Activation of AMPA/Kainate Receptors but Not Acetylcholine Receptors Causes Mg$^{2+}$ Influx into Retzius Neurones of the Leech Hirudo medicinalis

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ABSTRACT

In Retzius neurones of the medicinal leech, Hirudo medicinalis, kainate activates ionotropic glutamate receptors classified as AMPA/kainate receptors. Activation of the AMPA/kainate receptor–coupled cation channels evokes a marked depolarization, intracellular acidification, and increases in the intracellular concentrations of Na$^+$ ([Na$^+$]) and Ca$^{2+}$. Qualitatively similar changes are observed upon the application of carbachol, an activator of acetylcholine receptor-coupled cation channels. Using multibarrelled ion-selective microelectrodes it was demonstrated that kainate, but not carbachol, caused additional increases in the intracellular free Mg$^{2+}$ concentration ([Mg$^{2+}$]). Experiments were designed to investigate whether this kainate-induced [Mg$^{2+}$], increase was due to a direct Mg$^{2+}$ influx through the AMPA/kainate receptor–coupled cation channels or a secondary effect due to the depolarization or the ionic changes. It was found that: (a) Similar [Mg$^{2+}$], increases were evoked by the application of glutamate or aspartate. (b) All kainate-induced effects were inhibited by the glutamatergic antagonist DNQX. (c) The magnitude of the [Mg$^{2+}$], increases depended on the extracellular Mg$^{2+}$ concentration. (d) A reduction of the extracellular Ca$^{2+}$ concentration increased kainate-induced [Mg$^{2+}$], increases, excluding possible Ca$^{2+}$ interference at the Mg$^{2+}$-selective microelectrode or at intracellular buffer sites. (e) Neither depolarizations evoked by the application of 30 mM K$^+$, nor [Na$^+$], increases induced by the inhibition of the Na$^+$/K$^+$ ATPase caused comparable [Mg$^{2+}$], increases. (f) Inhibitors of voltage-dependent Ca$^{2+}$ channels did not affect the kainate-induced [Mg$^{2+}$], increases. Moreover, previous experiments had already shown that intracellular acidification evoked by the application of 20 mM propionate did not cause changes in [Mg$^{2+}$]. The results indicate that kainate-induced [Mg$^{2+}$], increases in leech Retzius neurones are due to an influx of extracellular Mg$^{2+}$ through the AMPA/kainate receptor-coupled cation channel. Mg$^{2+}$ may thus act as an intracellular signal to distinguish between glutamatergic and cholinergic activation of leech Retzius neurones.

KEY WORDS: magnesium • ion-selective microelectrode • glutamate receptor • ion channels • divalent cations

INTRODUCTION

During the past 20 yr, mechanisms for the extrusion of Mg$^{2+}$ from cells have been studied in a great variety of cell types (for review see Beyenbach, 1990; Flatman, 1991; Bijvelds et al., 1998; Günzel and Schlue, 2000). In contrast, in spite of some recent advances, comparatively little is known about the physiological pathways of Mg$^{2+}$ influx into eukaryotic cells (Freire et al., 1996; Bijvelds et al., 2001; Graschopf et al., 2001; Nadler et al., 2001; Schlingmann et al., 2002).

In neurones, obvious candidates for a specific and regulated Mg$^{2+}$ influx are neurotransmitter-activated cation channels. Increases in the intracellular free Mg$^{2+}$ concentration ([Mg$^{2+}$]) have been demonstrated to occur after activation of the NMDA receptor in cultured rat cortical neurones (Brocard et al., 1993; Stout et al., 1996). A [Mg$^{2+}$], increase may also be expected upon activation of certain subtypes (GluR-B(Q), GluR-D) of the AMPA receptor–coupled ion channel, as these channels exhibit a divalent cation permeability in patch-clamp studies (Burnashev et al., 1992).

Neurotransmitter action could cause a primary increase in [Mg$^{2+}$], due to Mg$^{2+}$ entering the cell through the activated channel. Secondary increases in [Mg$^{2+}$], could be induced by membrane depolarizations or increases in the intracellular concentrations of Ca$^{2+}$ ([Ca$^{2+}$]) and Na$^+$ ([Na$^+$]) caused by the transmitter. If depolarizations activated Ca$^{2+}$ channels, Mg$^{2+}$ could enter through these channels. An increase in [Ca$^{2+}$], could lead to an increase in [Mg$^{2+}$], by changing intracellular buffering through competition of Ca$^{2+}$ and Mg$^{2+}$ for common binding sites (Brocard et al., 1993; Handy et al., 1996). If [Na$^+$], increased, the Na$^+$/Mg$^{2+}$ antiport could reverse and induce an increase in [Mg$^{2+}$], (Handy et al., 1996; Stout et al., 1996).

