J. Cell. Mol. Med. Vol 15, No 3, 2011 pp. 593-601

Imipramine inhibition of TRPM-like plasmalemmal Mg^{2+} transport in vascular smooth muscle cells

Yukihisa Hamaguchi [a, #], Yasushi Tatematsu [a, †], Koichi Furukawa [b], Tatsuaki Matsubara [c], Shinsuke Nakayama [a, *]

[a] *Department of Cell Physiology, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[b] *Department of Biochemistry II, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[c] *Department of Internal Medicine, Aichi-Gakuin University School of Dentistry, Nagoya, Japan*

Received: November 22, 2009; Accepted: January 21, 2010

Abstract

Depression is associated with vascular disease, such as myocardial infarction and stroke. Pharmacological treatments may contribute to this association. On the other hand, Mg^{2+} deficiency is also known to be a risk factor for the same category of diseases. In the present study, we examined the effect of imipramine on Mg^{2+} homeostasis in vascular smooth muscle, especially *via* melastatin-type transient receptor potential (TRPM)-like Mg^{2+}-permeable channels. The intracellular free Mg^{2+} concentration ($[Mg^{2+}]_i$) was measured using ^{31}P-nuclear magnetic resonance (NMR) in porcine carotid arteries that express both TRPM6 and TRPM7, the latter being predominant. pH_i and intracellular phosphorus compounds were simultaneously monitored. To rule out Na^+-dependent Mg^{2+} transport, and to facilitate the activity of Mg^{2+}-permeable channels, experiments were carried out in the absence of Na^+ and Ca^{2+}. Changing the extracellular Mg^{2+} concentration to 0 and 6 mM significantly decreased and increased $[Mg^{2+}]_i$, respectively, in a time-dependent manner. Imipramine statistically significantly attenuated both of the bi-directional $[Mg^{2+}]_i$ changes under the Na^+- and Ca^{2+}-free conditions. This inhibitory effect was comparable in influx, and much more potent in efflux to that of 2-aminoethoxydiphenyl borate, a well-known blocker of TRPM7, a channel that plays a major role in cellular Mg^{2+} homeostasis. Neither $[ATP]_i$ nor pH_i correlated with changes in $[Mg^{2+}]_i$. The results indicate that imipramine suppresses Mg^{2+}-permeable channels presumably through a direct effect on the channel domain. This inhibitory effect appears to contribute, at least partially, to the link between antidepressants and the risk of vascular diseases.

Keywords: magnesium • vascular smooth muscle • tricyclic antidepressants • nuclear magnetic resonance • melastatin-type transient receptor potential channels

Introduction

Psychiatric disorders are associated with brain and heart diseases caused by ischaemia [1–4]. Epidemiological studies suggest that some behavioural factors of depressive disorders especially physical inactivity make a major contribution to this association [5]. Also, in the genetic aspect, malfunction of cell signalling, such as reduced serotonin transport caused by polymorphisms of this transporter gene, can affect not only the mental condition but also numerous peripheral functions, including platelet aggregation in blood vessels [6, 7]. Furthermore, pharmacological treatment can also affect vascular muscle contractility. Tricyclic antidepressants, such as imipramine, are known to have a number of unwanted side effects in addition to inhibition of serotonin and noradrenaline reuptake [8, 9].

Mg^{2+} deficiency is a well-known risk factor for vascular disease [10–13], and higher Mg^{2+} intake is recommended to prevent arteriosclerosis and hypertension. Lines of evidence have recently suggested that two isoforms of melastatin-type transient receptor potential (TRPM) isoforms, such as TRPM6 and TRPM7 act as Mg^{2+}-permeable channels, and play essential roles in Mg^{2+} homeostasis [14, 15]. TRPM6 and TRPM7 channels were initially thought to share roles of Mg^{2+} homeostasis by cell- and tissue-specific expression of either isoform [16, 17]. However, recent

[#]Present address: Tokai Memorial Hospital, Kasugai, Aichi 487–0031, Japan.
[†]Present address: Nagoya Daini Red Cross Hospital, Nagoya 466–8650, Japan.
*Correspondence to: Shinsuke NAKAYAMA,
Department of Cell Physiology,
Nagoya University Graduate School of Medicine,
Nagoya 466–8550, Japan.
Tel.: +81 52 744 2045
Fax: +81 52 744 2048
E-mail: h44673a@nucc.cc.nagoya-u.ac.jp

doi:10.1111/j.1582-4934.2010.01024.x

studies have revealed heteromeric formation of TRPM6/7 in Mg^{2+}-permeable channels [18–20].

