-selective cation channels in vascular smooth muscle cells. Here, we report that extracellular Mg^{2+} inhibits receptor-mediated non-selective cation currents, which may contribute to the relaxation effects of Mg^{2+} in vascular smooth muscle cells.

2. Materials and methods

2.1. Cell preparation

A7r5 cells (ATCC-7), an established vascular smooth muscle cell line obtained from embryonic rat aorta (Kimes and Brandt, 1976; Lorenz et al., 1994), were purchased from American Type Culture Collection through Dainippon Seiyaku (Kyoto, Japan). Cells were grown in Dulbecco's modified Eagle's medium (DMEM, Nissui Seiyaku, Tokyo, Japan) supplemented with 10% fetal bovine serum (M.A. Bioproducts, Walkertsville, MD, USA), 50 units/ml of penicillin and 50 μg/ml of streptomycin at 37°C in a fully humidified atmosphere of 5% CO_2 in air. Cells subcultured to passage number 10–20 were grown as monolayers on glass slides, and confluent cell layers were serum-derived by culturing in DMEM containing 0.3% bovine serum albumin for 24 h. Cells were isolated by an enzymatic procedure using trypsin, and used for the later experiments. All experiments were performed at 35–37°C.

2.2. Solutions and drugs

The composition of the standard extracellular solution was as follows (in mM): NaCl 136.5, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5, and HEPES-NaOH buffer 5.5 (pH 7.4). When external ($[Cl^-]_o$) or internal ($[Cl^-]_i$) concentration of Cl^- was changed, Cl^- was replaced with equimolar aspartate. The patch pipette contained (in mM): CsCl 140, EGTA 0.15, $MgCl_2$ 2, Na_2ATP 3, guanosine-5′-triphosphate (GTP, sodium salt, Sigma) 1 and HEPES-CsOH buffer 5 (pH 7.2). In some cells, guanosine-5′-*o*-(3-thiotriphosphate) (GTPγS, Boehringer-Mannheim, Mannheim, Germany) or EGTA (10 mM) was included in the patch pipette. Various concentrations of magnesium