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Abbreviation used in this paper: SLS, standard leech saline.
In this study, neurotransmitter-induced changes in [Mg^{2+}], were investigated in the Retzius neurones of the central nervous system of the medicinal leech, Hirudo medicinalis. Retzius neurones are known to possess cation channel-coupled receptors for the neurotransmitters glutamate (Dörner et al., 1990, 1994; Kilb and Schlue, 1999) and acetylcholine (ACH) (Szczupak et al., 1998). The glutamate receptors have been characterized to be of the AMPA/kainate receptor type and can be stimulated by kainate as well as glutamate, aspartate, and AMPA. Activation of these receptors with 500 μM kainate causes a marked depolarization, an increase in [Na^+], and an intracellular acidification (Dörner et al., 1990, 1994; Kilb and Schlue, 1999). The depolarization activates voltage-dependent Ca^{2+} channels and thus triggers an increase in [Ca^{2+}], Activation of the ACh receptors with 500 μM carbamoyl induces qualitatively similar changes, but of smaller amplitude (Dierkes et al., 1997a).

In neurones, increases in both [Mg^{2+}], and [Ca^{2+}], may occur simultaneously. When using mag-fura-2 to investigate changes in [Mg^{2+}], when there are concomitant changes in [Ca^{2+}], it has to be kept in mind that part of the measured signal could be due to an interaction between Ca^{2+} and mag-fura-2. Microelectrodes do not have this disadvantage as long as [Ca^{2+}] remains <1 μM. Because of this, multibarrelled ion-selective microelectrodes were used to investigate the possible effects of neurotransmitter receptor activation on [Mg^{2+}], and to distinguish between primary and secondary effects of transmitter action. The use of multibarrelled microelectrodes enabled the simultaneous measurement of membrane potential and up to three different ion species.

Our results show that Mg^{2+} can permeate AMPA/kainate receptor-coupled, but not ACh receptor-coupled cation channels of leech Retzius neurones. Some of the results presented here have been published in abstract form (Müller et al., 1997a,b, 1998).

**M A T E R I A L S A N D M E T H O D S**

**Preparation**

Experiments were performed on Retzius neurones in segmental ganglia of the leech Hirudo medicinalis. Segmental ganglia from the leech central nervous system were dissected as described by Schlue and Deitmer (1980). Isolated ganglia were transferred to an experimental chamber and fixed ventral side up by piercing the connectives with insect pins. During all experiments the experimental chamber (volume 0.2 ml) was continuously superfused with saline at room temperature (20–25°C) at a rate of ~20-chamber volumes per minute (5 ml/min).

**Solutions**

Standard leech saline (SLS) contained (in mM): NaCl 85, KCl 4, MgCl₂ 1, CaCl₂ 2, and HEPES (N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid]; AppliChem) 10, pH 7.4 adjusted with ~3 mM NaOH, bringing the total Na⁺ concentration up to 90 mM. For a nominally Mg^{2+}-free saline, MgCl₂ was omitted. For a 5 mM MgCl₂ saline MgCl₂ was added to a nominally Mg^{2+}-free saline. Additionally, Na⁺-free saline and saline with a Na⁺ content reduced to 45 mM were obtained by an equimolar substitution of Na⁺ with NMDG⁺ (N-methyl-D-glucamine⁺; Sigma-Aldrich), and pH was adjusted with NMDG-OH. In solutions with a Mg^{2+} content of 10, 20, and 30 mM, the Na⁺ concentration was kept at 45 mM while NMDG-Cl was partly or completely replaced with MgCl₂. Thus, it was possible to avoid changes in osmolarity and in the Na⁺ gradient across the cell membrane during alterations of [Mg^{2+}]. In the 30 mM K⁺ saline NaCl was replaced by KCl. In the 0.2 mM K⁺ saline, KCl was added to a nominally K⁺-free saline. The saline containing La³⁺ was prepared by adding appropriate amounts of an aqueous 0.5 M stock solution of LaCl₃, Ca²⁺ in Ca²⁺-free saline was buffered with 5 mM EGTA (ethylene glycol-bis(β-aminoethyl ether); Sigma-Aldrich). L-glutamic acid (Sigma-Aldrich), magnesium-L-aspartate-hydrochloride (Verla-Pharm), and kainic acid (2-Carboxy-3-carboxymethyl-4-isopropylpyrrrolidine; Sigma-Aldrich) were added from aqueous stock solutions. Magnesium aspartate (L-aspartic acid magnesium salt; FLUKA), the glutamatic acid antagonist DNQX (6,7-dinitroquinoxaline-2,3-dione; Sigma-Aldrich), the cyclic alcohol menthol and the cholinergic agonist carbachol ((2-hydroxyethyl)trimethylammonium chloride carbachol; Sigma-Aldrich) were added in solid form.