In the present study, we applied ^{31}P-NMR to examine the effects of imipramine on Mg^{2+}-permeable channels in the pig carotid artery, which is frequently used as a model to evaluate arteriosclerotic changes, and expresses both TRPM6 and TRPM7 despite a predominant expression of the latter [21]. We found that imipramine considerably attenuates transmembrane Mg^{2+} transport driven by the concentration gradient. The inhibitory effect of this drug on Mg^{2+} influx was comparable to the effect of 2-aminoethoxydiphenyl borate (2-APB), a well-known blocker of TRPM7 [22], but was much more potent on Mg^{2+} efflux. We discuss a possible scenario how tricyclic antidepressants and vascular diseases are associated *via* TRPM-like channels and Mg^{2+} homeostasis.

Methods

Preparation

Porcine carotid arteries were collected at an abattoir. The arteries were stripped of fat and connective tissue, and cut into segments of approximately 30 mm in length. After removing the endothelium by rubbing with cotton tips, pig carotid artery strips (~2 g wet weight) were mounted in a sample tube of 10 mm in diameter [23, 24]. This study was registered, and all procedures for sample collection and preparation were approved by the institutional committee of animal experiments.

^{31}P-NMR measurements

The methods employed for the ^{31}P-NMR measurements were essentially the same as those previously described [21]. An NMR spectrometer (UNITY-500plus: Varian, Tokyo, Japan) was operated at 202.3 MHz. The temperature of the sample was maintained at 32°C. Radio frequency pulses corresponding to a flip angle of 30° were applied every 0.6 sec. ^{31}P-NMR spectra were obtained by accumulating 2500 signals (free induction decays) over 25 min.

Control spectra of the carotid artery samples were acquired in the absence of Ca^{2+}. Then, extracellular Na^+ was removed by substituting with equimolar K^+ to rule out any contribution of Na^+-coupled Mg^{2+} transport, *i.e.* Na^+/Mg^{2+} exchange. This high extracellular K^+ condition also abolished the membrane potential and enabled us to easily compare the inhibitory effects of imipramine on Mg^{2+} influx and efflux.

Concentrations of phosphorus compounds were estimated by integrating the peak areas (Scion image; Scion Corp., Fredrick, MA, USA) and by correcting with their saturation factors (P_i, 1.60; PCr, 1.36; β-ATP, 1.07).

Estimation of $[Mg^{2+}]_i$ and pH_i

Intracellular pH (pH_i) was first estimated from the chemical shift observed for the Pi peak ($\delta_{o(Pi)}$) using a Henderson–Hasselbalch type equation:

$$pH_i = pK_a + \log_{10}[(\delta_{o(Pi)} - \delta_{p(Pi)})/(\delta_{d(Pi)} - \delta_{o(Pi)})], \quad (1)$$

where pK_a is the negative logarithm of the dissociation constant of P_i (=6.70), and $\delta_{p(Pi)}$ and $\delta_{d(Pi)}$ are the chemical shifts for $H_2PO_4^-$ (=3.15 p.p.m.) and HPO_4^{2-} (=5.72 p.p.m.), respectively. The pH_i value was used to correct the $[Mg^{2+}]_i$ estimation.

$[Mg^{2+}]_i$ can be estimated from the chemical shift observed for the β-ATP peak (δoβ), using the following equation [25]:

$$[Mg^{2+}]_i = K_D^{\text{"MgATP"}} (\delta_{o\beta} - \delta_{f\beta})/(\delta_{b\beta} - \delta_{o\beta}), \quad (2)$$

where $\delta_{f\beta}$ and $\delta_{b\beta}$ are the chemical shifts of metal-free and Mg^{2+}-bound forms of β-ATP, respectively. We have previously shown that $K_D^{\text{'MgATP'}}$, $\delta_{f\beta}$ and $\delta_{b\beta}$ can be described as functions of pH [26]. Thus, equation (2) is rewritten:

$$[Mg^{2+}]_i = K_D^{\text{"MgATP"}} (pH_i) (\delta_{o\beta} - \delta_{f\beta}(pH_i))/(\delta_{b\beta}(pH_i) - \delta_{o\beta}). \quad (3)$$

$K_D^{\text{'MgATP'}}$ at 25°C and 37°C are expressed as quadratic pH functions [27]:

$$K_D^{\text{"MgATP"}(25°C)}(pH) = K_{D.Z(TA)} = 841.793 - 142.399\ pH + 6.0\ pH^2; \quad (4A)$$

$$K_D^{\text{"MgATP"}(37°C)}(pH) = K_{D.Z(TB)} = 1311.055 - 311.355\ pH + 19.639\ pH^2. \quad (4B)$$

The pH function of $K_D^{\text{'MgATP'}}(pH_i)$ at 32°C is derived from those at 25°C and 37°C using the van't Hoff isochore:

$$K_D^{\text{"MgATP"}(32°C)}(pH) = K_{D.Z(TC)} = K_{D.Z(TB)}\ [K_{D.Z(TA)}/K_{D.Z(TB)}]^{\Psi} \quad (4C).$$

($\Psi = (1/T_C - 1/T_B)/(1/T_A - 1/T_B)$: T_A, T_B and T_C are absolute temperatures of 25°C, 37°C and 32°C, respectively.)