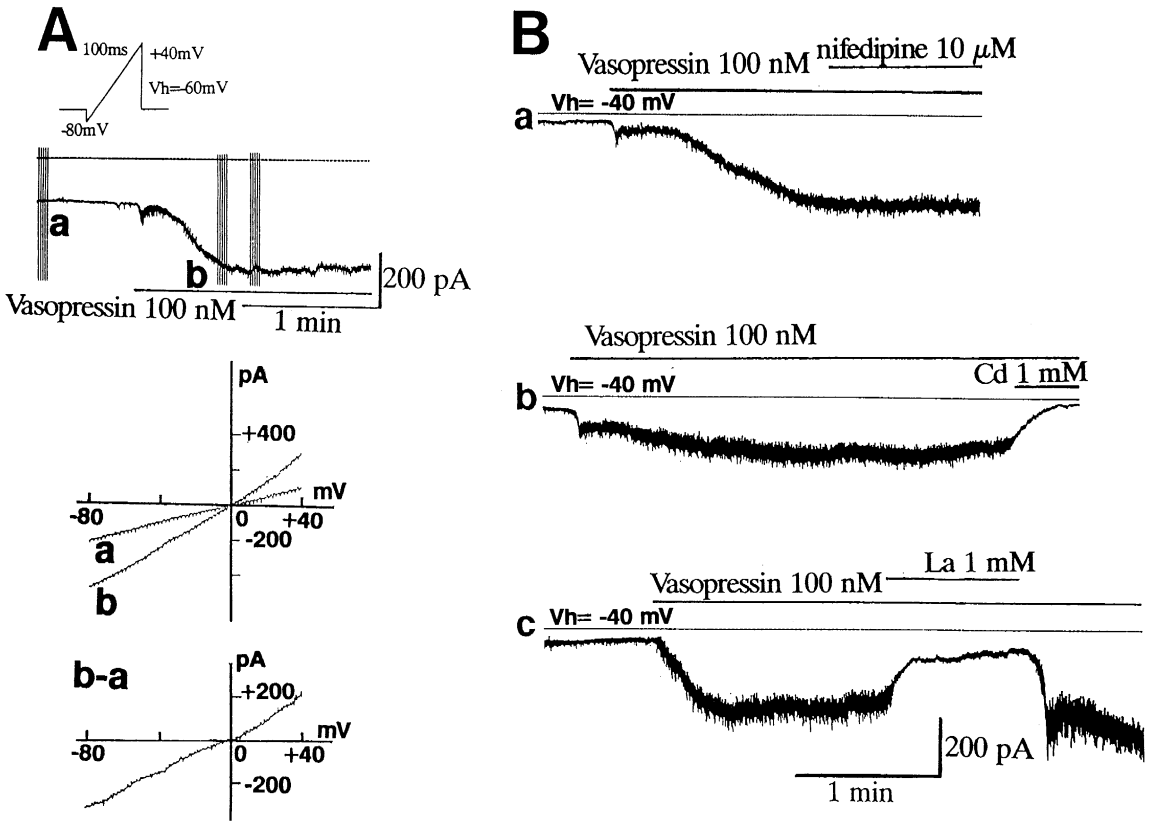

Fig. 1. Activation of a non-selective cation channel by vasopressin and the effects of nifedipine, Cd^{2+} or La^{3+} on the channel in aortic smooth muscle cells. (A) Activation of an inward current by vasopressin. The cell was held at −60 mV. The patch pipette contained the Cs^+-internal solution. Ramp-voltage pulses from −80 mV to +40 mV (100 ms in duration) were applied before (a), and during application of vasopressin (100 nM) (b). The current traces of the ramp pulses (a, b) were recorded at the times indicated in the upper part of the trace. The zero-current level is denoted by dotted lines. The current-voltage (I-V) relationships of the subtraction current from (b) to (a) are shown in the lower part of (A). (B) Effects of nifedipine (10 μM, a), Cd^{2+} (1 mM, b) and La^{3+} (1 mM, c) on vasopressin-activated non-selective cation currents. The cells were held at −40 mV. The zero-current level is illustrated by dotted lines.

were added to the standard bathing solution. [Arg^8]Vasopressin and endothelin-1 were obtained from Sigma (St. Louis, MO, USA).

2.3. Recording technique and data analysis

Membrane currents were recorded with glass pipettes in the whole-cell voltage-clamp condition (Hamill et al., 1981; Nakajima et al., 1992), using a patch-clamp amplifier (EPC-7, List Electronics, Darmstadt, Germany). Heat-polished patch pipettes, filled with the artificial internal solution (for composition, see above), had a tip resistance of 2–4 MΩ. Membrane currents were monitored with a high-gain storage oscilloscope (COS 5020-ST, Kikusui Electronics, Tokyo, Japan). At the start of the experiment, the series resistance was compensated. The data were stored on video tapes using the PCM converter system (RP-880, NF Electronic Circuit Design, Tokyo, Japan). Later, the data were reproduced, low-passed filtered at 2 kHz (−3 dB) with a Bessel filter (FV-665, NF, 48 dB/octave slope attenuation), sampled at 5 kHz, and analysed off-line on a computer using p-Clamp software (Axon Instruments, CA, USA). Voltage-ramp command pulses were used to generate a quasi-steady-state current-voltage (I-V) relationships. Results are expressed as mean ± S.D. Student's *t*-test was used for statistical evaluation, and $P < 0.05$ was considered to be significant.