**Ion-selective Microelectrodes**

Experiments were performed using ion-selective double-, triple-, or four-barrelled microelectrodes. Microelectrodes were pulled from borosilicate glass-capillaries (double-barrelled, TGC200–15; triple-barrelled, TGC200–15 and GC150F-15; four-barrelled, TGC200–15 and GC150–15; Clark) and silanized as described by Günzel et al. (1997, 1999). Mg^{2+}, Na⁺, and pH-selective barrels were filled with ion sensors based on the neutral carriers ETH 5214, ETH 227 and ETH 1907 (Fluka), respectively. The respective backfill solutions were 100 mM MgCl₂, 100 mM NaCl, and the pH 7.67 calibration solution (composition see below). One barrel of each microelectrode was filled with 3 M KCl and served as an intracellular reference. K⁺-selective barrels were filled with a sensor based on the K⁺ ionophore valinomycin (K⁺-ionophore I, Fluka) while Cl⁻-selective barrels were filled with the FLUKA Cl⁻ sensor I. In both cases, the ion-selective barrels were backfilled with 100 mM KCl, whereas the reference channel was filled with 3 M Na-acetate (+8 mM KCl).

**Calibration Procedure**

Before and after each experiment, microelectrodes were calibrated in solutions that mimicked the ionic background of intracellular conditions. Mg^{2+} calibration solutions contained (in mM): KCl 110, NaCl 10, HEPES 10, and 10, 2.5, 0.5, or 0 MgCl₂, added from a 1 M stock solution (Fluka), pH 7.5 adjusted with KOH. pH calibration solutions were identical to the 0.5 mM Mg^{2+} calibration solutions except that they were buffered either with 10 mM HEPES or 10 mM MES (2-[N-morpholino]ethanesulfonic acid; Sigma-Aldrich) to a pH of 7.67 and 6.22, respectively, by the addition of equal amounts of KOH. Na⁺ calibration solutions contained (in mM): KCl 110, MgCl₂ 0.5, HEPES 10, and 50, 10, 2.5, or 0 NaCl, pH 7.3 adjusted with KOH. In addition, Ca²⁺ was buffered to a free concentration of ~10⁻⁷ M by adding 0.73 mM CaCl₂ and 1 mM EGTA (calculation of CaCl₂ and EGTA concentrations based on Pershadsingh and McDonald, 1980). K⁺ calibration solutions contained a background of (in mM): MgCl₂ 0.5, HEPES 10, pH 7.3 adjusted with NaOH. The concentrations of both KCl and NaCl were varied to keep...
the ionic strength of the solutions constant. KCl amounted to 100, 50, 20, 10, 5, 2.5, and 0 mM with NaCl being 10, 60, 100, 105, 107.5, and 110 mM, respectively. In Cl−-calibration solutions, the concentrations of KCl and Kgluconate were varied (mM): KCl 50, 10, 2.5, 0 with Kgluconate 60, 100, 107.5, 110, respectively, over a background of Na-gluconate 10, Mg-gluconate 0.5, HEPES 10, pH 7.3 adjusted with KOH.

The potential differences between the ion-selective channels and the reference channel were plotted against $p_{\text{Ion}} (-\log[I_{\text{on}}])$, and from the calibration procedure resulting calibration curves were fitted with the Nicolsky-Eisenman equation ($M_{\text{g}}^{2+}$, $Na^{+}$, $K^{+}$, $Cl^{-}$), or the Nernst equation ($pH$). Microelectrodes were only used if their detection limit (as defined by Ammann, 1986) was below the values recorded during an experiment.

**Measuring Procedure**

All potentials were measured against the potential of an extracellular reference electrode (agar bridge containing 3 M KCl and Ag/AgCl cell), using voltmeters with an input resistance of 10$^{15}$ Ω (two-channel voltmeter FD223, WPI; or four-channel voltmeter, Institute of Electrochemistry, University of Düsseldorf, Germany). The actual ionic signals, i.e., the differences between the potentials of the ion-selective channels and the reference channel, were obtained directly by means of the built-in differential amplifier of the voltmeter. The output signals were AD-converted and continuously recorded on a personal computer.

Since the values of the transformed ion concentrations were not normally distributed, means ± SD are always given in connection with $p_{\text{Ion}}$ as suggested by Fry et al. (1990). Mean ion concentrations ($I_{\text{on}}$) were then calculated from the mean $p_{\text{Ion}}$ values. Similarly, the rate of changes in $[Mg^{2+}]_{\text{i}}$, was quantified as $\Delta p_{\text{Mg}}/\text{min}$. To be able to compare mean results from different preparations, data were normalized with respect to effects of kainate in SLs. Statistical analysis was performed using Student’s t test (P < 5%).