Also, the pH functions of $\delta_{f\beta}$ and $\delta_{b\beta}$ are constructed by fitting the data points of model solutions with sigmoid curves [26]:

$$\delta_{f\beta}(pH_i) = (\delta_{f\beta,p} - \delta_{f\beta,d})/\{1 + 10^{[HL(pK-pH)]}\} + \delta_{f\beta,d}$$
$$(\delta_{f\beta,d} = -18.59;\ \delta_{f\beta,p} = -19.79;\ HL = -1.00;\ pK = 6.48); \quad (5A)$$

$$\delta_{b\beta}(pH_i) = (\delta_{b\beta,p} - \delta_{b\beta,d})/\{1 + 10^{[HL(pK-pH)]}\} + \delta_{b\beta,d}$$
$$(\delta_{b\beta,d} = -15.79;\ \delta_{b\beta,p} = -19.12;\ HL = -0.90;\ pK = 4.84), \quad (5B)$$

where p and d represent protonated and deprotonated forms, respectively.

$[Mg^{2+}]_i$ was thus estimated from the chemical shift of the β-ATP peak using equation (3), with correction of pH_i from the chemical shift of the P_i peak (equation 1).

Solutions and chemicals

The extracellular solution used for the 'normal' solution had the following composition in mM: NaCl, 137.9; $KHCO_3$ 5.9; $CaCl_2$ 2.4; $MgCl_2$ 1.2; glucose 11.8; HEPES 5 (pH adjusted to 7.4–7.5 at 32°C). The ionic composition was modified iso-osmotically. Also, divalent cation-free solutions contained 1 mM ethylenediaminetetraacetic acid. The solutions used for ^{31}P-NMR measurements were normally aerated with 95%O_2/5%CO_2. Imipramine was purchased from Sigma (St. Louis, MO, USA).