3. Results

Fig. 1A shows the effects of vasopressin on membrane currents in rat aortic smooth muscle cells (A7r5 cells). Under the condition with Cs^+-internal solution, vasopressin (100 nM) induced a long-lasting inward current with a high noise level at a holding potential of −40 mV. The current-voltage relationships of the inward current were investigated with the ramp-voltage steps (−80 mV to +40 mV, 100 ms in duration). In control bathing solution, the current-voltage relationships of the vasopressin-induced current reversed at −2 ± 3 mV ($n = 12$, Fig. 1A, lower part and Table 1). The reversal potential (E_{rev}) of the current was unchanged by decreasing $[Cl^-]_o$ from 140 mM to 0 mM or $[Cl^-]_i$ from 140 mM to 0 mM (Table 1). These results suggest that vasopressin activates a non-selective cation current, but not a Cl^- current (Nakajima et al., 1995; Hazama et al., 1996) under our conditions as described previously (Van Renterghem et al., 1988; Krautwurst et al., 1994; Nakajima et al., 1996). Similarly, vasopressin induced the inward current with 10 mM EGTA

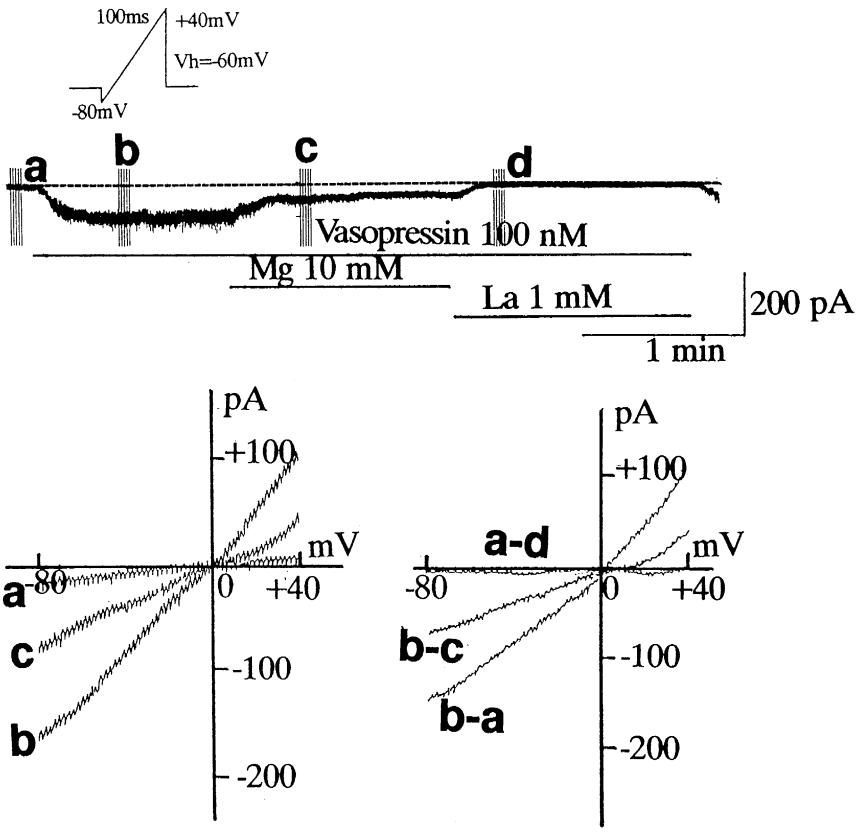

Fig. 2. Effects of extracellular Mg^{2+} on the current-voltage (I-V) relationships of the vasopressin-induced current. The cell was held at −60 mV, and the ramp-voltage command steps (−80 mV to +40 mV in 100 ms duration) were applied in control (a), during application of vasopressin (100 nM, b), vasopressin plus Mg^{2+} (10 mM, c) and vasopressin plus La^{3+} (1 mM, d). The original current traces of a–c are shown in the left side of the lower part. The I-V relationships of the subtraction current from (b) to (a), (b) to (c) and (a) to (d) are shown in the right side of the lower part.