**Fluorescence Recording of $[Ca^{2+}]_{\text{i}}$, and Changes in Cell Volume**

In some control experiments changes in $[Ca^{2+}]_{\text{i}}$, and in the cell volume were recorded. To this end, microfluorimetric measurements with the fluorescent calcium indicator Fura-2 (Molecular Probes) were performed. The experimental procedures and the set-up have been described in detail in previous papers (Hochstrate and Schlue, 1994; Hochstrate et al., 1995; Dierkes et al., 1996). Briefly, dye fluorescence of the iontophoretically Fura-2-loaded cells was alternately excited with wavelengths of 340, 360, and 380 nm, using a commercial microspectrophotometer (Deltascan 4000; Photon Technology International) with an objective of high numerical aperture (Fluor 40 Ph3DL; Nikon). The fluorescence light emitted from the preparation was collected by the objective, filtered through a 510/540 nm barrier filter, and measured by a photon-counting photomultiplier tube. The Fura-2 fluorescence ($F_{340}$, $F_{360}$, $F_{380}$) was obtained by correcting the raw data for the autofluorescence of the preparation, which was either measured in the same ganglion in a neighboring non-injected position, or in an untreated ganglion. The fluorescence of the injected Fura-2 was 10–50 times larger than the autofluorescence of the preparations.

$[Ca^{2+}]_{\text{i}}$ was calculated from the ratio $R = F_{360}/F_{340}$, obtained with Fura-2, according to the equation given by Grynkiewicz et al. (1985) and as previously described in detail (Hochstrate and Schlue, 1994; Hochstrate et al., 1995; Dierkes et al., 1996).

Relative changes in cell volume during the application of kainate were calculated from the changes in $F_{360}$ (Muallem et al., 1992; Kees et al., 2002). At a wavelength of 360 nm, Fura-2 fluorescence is independent of $[Ca^{2+}]_{\text{i}}$. Thus, decreases in $F_{360}$ reflect cell swelling (uptake of water causing dilution of the dye) while increases in $F_{360}$ indicate cell shrinking. Data were corrected for a slow decrease in fluorescence (bleaching, loss of dye), by fitting the traces with a single exponential function. Kainate-induced changes were evaluated relative to this curve.

**RESULTS**

**Steady-state Values of the Membrane Potential and Intracellular Ion Concentrations**

The mean steady-state values of $[Mg^{2+}]_{\text{i}}$, $[Na^{+}]_{\text{i}}$, $[Ca^{2+}]_{\text{i}}$, the intracellular concentrations of $K^{+}$ and $Cl^{-}$ ($[K^{+}]_{\text{i}}$, $[Cl^{-}]_{\text{i}}$) and the intracellular pH ($pH_{i}$) in leech Retzius neurones are summarized in Table I. The mean membrane potential was found to be $-36.6 ± 7.8$ mV ($n = 105$, 95% confidence limit $-37.9$ to $-35.3$ mV). There were no significant differences in values determined with double-, triple-, or four-barreled microelectrodes.

**Effects of L-glutamate and Kainate on $E_{\text{m}}$**

The application of the glutamatergic agonist kainate (100 μM) for 1 min evoked a prominent depolarization of the neuronal membrane, an increase in $[Na^{+}]_{\text{i}}$, and $[Ca^{2+}]_{\text{i}}$, a decrease in $[K^{+}]_{\text{i}}$, and an intracellular acidification (Fig. 1, A–C). In addition, a small but significant increase in $[Mg^{2+}]_{\text{i}}$, was observed (Table II, Fig. 1 A).

The application of the neurotransmitter L-glutamate (5 mM) induced a less prominent membrane depolarization of the neuronal membrane, an increase in $[Na^{+}]_{\text{i}}$, $[Ca^{2+}]_{\text{i}}$, $[K^{+}]_{\text{i}}$, $[Cl^{-}]_{\text{i}}$, and $pH_{i}$. Table II summarizes the effects of L-glutamate and kainate applications. Since kainate showed significantly larger effects on $E_{\text{m}}$ and $[Mg^{2+}]_{\text{i}}$, in Retzius neurones of the medicinal leech, this agonist was preferred for the further investigation.

**Effects of Carbachol on $E_{\text{m}}$ and $[Mg^{2+}]_{\text{i}}$**

In contrast to the application of L-glutamate and kainate, the application of the cholinergic neurotransmitter agonist carbachol (0.5 mM for 30 s) had no significant
The changes in intracellular ion concentrations upon the application of kainate were accompanied by a significant cell swelling, as determined by the transient decrease in F360 of the fluorescent dye Fura-2. F360 decreased by 10.2 ± 1.9% (n = 9, P < 0.01), which corresponded to a mean increase in cell volume by 11.4 ± 2.5% (Fig. 1 D).

Influences of Changes in [Mg2+]i on Kainate- and Carbachol-induced Effects

The size of kainate-induced [Mg2+]i increases should depend on the extracellular Mg2+ concentration ([Mg2+]o), if they were due to Mg2+ influx, as long as [Mg2+]o remains below the saturation level of the influx pathway. In contrast, [Mg2+]i increases should be independent of [Mg2+]o, if they were evoked by a release of Mg2+ from intracellular buffers/stores and if factors that might modulate intracellular Mg2+ buffering (such pHi or [Ca2+]i) are not in themselves affected by changes in [Mg2+]o.