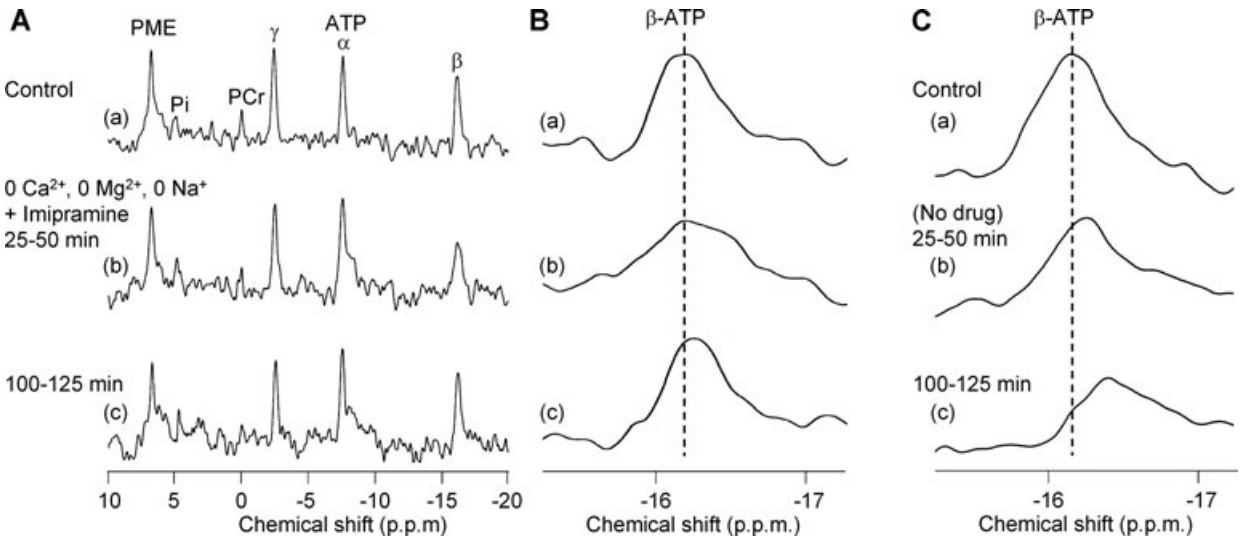

Fig. 1 Changes in the ^{31}P-NMR spectrum during exposure to a divalent-cation-free, Na^+-free solution in the presence of imipramine. After acquiring the control spectrum in a Ca^{2+}-free solution (a), extracellular Mg^{2+} and Na^+ were simultaneously removed (0 Ca^{2+}, 0 Mg^{2+}, 0 Na^+: K^+ substitution) and 100 μM imipramine was added for 125 min. The spectra (b) and (c) were obtained during 25–50 min. and 100–125 min. periods, respectively. Each spectrum was obtained with 2500 signals accumulated over 25 min. The whole spectrum is shown in (**A**), and the β-ATP peaks are shown expanded in (**B**). The vertical line indicates the initial chemical shift of the β-ATP peak. The expanded β-ATP peaks in (**C**) show an experiment carried out in the absence of imipramine (reproduced from Hamaguchi *et al.*). Note that imipramine markedly suppressed the shift of the β-ATP peak towards lower frequency.

Statistics

Numerical data are expressed as the mean ± S.D. Differences between groups with different experimental protocols were evaluated by use of ANOVA for repeated measures. When a significant difference was identified between the groups ($P < 0.05$), individual comparisons at the same time-point were performed with an unpaired t-test.

Results

Imipramine inhibition of Na^+-independent depletion of intracellularMg^{2+}

$[Mg^{2+}]_i$ was continuously measured using ^{31}P-NMR in the pig carotid artery, in which both active and passive Mg^{2+} transport (Na^+/Mg^{2+} exchange and TRPM-like channels, respectively) are operating. After observing control spectra in Ca^{2+}-free solution, removal of Mg^{2+} and Na^+ resulted in a chemical shift of the β-ATP peak towards a lower frequency, indicating a large decrease in $[Mg^{2+}]_i$ to approximately 50% through TRPM-like Mg^{2+}-permeable channels. Application of imipramine (100 μM) prevented the shift of the β-ATP peak in the absence of Na^+ (Fig. 1). This reflected a nearly complete inhibition of Mg^{2+} efflux down the concentration gradient.

Figure 2 summarizes the inhibitory effect of imipramine on Na^+-independent Mg^{2+} efflux (A) and changes in pH_i (B). Even in the absence of Na^+, simultaneous removal of Mg^{2+} and Ca^{2+} depleted $[Mg^{2+}]_i$ from 0.75 ± 0.09 mM to 0.46 ± 0.05 mM after 125 min. ($n = 7$; data from Hamaguchi *et al.*). When imipramine (100 μM) was applied to the preparations, $[Mg^{2+}]_i$ was stable throughout exposure to the divalent-cation and Na^+-free solution (0.74 ± 0.07 mM after 125 min., $n = 5$), but in the absence of imipramine $[Mg^{2+}]_i$ was significantly lower after 50 min. (A), whereas pH_i decreased by approximately 0.2 units irrespective of imipramine application (B).

Inhibitory effect of imipramine on the $[Mg^{2+}]_i$ increase *via* Na^+-independent Mg^{2+}influx

Exposure to a Ca^{2+}-, Na^+-free solution containing high Mg^{2+} (6.0 mM), increased $[Mg^{2+}]_i$ approximately 2-fold after 125 min. (from 0.78 ± 0.08 to 1.79 ± 0.18 mM after 125 min., $n = 7$; Fig. 3A open squares). Application of imipramine (100 μM) strongly attenuated the increase in $[Mg^{2+}]_i$ (filled squares). The changes in $[Mg^{2+}]_i$ in the absence and presence of imipraime differ significantly during 25–125 min. When imipramine was applied $[Mg^{2+}]_i$ increased to only 1.22 ± 0.14 mM after 125 min. ($n = 4$), whereas changes in pH_i were nearly to the same degree, irrespective of the application of imipramine (B).