Table 1
Effects of extracellular or intracellular Cl^- concentration on the reversal potential (E_{rev}) of the vasopressin-induced current in A7r5 cells

Bath/pipette solution	E_{rev} (mean ± S.D.)
140 mM $[Cl^-]_o$ /140 mM $[Cl^-]_i$	−2 ± 3 mV (n = 12)
140 mM $[Cl^-]_o$ / 0 mM $[Cl^-]_i$	−3 ± 5 mV (n = 5)
0 mM $[Cl^-]_o$ /140 mM $[Cl^-]_i$	−2 ± 4 mV (n = 5)

Extracellular or intracellular Cl^- was replaced by aspartate. The data represent the mean ± S.D. and the number of cells tested is shown. No significant difference ($P < 0.05$) was observed when compared with the control (140 mM $[Cl^-]_o$ /140 mM $[Cl^-]_i$).

in the patch pipette to chelate intracellular Ca^{2+}. Endothelin-1 (100 nM) also activated the currents in A7r5 cells as indicated in Fig. 3B. In addition, even when extracellular Na^+ was totally replaced by Ca^{2+}, vasopressin (100 nM) still activated the non-selective cation currents (data not shown), suggesting that Ca^{2+} is also a charge carrier of the vasopressin-induced non-selective cation current. These results are compatible with previous papers showing that both vasopressin and endothelin-1 activate Ca^{2+}-permeable non-selective cation channels in aortic smooth muscle cells (A7r5 cells) (Van Renterghem et al., 1988; Krautwurst et al., 1994; Nakajima et al., 1996). Fig. 1B shows the effects of nifedipine, Cd^{2+}, or La^{3+} on vasopressin-induced Ca^{2+}-permeable non-selective cation currents. Nifedipine (10 μM) failed to affect the holding current, and to inhibit the vasopressin-induced non-selective cation currents significantly (Fig. 1Ba). However, Cd^{2+} (1 mM, Fig. 1Bb) and La^{3+} (1 mM, Fig. 1Bc) almost completely inhibited the vasopressin-induced non-selective cation currents. Similar results were obtained from four different cells in each case. The reduction of the non-selective cation channels by these agents was associated with a decrease in the noise level and the inhibitory effects of these agents were reversible.

Figs. 2 and 3 (A, B) illustrate the effects of extracellular Mg^{2+} on the vasopressin- or endothelin-induced non-selective cation currents. Mg^{2+} (3–20 mM) by itself failed to affect the holding current significantly (data not shown), but Mg^{2+} (3–20 mM) dose-dependently inhibited the vasopressin- (Fig. 3A) or endothelin-1-induced non-selective cation currents (Fig. 4, and Table 2). The inhibitory effect of extracellular Mg^{2+} was reversible. The Mg^{2+}-induced suppression of the current was examined with ramp-voltage steps (Fig. 2). Mg^{2+} (10 mM) suppressed the vasopressin-induced non-selective cation currents at any voltages (Fig. 2, left side of the lower part). The reversal potential of the current was not altered by Mg^{2+} (10 mM) significantly (Fig. 2, right side of the lower part), suggesting that ionic selectivity of the channels was not altered by Mg^{2+}. Fig. 4 and Table 2 show the effects of various concentrations of extracellular Mg^{2+} on the vasopressin- or endothelin-1-activated non-selective cation currents. Mg^{2+} (3–20 mM) inhibited the vasopressin- or endothelin-1-induced current in a concentration-dependent manner, and the half-maximal inhibitory dose (IC_{50}) of Mg^{2+} was about 7 mM in both cases.

To investigate the inhibitory site of Mg^{2+} on the non-selective cation currents, the effects of Mg^{2+} on GTPγS-induced non-selective cation currents were also examined. The patch pipette contained the non-hydrolysable GTP analogue (GTPγS, 1 mM). Non-selective cation currents were activated by the addition of GTPγS intracellularly through the patch pipette. Immediately after the rupture of the membrane (Fig. 3C), the holding current was gradually increased into the inward direction at a holding potential of −40 mV even in the absence of agonists (vasopressin or endothelin-1). In addition, inclusion of GDPβS (2 mM) in the patch pipette completely inhibited the activation of the current by vasopressin and endothelin-1, suggesting that GTP-binding proteins were involved in activating the current (Fig. 3E). La^{3+} (1 mM) inhibited the GTPγS-induced current (Fig. 3C and 3D).