During exposure to a nominally Mg2+-free saline for up to 20 min E_m, [Na+]i, pH_i, [Ca2+]i, and [Mg2+]i remained almost unchanged. As shown in Table III, bath applications of 100 μM kainate in nominally Mg2+-free saline did not result in any significant difference in the kainate-induced membrane depolarization, [Ca2+]i increase, and intracellular acidification. Kainate-induced [Na+]i increases were significantly amplified compared with kainate-induced effects in SLS, whereas kainate-induced [Mg2+]i increases were significantly reduced and in many cases even completely abolished (Fig. 3 A, Table III).

As it was not clear whether the remaining [Mg2+]i increase in some experiments was due to incomplete removal of the extracellular Mg2+ or due to secondary effects on [Mg2+]i, an attempt was made to remove the extracellular Mg2+ completely by a brief (1 min) exposure of the cells to a Mg2+-free bath solution in which all remaining Mg2+ was buffered with 5 mM EDTA. As the cells did not tolerate long exposure to the EDTA-buffered Mg2+-free saline, the experiments were then continued in a nominally Mg2+-free solution. If kainate was applied after such treatment with EDTA-buffered Mg2+-free saline, the [Mg2+]i increase was almost completely suppressed (14 ± 11.6%, n = 5).
If \([\text{Mg}^{2+}]_o\), was increased from 1 to 5 mM, \([\text{Mg}^{2+}]_o\), increased slightly by 0.04 mM (ΔpMg -0.05 ± 0.05, \(n = 20, \ P = 0.0003\)). These \([\text{Mg}^{2+}]_o\), increases were significantly smaller (44 ± 20%, \(n = 20, \ P < 10^{-5}\)) and slower (20 ± 9%, \(n = 20, \ P < 10^{-3}\)) than the \([\text{Mg}^{2+}]_o\), increases induced by the application of kainate. In 5 mM \([\text{Mg}^{2+}]_o\), \(E_m\) was slightly depolarized and \([\text{Na}^+]_i\), and pH remained unchanged. In addition, increasing \([\text{Mg}^{2+}]_o\), to 5 mM reduced the spontaneous generation of action potentials, suggesting suppression of synaptic transmission in the tissue caused by \(\text{Mg}^{2+}\) (Nicholls and Purves, 1970; Stuart, 1970).

When kainate was applied in the presence of 5 mM \([\text{Mg}^{2+}]_o\), the kainate-induced \([\text{Mg}^{2+}]_o\), increase was significantly greater than the kainate-induced \([\text{Mg}^{2+}]_o\), increase in the presence of 1 mM \([\text{Mg}^{2+}]_o\), (see Table III, Fig. 1 A). The kainate-induced changes in \(E_m\), \([\text{Na}^+]_i\), and pH showed no significant differences compared with kainate-induced changes in SLS while the kainate-induced \([\text{Ca}^{2+}]_i\), increases in 5 mM \([\text{Mg}^{2+}]_o\), were significantly reduced (Table III).

Increasing \([\text{Mg}^{2+}]_o\), to 10, 20, or 30 mM for 8 min at a constant \([\text{Na}^+]_o\) of 45 mM only caused minor changes in \([\text{Mg}^{2+}]_o\), and \(E_m\) (Table IV). In these bath solutions kainate-induced \([\text{Mg}^{2+}]_o\), increases were significantly enhanced relative to kainate-induced \([\text{Mg}^{2+}]_o\), increases in a bath solution with reduced \(\text{Na}^+\) content (45 mM) and a \([\text{Mg}^{2+}]_o\) of 1 mM, whereas kainate-induced membrane depolarization was significantly reduced (Table III, compare e.g., Fig. 2).

Application of the cholinergic agonist carbachol to leech Retzius neurones in the presence of 10 mM \([\text{Mg}^{2+}]_o\), again had no significant effect on \([\text{Mg}^{2+}]_o\), (Δ[\text{Mg}^{2+}]_o, 0.04 mM, ΔpMg -0.1 ± 0.09, \(n = 4\)), whereas the carbachol-induced depolarization was significantly reduced by 43.6 ± 19.6% (\(n = 4; \ P = 0.011\), Fig. 2), relative to the carbachol-induced depolarization at 1 mM \([\text{Mg}^{2+}]_o\).

**Kainate but Not Carbachol-induced Cobalt Hexamine Influx**

Cobalt hexamine (Co(OH)\(_3\))\(^{3+}\) is similar in size to the hydrated \(\text{Mg}^{2+}\) ion and has therefore been used to distinguish between ion channels that are interacting.
with the hydrated rather than the unhydrated form of Mg\(^{2+}\) (Kucharski et al., 2000; Bijvelds et al., 2001). In this study it was found that Mg\(^{2+}\)-selective microelectrodes based on the sensor ETH 5214 are more sensitive to Co(NH\(_3\))\(_6\)\(^{3+}\) than to Mg\(^{2+}\) itself, so that they could be calibrated and used as Co(NH\(_3\))\(_6\)\(^{3+}\)-selective microelectrodes. 13 of those electrodes calibrated for Co(NH\(_3\))\(_6\)\(^{3+}\) had a mean maximum slope of \(-21.9 \pm 4.8\) mV/decade and a detection limit of 3.5 \pm 2.6 \mu M.