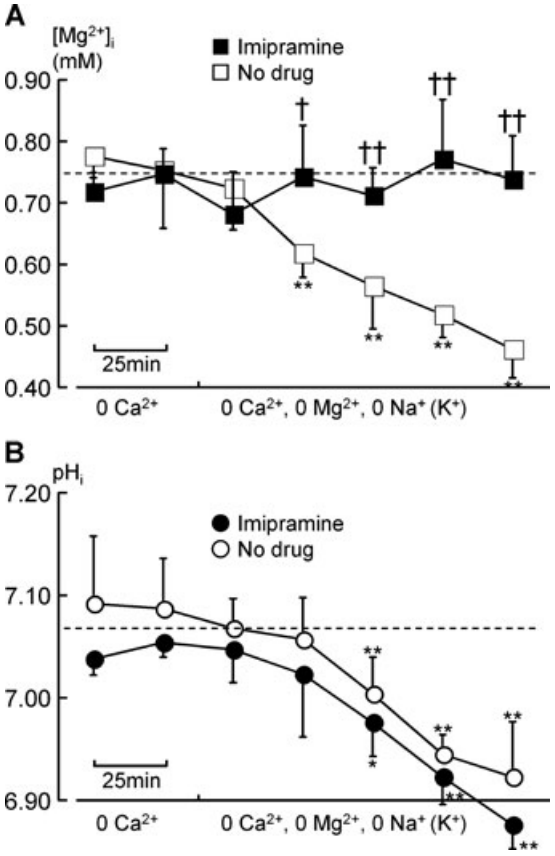

Fig. 2 The inhibitory effect of imipramine on $[Mg^{2+}]_i$ depletion during exposure to a Na^+-free, divalent-cation-free solution (0 Ca^{2+}, 0 Mg^{2+}, 0 Na^+). Changes in $[Mg^{2+}]_i$ and pH_i are plotted in (**A**) (■) and (**B**) (●), respectively. After acquiring the control data in a Ca^{2+}-free solution, extracellular Mg^{2+} and Na^+ were simultaneously removed, and 100 μM imipramine was added. Results previously obtained in the absence of imipramine (open symbols: □, ○; from Hamaguchi *et al.*) are also plotted to show clearly the inhibitory effect of imipramine. Asterisks indicate statistically significant differences compared to the $[Mg^{2+}]_i$ and pH_i values before removal of extracellular Na^+ (*, $P < 0.05$; **, $P < 0.01$). Crosses on filled symbols indicate statistically significant differences compared to the open symbols at the same time-point (†, $P < 0.05$; ††, $P < 0.01$).

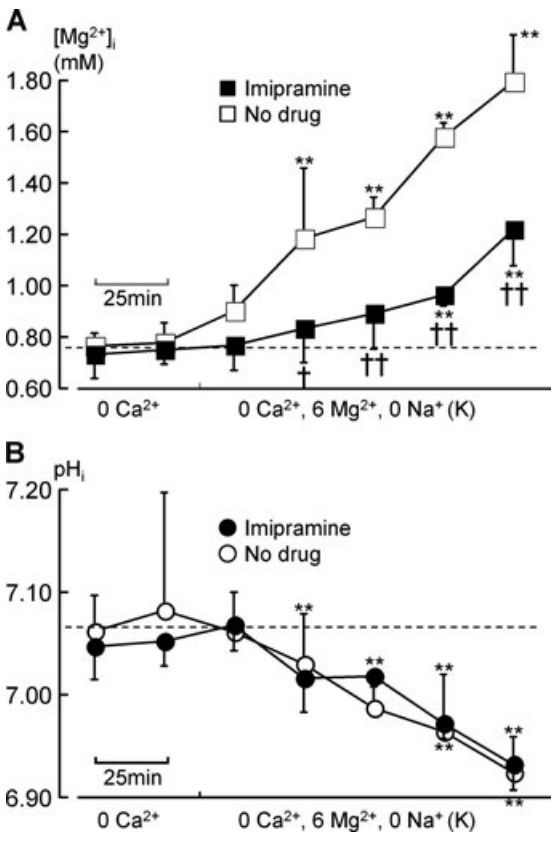

Fig. 3 The inhibitory effect of imipramine on $[Mg^{2+}]_i$ rise during exposure to a Na^+-free, Na^{2+}-free solution containing high Mg^{2+} (0 Ca^{2+}, 6.0 Mg^{2+}, 0 Na^+). Changes in $[Mg^{2+}]_i$ and pH_i are plotted in (**A**) (■) and (**B**) (●), respectively. After acquiring the control data in a Ca^{2+}-free solution, the extracellular Mg^{2+} was increased to 6.0 mM, and simultaneously Na^+ was removed, and 100 μM imipramine was added. The data obtained in the absence of imipramine (open symbols: □, ○; reproduced from Hamaguchi *et al.*) are also plotted to show the inhibitory effect of imipramine. Asterisks indicate statistically significant differences compared to the $[Mg^{2+}]_i$ and pH_i values before removal of extracellular Na^+ (*, $P < 0.05$; **, $P < 0.01$). Crosses on filled symbols indicate statistically significant differences compared to the open symbols at the same time-point (†, $P < 0.05$; ††, $P < 0.01$).

High-energy phosphates

TRPM6 and TRPM7 are primitive large molecules that contain multiple domains of different functions, *i.e.* channel pore and enzyme (α-kinase) domains [14]. These proteins are thus referred to as chanzymes. ATP acts as a major buffer for intracellular Mg^{2+}, and PCr plays an important role in keeping the phosphorylation potential and kinase activity stable. Also, it has been reported that Mg^{2+} and MgATP regulate the activity of TRPM7 *via* the kinase domain [28, 29].