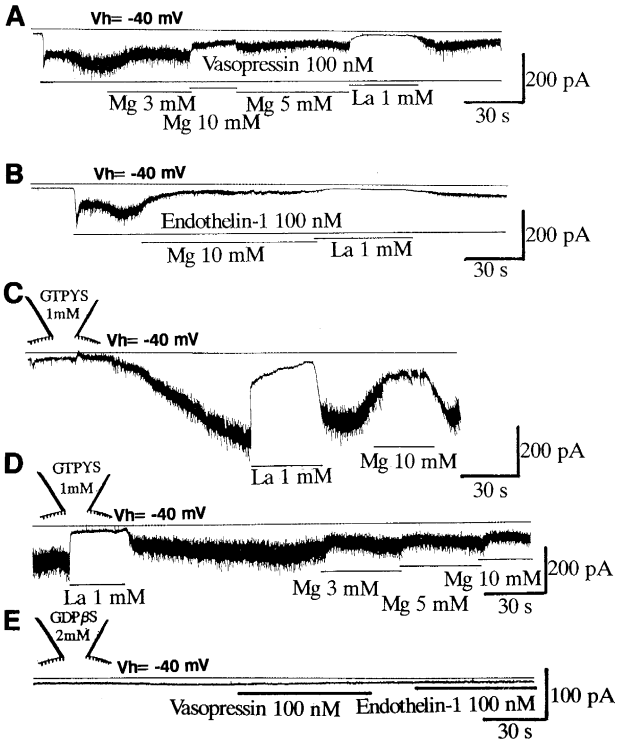

Fig. 3. Effects of extracellular Mg^{2+} on the vasopressin-, endothelin-1- or GTPγS-induced non-selective cation currents. (A and B) Effects of extracellular Mg^{2+} on the vasopressin- or endothelin-1-activated non-selective cation currents. The cells were held at −40 mV. Various concentrations of Mg^{2+} were added into the bath solution. The zero-current level is denoted by the dotted lines. (C and D) Effects of extracellular Mg^{2+} on GTPγS-induced non-selective cation currents. The patch pipette contained the Cs^+-internal solution with GTPγS (1 mM). Immediately after rupture of the membrane, the continuous holding current is shown in (C). In (D), the holding current is shown after the increased current reached to a steady-state level. The zero current is denoted by the dotted lines. (E) Effects of GDPβS on vasopressin or endothelin-1-induced non-selective cation currents. The patch pipette contained GDPβS (2 mM).

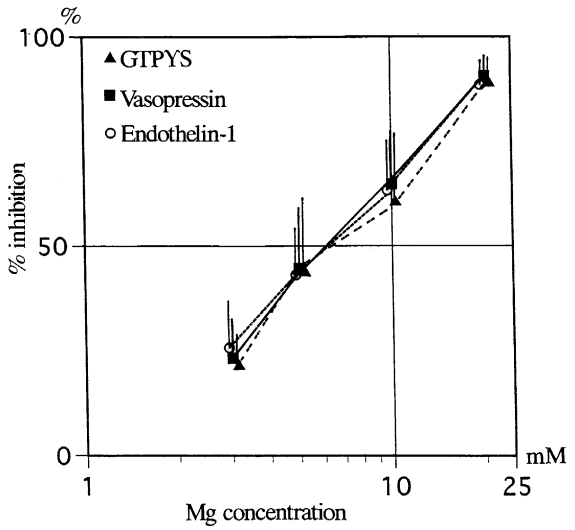

Fig. 4. The dose-response curves for extracellular Mg^{2+}-induced suppression of vasopressin-, endothelin-1- or GTPγS-induced non-selective cation currents. The per cent inhibition by extracellular Mg^{2+} (3–20 mM) on vasopressin- (closed squares), endothelin-1- (open circles) or GTPγS- (closed triangles) induced currents is illustrated. Each value represents the mean ± S.D. of six different cells. No statistical significance between the per cent inhibition by Mg^{2+} on vasopressin- or endothelin-1-induced currents and that by Mg^{2+} on GTPγS-induced currents at each Mg^{2+} concentration was observed.