Exposure of leech Retzius neurones to a nominally Mg\(^{2+}\)-free saline containing 1 mM Co(NH\(_3\))\(_6\)\(^{3+}\) led to a slow increase in the signal of the Mg\(^{2+}\)/Co(NH\(_3\))\(_6\)\(^{3+}\)-selective microelectrode, indicating a minor influx of Co(NH\(_3\))\(_6\)\(^{3+}\) under these conditions. During the application of kainate in the presence of 1 mM Co(NH\(_3\))\(_6\)\(^{3+}\), the rate of Co(NH\(_3\))\(_6\)\(^{3+}\) influx increased severalfold (from 0.29 \pm 0.21 \mu M/min to 1.26 \pm 1.38 \mu M/min, n = 17, P = 0.007, Fig. 3 B). In contrast, application of carbachol in the presence of 1 mM Co(NH\(_3\))\(_6\)\(^{3+}\) had no significant effect on the rate of Co(NH\(_3\))\(_6\)\(^{3+}\) influx into Retzius neurones (from 0.25 \pm 0.23 \mu M/min to 0.29 \pm 0.28 \mu M/min, n = 6).

After removing extracellular Co(NH\(_3\))\(_6\)\(^{3+}\), the intracellular Co(NH\(_3\))\(_6\)\(^{3+}\) concentration remained high, indicating that the Na\(^+\)/Mg\(^{2+}\) antiport does not transport Co(NH\(_3\))\(_6\)\(^{3+}\).

**Action of the Glutamatergic Antagonist DNQX on Kainate-induced Effects**

To confirm that the observed [Mg\(^{2+}\)] increase was due to the activation of glutamate receptors, kainate was applied in the presence of the glutamatergic antagonist DNQX. Under these conditions, Kainate-induced membrane depolarizations and [Na\(^+\)] increases were reduced significantly to 41 \pm 38% (n = 5, P = 0.025), and 25 \pm 42% (n = 5, P = 0.016), respectively, [Mg\(^{2+}\)].

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**Table 111**

| [Mg\(^{2+}\)] \(\text{E}_{\text{m}}\) | [Na\(^{+}\)] | pH | [Ca\(^{2+}\)] | [Mg\(^{2+}\)] \(\text{E}_{\text{m}}\) |
|----------------|-----------|---|----------|----------------|
| 0 mM [Mg\(^{2+}\)] | 14 \pm 12 (n = 5) | | | 111 \pm 51 (n = 5) |
| 5 mM EDTA (nominally) | P = 8 \times 10^{-5} | | | P = 0.65 |
| 0 mM [Na\(^{+}\)] | 43 \pm 39 (n = 8) | 144 \pm 45 (n = 8) | 164 \pm 85 (n = 7) | 155 \pm 88 (n = 8) | 101 \pm 20 (n = 9) |
| (90 mM [Na\(^{+}\)] (nominally) | P = 0.0044 | P = 0.03 | P = 0.90 | P = 0.12 | P = 0.88 |
| 10 mM [Mg\(^{2+}\)] | 183 \pm 83 (n = 9) | 104 \pm 71 (n = 9) | 127 \pm 84 (n = 6) | 137 \pm 45 (n = 8) | 41 \pm 12 (n = 4) |
| (90 mM [Na\(^{+}\)] (nominally) | P = 0.017 | P = 0.87 | P = 0.47 | P = 0.053 | P = 0.002 |
| 10 mM [Mg\(^{2+}\)] | 206 \pm 132 (n = 3) | | | 84.4 \pm 1.1 (n = 3) |
| (45 mM [Na\(^{+}\)] (nominally) | P = 0.30 | | | P = 0.062 |
| 20 mM [Mg\(^{2+}\)] | 320 \pm 163 (n = 4) | | | 78.1 \pm 5.5 (n = 4) |
| (45 mM [Na\(^{+}\)] (nominally) | P = 0.024 | | | P = 0.0004 |
| 30 mM [Mg\(^{2+}\)] | 407 \pm 90 (n = 4) | | | 74.2 \pm 1.8 (n = 4) |
| (45 mM [Na\(^{+}\)] (nominally) | P = 0.086 | | | P = 9 \times 10^{-5} |

**AMPA/Kainate Receptor Activation Causes Mg\(^{2+}\) Influx**

732
increases were highly significantly reduced to 16 ± 20% \((n = 5, P = 0.0007)\) (see Fig. 4 A). Control applications of kainate after wash-out showed that the reduction of the kainate-induced effects was fully reversible.