Table 1 summarizes changes in the concentrations of ATP and PCr ([ATP] and [PCr]) during the experiments shown in Figs 2–3. Throughout the experiments, no significant change was observed in these phosphorus compounds, irrespective of the ionic environments and application of imipramine. [ATP] represents total [ATP], *i.e.* [metal-free ATP] + [MgATP]; therefore [MgATP] decreases as $[Mg^{2+}]_i$ decreases, while [MgATP] increases as $[Mg^{2+}]_i$ increases. Together with the fact that imipramine inhibited both Mg^{2+} efflux and influx consistently (Figs 2 and 3), it is suggested that this drug may directly affect the channel domain.

Table 1 Changes in the concentration of high-energy phosphates. Values are expressed relative to [ATP] in the control (before the application of drugs)

(A) Exposure to divalent-cation-free, Na^+-free solutions ($n = 7$)		
	[ATP]	**[PCr]**
Control (0 Ca^{2+} solution)	1.00	0.28 ± 0.02
0 Ca^{2+}, 0 Mg^{2+}, 0 Na^+ (K^+)	0.93 ± 0.08	0.30 ± 0.02
25–50 min.		
0 Ca^{2+}, 0 Mg^{2+}, 0 Na^+ (K^+)	0.92 ± 0.09	0.31 ± 0.01
100–125 min.		
(B) Exposure to divalent-cation-free, Na^+-free solutions containing 100 μM imipramine ($n = 4$)		
	[ATP]	**[PCr]**
Control (0 Ca^{2+} solution)	1.00	0.31 ± 0.02
0 Ca^{2+}, 0 Mg^{2+}, 0 Na^+ (K^+)	1.10 ± 0.10	0.31 ± 0.03
+100 μM imipramine 25–50 min.		
0 Ca^{2+}, 0 Mg^{2+}, 0 Na^+ (K^+)	1.10 ± 0.13	0.29 ± 0.07
+100 μM imipramine 100–125 min.		
(C) Exposure to Ca^{2+}-, Na^+-free, high-Mg^{2+} (6.0 mM) solutions ($n = 7$).		
	[ATP]	**[PCr]**
Control (0 Ca^{2+} solution)	1.00	0.30 ± 0.02
0 Ca^{2+}, 6.0 Mg^{2+}, 0 Na^+ (K^+)	1.05 ± 0.05	0.29 ± 0.01
25–50 min.		
0 Ca^{2+}, 6.0 Mg^{2+}, 0 Na^+ (K^+)	1.02 ± 0.06	0.28 ± 0.01
100–125 min.		
(D) Exposure to Ca^{2+}-, Na^+-free, high-Mg^{2+} (6.0 mM) solutions containing 100 μM imipramine ($n = 4$).		
	[ATP]	**[PCr]**
Control (0 Ca^{2+} solution)	1.00	0.32 ± 0.05
0 Ca^{2+}, 6.0 Mg^{2+}, 0 Na^+ (K^+)	1.09 ± 0.07	0.31 ± 0.06
+100 μM imipramine 25–50 min.		
0 Ca^{2+}, 6.0 Mg^{2+}, 0 Na^+ (K^+)	1.05 ± 0.10	0.30 ± 0.06
+100 μM imipramine 100–125 min.		

Discussion

Cellular Mg^{2+} is believed to be regulated by Na^+-dependent and -independent Mg^{2+} transport (Fig. 4) [30, 31]. Although no corresponding molecule has yet been identified in mammals, Na^+-dependent transport is ascribed to Na^+/Mg^{2+} exchangers that can transport Mg^{2+} against the electrochemical gradient using the energy of the Na^+ concentration gradient across the plasma membrane. On the other hand, Na^+-independent Mg^{2+} transport is attributed to Mg^{2+}-permeable channels. These have recently been identified [14]. The present investigation on the effect of imipramine on $[Mg^{2+}]_i$ regulation revealed that this drug inhibits both influx and efflux of Mg^{2+} through Na^+-independent mechanisms in vascular smooth muscle (Figs 2 and 3). Mg^{2+} efflux in particular was nearly completely blocked. The inhibitory effects of imipramine on influx are comparable to and much more potent in efflux than those of 2-APB [21] (Fig. 5), a well-known TRPM7 blocker [22].

The vascular smooth muscles used in this study (pig carotid artery) express TRPM6 and TRPM7, particularly the latter [21].