The reversal potential of the GTPγS-induced current was -1 ± 3 mV ($n = 5$), and was unaffected by the alteration of the external or internal concentration of Cl^-. These results suggest that GTPγS activates the non-selective cation currents even in the absence of agonists, possibly by the direct activation of the GTP-binding proteins coupled to the receptors (vasopressin and endothelin receptors). Mg^{2+} also inhibited the GTPγS-activated non-selective cation current in a concentration-dependent manner (Fig. 3D). The concentration-dependence curve of extracellular Mg^{2+} on GTPγS-induced non-selective cation currents is indicated in Fig. 4 (closed triangles) and Table 2. The IC_{50} value was approximately 7 mM, and the concentration-dependence curves of extracellular Mg^{2+} on the vasopressin-, endothelin-1- and GTPγS-induced currents are almost superimposable.

Table 2
Per cent inhibition by extracellular Mg^{2+} on vasopressin-, endothelin-1- and GTPγS-induced currects in A7r5 cells

$[Mg^{2+}]_o$	% Inhibition (mean ± S.D.)		
	Vasopressin	Endothelin-1	GTPγS
3 mM	23 ± 9 ($n = 6$)	26 ± 11 ($n = 6$)	22 ± 7 ($n = 6$)
5 mM	44 ± 15 ($n = 6$)	43 ± 11 ($n = 6$)	46 ± 16 ($n = 6$)
10 mM	66 ± 11 ($n = 6$)	63 ± 12 ($n = 6$)	60 ± 17 ($n = 6$)
20 mM	90 ± 5 ($n = 6$)	88 ± 6 ($n = 6$)	91 ± 4 ($n = 6$)

The per cent inhibition by extracellular Mg^{2+} (3–20 mM) on vasopressin-, endothelin-1- or GTPγS-induced non-selective cation currents is indicated. The mean ± S.D. value is shown, and the data were obtained from six different cells.

4. Discussion

The present study indicates that extracellular Mg^{2+} (3–20 mM) inhibited the receptor (vasopressin and endothelin)-mediated Ca^{2+}-permeable non-selective cation channels in aortic smooth muscle cells. The functional importance of this channel is considered as follows. (1) It depolarizes the membrane potential, which indirectly activates the voltage-dependent Ca^{2+} channels, and then increases Ca^{2+} influx. (2) In various kinds of cells including vascular smooth muscle cells, the activation of the channel itself can evoke Ca^{2+} influx through the channel that is highly permeable to divalent cations, i.e. Ca^{2+} (Benham and Tsien, 1987; Van Renterghem et al., 1988; Matsunaga et al., 1994; Nakajima et al., 1996), which can contribute to the sustained rise of $[Ca^{2+}]_i$ evoked by contractile agonists, such as vasopressin, endothelin or platelet-derived growth factor (PDGF). Therefore, the inhibitory effects of extracellular Mg^{2+} on the channels are thought to contribute to the vasorelaxant effects of Mg^{2+}. Nifedipine, a dihydropyridine Ca^{2+} antagonist, failed to inhibit the receptor-mediated non-selective cation channels, proposing that Mg^{2+} has a unique therapeutic effect which is different from that of organic L-type Ca^{2+} channel blockers.