**TABLE IV**

| [Mg^{2+}] | \(\Delta p_{Mg} \pm SD\) | \(\Delta E_m \pm SD\) |
|-----------|------------------|------------------|
| 10 mM [Mg^{2+}] | -0.0035 ± 0.0049 (\(n = 3\)) | 2 ± 0.5 (\(n = 3\)) |
|           | P = 0.34         | P = 0.02         |
| 20 mM [Mg^{2+}] | -0.0052 ± 0.0232 (\(n = 4\)) | 4 ± 1.5 (\(n = 4\)) |
|           | P = 0.45         | P = 0.009        |
| 30 mM [Mg^{2+}] | -0.2033 ± 0.0646 (\(n = 4\)) | 4.8 ± 3.5 (\(n = 4\)) |
|           | P = 0.008        | P = 0.07         |

P values <0.05 indicate changes significantly different from zero.

Since kainate applications are accompanied by considerable membrane depolarizations, voltage-dependent ion channels such as voltage-dependent Ca^{2+} channels are activated in leech Retzius neurones (Hochstrate and Schlue, 1994; Dierkes et al., 1996). The activation of voltage-dependent ion channels might contribute to the kainate-induced [Mg^{2+}] increase. Therefore, membrane depolarization was induced by increasing the extracellular K^{+} concentration \((\left[K^+\right]_o)\) from 4 to 30 mM. 30 mM \([K^+]_o\) evoked a membrane depolarization of 13.6 ± 5.9 mV \((n = 28)\), a [Na^{+}], decrease of 2.5 mM \((\Delta p_{Na} = 0.10 ± 0.25, n = 9)\) caused by the replacement of NaCl in the solution, and nonuniform changes in [Mg^{2+}]. During 28 such experiments, [Mg^{2+}], decreased 7 times, increased 8 times, and remained unchanged 13 times. In total, 30 mM \([K^+]_o\)-induced [Mg^{2+}] changes amounted only to 8 ± 49% \((n = 28)\) of the kainate-induced [Mg^{2+}] changes, al-

**FIGURE 4**. Kainate-induced [Mg^{2+}], increase are inhibited by DNQX, cannot be mimicked by kainate-independent membrane depolarizations or [Na^{+}], increases, and are not inhibited in nominally Na^{+}-free bath solutions. Simultaneous recording of \(E_m\), [Mg^{2+}], and [Na^{+}], (A and B), \(E_m\), [Mg^{2+}], [Na^{+}], and pH, (C), and \(E_m\) and [Mg^{2+}], (D). (A) In the presence of 0.5 mM DNQX, application of kainate caused a much reduced membrane depolarization. The changes in [Mg^{2+}], and [Na^{+}], were completely abolished. All effects were reversible upon washout. (B) Increasing \([K^+]_o\) from 4 mM to 30 mM caused a membrane depolarization that was even larger than the kainate-induced depolarization. There was no increase in [Mg^{2+}], similar to that elicited by kainate. The decrease in [Na^{+}], at 30 mM \([K^+]_o\), was caused by the reduction in [Na^{+}]. (C) A reduction of \([K^+]_o\), from 4 to 0.2 mM caused an increase in [Na^{+}]. Although [Na^{+}], reached a similar maximum value as in the presence of kainate, it was not accompanied by an increase in [Mg^{2+}]. A small decrease in pH was also seen. (D) In the absence of extracellular Na^{+}, the amplitude of the kainate-induced [Mg^{2+}] increase was unchanged. [Mg^{2+}], remained elevated until Na^{+} was reintroduced to the bath solution.
though 30 mM [K+]o-induced membrane depolarization was significantly larger (158 ± 47%, n = 28, P = 0.0002) than kainate-induced membrane depolarization. Moreover, in those experiments in which [Mg2+]i increases did occur, these increases were significantly slower (43 ± 33%, n = 8, P = 0.002) than [Mg2+]i increases induced by kainate (Fig. 4 B).

**Contribution of Increased [Na+]i, to the Kainate-induced [Mg2+]i, Increase**

Since [Mg2+]i in Retzius neurones is regulated by a Na+/Mg2+ antiport (Günzel and Schlue, 1996), [Mg2+]i, extrusion should be reduced during an increase in [Na+]i. Such an inhibition of the Na+/Mg2+ antiport might be caused by the kainate-induced Na+ influx and could thus be responsible for the kainate-induced [Mg2+]i increase.

In Retzius neurones, kainate-independent [Na+]i increases can be evoked by a reduction of [K+]o to 0.2 mM, which leads to an inhibition of the Na+/K+-ATPase (Deitmer and Schlue, 1983). The reduction of [K+]o caused a mean hyperpolarization of −5.9 ± 2.0 mV (n = 5) and evoked an average increase in [Na+]i of 9.9 mM (Δ[Na+]i: −0.24 ± 0.08, n = 4), which was not significantly different from kainate-induced [Na+]i increases (10.3 mM, Δ[Na+]i: −0.32 ± 0.20, n = 4). [Mg2+]i increases at 0.2 mM [K+]o were marginal (0.03 mM, Δ[Mg2+]i: −0.035 ± 0.015, n = 5) and highly significantly less than the kainate-induced [Mg2+]i increases (0.14 mM, Δ[Mg2+]i: −0.130 ± 0.028, n = 5, P = 0.0002), excluding both a contribution of the Na+/Mg2+ antiport to the kainate-induced [Mg2+]i increase and a Na+ interference with the Mg2+-selective ionophore (Fig. 4 C).