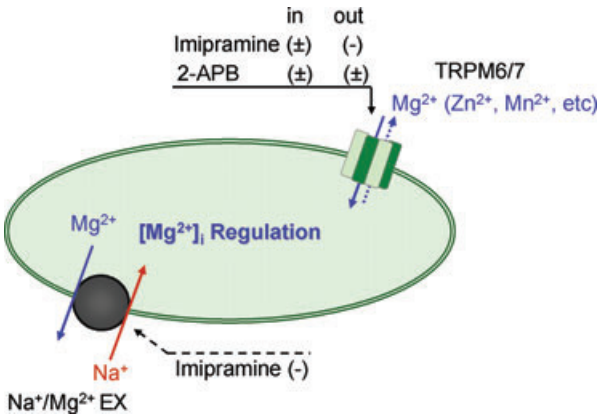

Fig. 4 Diagram summarizing cellular Mg^{2+} homeostasis and pharmacological inhibition. $[Mg^{2+}]_i$ is regulated by Na^+-dependent and -independent transporters. The former is a Na^+/Mg^{2+} exchanger (EX) and the latter are Mg^{2+}-permeable heteromeric TRPM6/7 channels. 2-APB incompletely inhibits both inward and outward Mg^{2+} transport in TRPM6/7 channels [21]. The present study demonstrates that the inhibitory effect of imipramine is comparable in influx to and much more potent in efflux than that of 2-APB in vascular smooth muscle cells. Imipramine is also known to inhibit Na^+/Mg^{2+} exchange. (–) and (±) represent strong and partial inhibition, respectively.

These two TRPM isoforms are non-selective cation channels permeable to Mg^{2+}, and are proposed to play essential roles in Mg^{2+} homeostasis in the complete organism and at cellular levels, respectively [13, 14, 19]. TRPM6 is abundant in the small intestine and kidney, and loss-of-function mutations are known to cause familial hypomagnesemia by reducing Mg^{2+} absorption and reabsorption [32, 33]. On the other hand, TRPM7, ubiquitously distributed in the body, is required for cell growth and viability [16, 34, 35]; 2-APB inhibition of TRPM7 has been shown to cause cell death in cancer cells [36, 37]. These TRPM channels had initially been thought to share the roles of Mg^{2+} homeostasis by cell- and tissue-specific expression of either isoform [16, 17]. Recent studies have, however revealed heteromeric formation of TRPM6/7 in numerous cells and tissues [18–21].

In cultured cells in which either TRPM6 or TRPM7 are transfected, patch clamp experiments have revealed interesting pharmacological effects: 2-APB at several tens of μM largely suppresses plasma membrane currents corresponding to TRPM7, whereas it potentiates ionic currents of TRPM6 [38]. These opposing effects of 2-APB can explain our previous observations. Presumably, heterometic TRPM6/7 channels with a minor component of TRPM6 are responsible for the incomplete inhibition of TRPM-like Na^+-independent Mg^{2+} transport at a high concentration (150 μM) of 2-APB.

The fact that the inhibitory effect of imipramine is comparable or more potent than that of 2-APB suggests that tricyclic antidepressants impair Mg^{2+} homeostasis. Furthermore, imipramine is also known to inhibit Na^+/Mg^{2+} exchange [39–41], suggesting that tricyclic antidepressants impair $[Mg^{2+}]_i$ homeostasis much more severely due to synergic inhibition of both Mg^{2+} transporters. Imipramine is frequently used as a 'selective' blocker of Na^+/Mg^{2+} exchange, in comparison to amiloride and its derivatives that also inhibit Na^+-coupled transporters, including Na^+/H^+ exchange, Na^+/Ca^{2+} exchange, etc. [42–45]. However, we found an inhibitory effect of imipramine on TRPM-like Mg^{2+}-permeable channels during a 'control' experiment designed to inhibit only Na^+/Mg^{2+} exchange.

The possible impairment of Mg^{2+} homeostasis with tricyclic antidepressants may, at least partially, account for the risk of several functional disorders induced by such drugs. For example, overdose of tricyclic antidepressants is known to slow ventricular conduction, and cause re-entry arrhythmia and complete heart block [9, 46, 47]. Tricyclic antidepressants are therefore dangerous in patients with pre-existing conduction defect and under treatment of class I anti-arrhythmic agents, and are contraindicated for use during the acute recovery phase following myocardial infarction. On the other hand, a similar association is known for Mg^{2+}. Epidemiological studies suggest that drinking soft water and Mg^{2+} deficiency increase the risk of cerebrovascular and cardiovascular disease [10, 11, 13]. It is also known that during myocardial infarction β-adrenoceptor activation causes loss of Mg^{2+} [48, 49].

Intracellular ATP bound to Mg^{2+} is the universal currency of energy metabolism. Therefore, it is possible that tricyclic antidepressants impair cellular energy metabolism by lowering Mg^{2+} concentration. Such metabolic conditions are favourable to heart failure and orthostatic hypotension. Also, intracellualr Mg^{2+} plays many other important roles in cellular metabolism, *i.e.* as a co-factor of many enzymes, other than forming energy currency, MgATP. Interestingly, there is a known association between Mg^{2+} deficiency, type 2 diabetes and metabolic syndrome [50–53]. Conversely, dietary Mg^{2+} prevents such disease [54]. In line with this evidence, we have previously shown in animal studies that catecholamines, which increase the mortality of myocardiac infarction, significantly decrease $[Mg^{2+}]_i$ in the heart, and that insulin prevents this intracellular Mg^{2+} deficiency [55, 56]. Furthermore, it has been shown that Mg^{2+} and Zn^{2+} alter the promoter activity of apolipoproteins that are good risk markers for myocardial infarction [57, 58].