The inhibitory effects of Mg^{2+} on the channels can occur at any of the steps that exists between receptor binding and channel opening. High EGTA in the patch pipette could not block the activation of the channel by vasopressin or endothelin-1, suggesting that it is not directly mediated by the rise of $[Ca^{2+}]_i$, which is consistent with earlier papers (Van Renterghem et al., 1988; Krautwurst et al., 1994; Nakajima et al., 1996). Therefore, the inhibitory action of Mg^{2+} on the receptor-mediated non-selective cation channels does not seem to be mediated secondarily by the alteration of $[Ca^{2+}]_i$. Extracellular Mg^{2+} has been shown to affect binding of pressor hormones (including histamine) to vascular smooth muscle (Howell and Carrier, 1986; Karaki, 1989). However, it seems unlikely that the direct inhibition of receptor (vasopressin or endothelin-1) is mainly involved in Mg^{2+} actions on the non-selective cation channels. As indicated in Fig. 3 and previous papers (Krautwurst et al., 1994; Nakajima et al., 1996), the activation of the non-selective cation channels by vasopressin and endothelin-1 is mediated by GTP-binding proteins. Inclusion of GDPβS in the patch pipette abolished the activation of the current, and the addition of GTPγS, a non-hydrolysable GTP analogue, activated the channels even in the absence of agonists, probably due to the direct activation of GTP-binding proteins coupled to the receptors. Extracellular Mg^{2+} inhibited both the agonist-dependent activation of non-selective cation channels and the activation of the channels by GTPγS in a similar manner, suggesting that the receptor sites are not mainly involved in the Mg^{2+} effects. Furthermore, the inhibitory effect of extracellular Mg^{2+} was quite rapid, and easy to wash out. Also, D'Angelo et al. (1992)

have reported that increased extracellular Mg^{2+} concentration $[Mg^{2+}]_o$ (1.2–10 mM) induces relaxation by decreasing myoplasmic $[Ca^{2+}]$ without changing intracellular Mg^{2+} concentration $[Mg^{2+}]_i$ in carotid arteries. From these findings, extracellular Mg^{2+} does not appears to inhibit the channel by affecting $[Mg^{2+}]_i$ indirectly, but appears to block it from the outside.

The voltage-dependent Ca channels (L- and T-type) can be blocked by a wide variety of multivalent cations including Cd^{2+}, Co^{2+} and La^{3+}. External Mg^{2+} (1–10 mM) also blocks the voltage-dependent L-type Ca^{2+} channels (Lansman et al., 1986; Ohya and Sperelakis, 1990) with an IC_{50} of about 12 mM (Ohya and Sperelakis, 1990). Recently, Krautwurst et al. (1994) showed that multivalent cations can inhibit receptor (vasopressin)-mediated non-selective cation channels with a rank of potency ($Gd^{3+} > La^{3+} > Cd^{2+}$) in A7r5 cells where the IC_{50} values for these cations were 0.09 μM, 27 μM, and 243 μM, respectively. The present study illustrated that extracellular Mg^{2+} can also inhibit the receptor-mediated non-selective cation channels with IC_{50} values of approximately 7 mM. Therefore, Mg^{2+} seems to be a less potent blocker than some other multivalent cations, but appears to work as blockers as other multivalent cations. In aortic smooth muscle cells (Ruegg et al., 1989; Wallnofer et al., 1989), extracellular Mg^{2+} (1–10 mM) inhibits agonist (vasopressin, angiotensin II or purinergic agonist)-induced Ca^{2+} influx with a rank of potency ($La^{3+} > Cd^{2+} > Mg^{2+}$) which is somewhat compatible with the potency of extracellular Mg^{2+} on receptor-operated Ca^{2+}-permeable non-selective cation channels in the present study.

In conclusion, extracellular Mg^{2+} inhibits receptor-mediated non-selective cation channels in a physiological range of 3–10 mM in vascular smooth muscle cells, probably due to the direct blockade of the channels, which may contribute to the vasorelaxant effects of Mg^{2+}.

Acknowledgements

This work was partly supported by grants from the Ministry of Education, Science and Culture of Japan and Grant-in-Aid for Scientific Research on Priority Areas of 'Channel-Transporter Correlation' to T.N.

References

Altura, B.M. and B.T. Altura, 1981, Magnesium ions and contraction of vascular smooth muscles: relationship to some vascular diseases, Fed. Proc. 40, 2672.

Benham, C.D. and R.W. Tsien, 1987, A novel receptor-operated Ca^{2+}-permeable channel activated by ATP in smooth muscle, Nature 328, 275.

D'Angelo, E.K.G., H.A. Singer and C.M. Rembold, 1992, Magnesium relaxes arterial smooth muscle by decreasing intracellular Ca^{2+} without changing intracellular Mg^{2+}, J. Clin. Invest. 89, 1988.

Hamill, O.P., A. Marty, E. Neher, B. Sakmann and F.J. Sigworth, 1981, Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches, Pflüg. Arch. 391, 85.

Hazama, H., T. Nakajima, E. Hamada, M. Omata and Y. Kurachi, 1996, Neurokinin A and Ca^{2+} current induce Ca^{2+}-activated Cl^- currents in guinea-pig tracheal myocytes, J. Physiol. (London) 492, 377.

Howell, R.E. and G.O. Carrier, 1986, Influence of magnesium on norepinephrine- and histamine-induced contractions of pulmonary vascular smooth muscle, Pharmacology 33, 27.

Karaki, H., 1989, Magnesium as a modification of smooth muscle contractility, Microcirc. Endothelium Lymphatics 5, 77.

Kimes, B.W. and B.L. Brandt, 1976, Characterization of two putative smooth muscle cell lines from rat thoracic aorta, Exp. Cell. Res. 98, 349.

Krautwurst, D., V.E. Degtiar, G. Schultz and J. Hescheler, 1994, The isoquinoline derivative LOE 908 selectively blocks vasopressin-activated nonselective cation currents in A7r5 aortic smooth muscle cells, Naunyn-Schmiedeberg's Arch. Pharmacol. 349, 301.

Lansman, J.B., P. Hess and R.W. Tsien, 1986, Blockade of current through single calcium channels by Cd^{2+}, Mg^{2+}, and Ca^{2+}. Voltage and concentration dependence of calcium entry into the pore, J. Gen. Physiol. 88, 321.

Lorenz, J.N., D.R. Bielefeld and N. Sperelakis, 1994, Regulation of calcium channel current in A7r5 vascular smooth muscle cells by cyclic nucleotides, Am. J. Physiol. 266, C1656.

Matsunaga, H., B.N. Ling and D.C. Eaton, 1994, Ca^{2+}-permeable channel associated with platelet-derived growth factor receptor in mesangial cells, Am. J. Physiol. 267, C456.

Nakajima, T., T. Sugimoto and Y. Kurachi, 1992, Effects of anions on the G protein-mediated activation of the muscarinic K^+ channel in the cardiac atrial cell membrane: intracellular chloride inhibition of the GTPase activity of G_K, J. Gen. Physiol. 99, 665.

Nakajima, T., H. Hazama, E. Hamada, M. Omata and Y. Kurachi, 1995, Ionic basis of neurokinin A-induced depolarization in single smooth muscle cells isolated from the guinea-pig trachea. Pflügers Arch. 430, 552.

Nakajima, T., H. Hazama, E. Hamada, S.-N. Wu, K. Igarashi, T. Yamashita, Y. Seyama, M. Omata and Y. Kurachi, 1996, Endothelin-1 and vasopressin activate Ca^{2+}-permeable non-selective cation channels in aortic smooth muscle cells: mechanism of receptor-mediated Ca^{2+}-influx, J. Mol. Cell. Cardiol. 28, 707.

Ohya, Y. and N. Sperelakis, 1990, Tocolytic agents act on calcium channel current in single smooth muscle cells of pregnant rat uterus, J. Pharmacol. Exp. Ther. 253, 580.

Ruegg, U.T., A. Wallnofer, S. Weir and C. Cauvin, 1989, Receptor-operated calcium-permeable channels in vascular smooth muscle, J. Cardiovasc. Pharmacol. 14, S49.

Van Renterghem, C., G. Romey and M. Lazdunski, 1988, Vasopressin modulates the spontaneous electrical activity in aortic cells (line A7r5) by acting on three different types of ionic channels, Proc. Natl. Acad. Sci. USA 85, 9365.

Wallnofer, A., C. Cauvin, T.W. Lategan and U.T. Ruegg, 1989, Differential blockade of agonist- and depolarization-induced $^{45}Ca^{2+}$ influx in smooth muscle cells, Am. J. Physiol. 257, C607.