A broad range of divalent cations can also permeate TRPM6 and TRPM7, including trace metals such as Zn^{2+}, Co^{2+} and Mn^{2+} [19, 38]. Since these ions are required for activation of metalloenzymes, impairment of TRPM6/7 channels is likely to affect cellular metabolism *via* these divalent cations. Disorders relating to the inhibitory effects of imipramine on the transport of such metal ions are considered to become noticeable after long-term administration.

Constipation and urinary retention are common unwanted side effects of imipramine and tricyclic antidepressants [8]. These effects can be attributed to atropine-like inhibition of muscarinic receptor signalling. In addition, it has recently been revealed that interstitial cells of Cajal (ICC) act as gut pacemaker cells employing Ca^{2+}-associated mechanisms [59]. Together with the fact that the knockdown of *trpm7* by use of siRNA eliminates ICC pacemaking in cultured preparations [60], it is considered that tricyclic

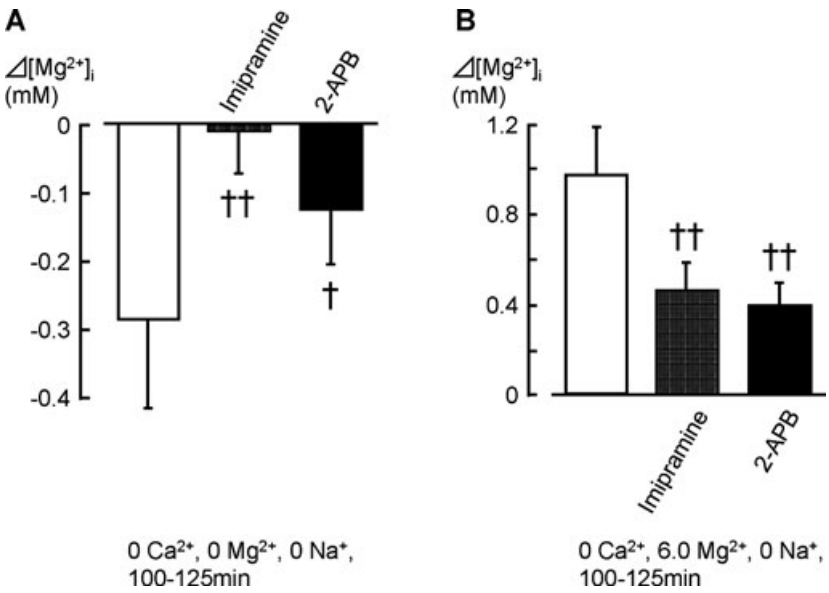

Fig. 5 Comparison of inhibitory effects of imipramine (100 μm) and 2-APB (150 μm) on Mg^{2+} transport during exposure to 0 Na^{+}, 0 Ca^{2+}, 0 Mg^{2+} (**A**) and 0 Na^{+}, 0 Ca^{2+}, 6.0 Mg^{2+} (**B**). Crosses indicate statistically significant differences compared to the experiments with no drugs (†, $P < 0.05$; ††, $P < 0.01$). (Data without drugs and with 2-APB reproduced from Hamaguchi *et al.*)

antidepressants block the TRPM-like Ca^{2+} permeability, essential for maintaining basal Ca^{2+} influx in pacemaker cells, and thereby cause bowel motility disorders and constipation. In addition, there is an accumulating body of evidence that ICC-like cells exist outside the gastrointestinal tract including in the urinary bladder [61–63]. Imipramine and antidepressants may cause urinary retention by similar mechanisms.

In conclusion, the present study revealed an inhibitory effect of imipramine on TRPM-like Mg^{2+} transport. This effect is comparable to or more potent than that of 2-APB, a well known blocker of TRPM7, and can, at least partially, account for the risk of imipramine and other tricyclic antidepressants in vascular disease and functional disorders such as constipation. In future studies, the possible presence of TRPM6/7 isoforms leading to impairment of the transport of Mg^{2+} and other cations should be taken into consideration.

Acknowledgements

The authors are grateful to Dr. Lorraine M. Smith (Edinburgh University, UK) for useful discussions, and to the Nagoya Meat Hygiene Laboratory (Nagoya, Japan) for continuous supply of porcine carotid arteries.

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