**Effect of Na+-free Solution on the Kainate-induced [Mg2+]i, Increase**

To suppress the kainate-induced Na+ influx and the accompanying membrane depolarization, NaCl in the SLS was replaced by NMDG-Cl. During exposure to this solution (maximum 15 min before the application of kainate), [Mg2+]i decreases significantly by 0.05 mM (Δ[Mg2+]i: 0.09 ± 0.05, n = 5, P = 0.02). In Na+-free saline E_m transiently hyperpolarized (−5.9 ± 1.6 mV, n = 5) but then eventually depolarized (3.8 ± 1.8 mV, n = 5).

Application of kainate in the nominal absence of Na+ altered neither the kainate-induced [Mg2+]i increases (90 ± 34%, n = 5) nor the rates of these [Mg2+]i increases (82 ± 25%, n = 5) relative to the kainate-induced [Mg2+]i increases in SLS. Due to the inhibition of the Na+/Mg2+ antiport in the absence of extracellular Na+, [Mg2+]i remained increased after the application of kainate until [Na+]i was restored (Fig. 4 D).

**Figure 5.** Effects of Ca2+-free bath solutions and blockers of the voltage-dependent Ca2+ channels on the kainate-induced [Mg2+]i increase. Simultaneous recording of E_m and [Mg2+]i. (A) Exposure of the cells to nominally Ca2+-free bath solutions caused a significant enhancement of the kainate-induced [Mg2+]i increase. (B) 2 mM menthol did not influence the kainate-induced [Mg2+]i increase.

**Effect of Ca2+-free Solutions on the Kainate-induced [Mg2+]i, Increase**

Kainate-induced membrane depolarization activates voltage-dependent Ca2+ channels, and thus causes a Ca2+ influx into the cells (Hochstrate and Schlue, 1994; Dierkes et al., 1996). Therefore, the influence of the extracellular Ca2+ concentration ([Ca2+]o) on the kainate-induced [Mg2+]i increase was examined in a further set of experiments. When [Ca2+]o was reduced from 2 to 0.2 mM, the kainate-induced [Mg2+]i increase was significantly enhanced to 175 ± 33% (n = 3, P = 0.05). Furthermore, removing [Ca2+]o completely by buffering the nominally Ca2+-free saline with 5 mM EGTA enhanced the kainate-induced [Mg2+]i increase significantly to 195 ± 55% (n = 4; P = 0.04; Fig. 5 A), even though [Mg2+]i under these conditions was reduced to ~0.7 mM as calculated from the EGTA association constants given by Caldwell (1970).

**Effects of an Inhibition of Voltage-dependent Ca2+ Channels on the Kainate-induced [Mg2+]i, Increase**

Although a contribution of voltage-dependent ion channels to the kainate-induced [Mg2+]i increase already seemed unlikely from the results obtained during the depolarization of the membrane potential with high [K+]o, kainate applications in Ca2+-free solution indicated a possible correlation between kainate-induced [Mg2+]i and [Ca2+]i increase. To investigate the contribution of voltage-dependent Ca2+ channels to the kainate-induced [Mg2+]i increase, inhibitors of voltage-dependent Ca2+ channels were applied. Polyvalent cations like lanthanum (La3+), and the cyclic alcohol menthol are potent inhibitors of voltage-dependent Ca2+ channels of leech Retzius neurones (Dierkes et al., 1997b) and have been reported to completely in-
Kainate pHi, and Em in leech Retzius neurones were compared, mM MgAsp2 plus 2.5 mM MgCl2. The responses to 5 mM MgAspHCl or the solution containing 2.5 mM MgCl2. In addition, it was tested whether the effect of 5 mM MgAspHCl could be mimicked by 2.5 mM MgAsp2 plus 2.5 mM MgCl2. As summarized in Table VI, both magnesium aspartate compounds turned out to be considerably less potent agonists of AMPA/kainate receptors in leech Retzius neurones than kainate. However, [Mg2+]i changes were significantly larger upon the application of magnesium aspartate compounds than upon the application of 5 mM MgCl2.

As expected from the higher (10 mM) aspartate content, 5 mM MgAsp2 evoked a larger response than either 5 mM MgAspHCl or the solution containing 2.5 mM MgAsp2 plus 2.5 mM MgCl2. The responses to applications of the latter two solutions did not differ significantly from each other with respect to any of the parameters tested.

D I S C U S S I O N

Multibarrelled Ion-selective Microelectrodes

While multibarrelled ion-selective microelectrodes are time consuming to make, they are extremely useful when interactions between different ion species are investigated, especially if the expected concentration changes are either small or variable in magnitude (Günzel et al., 1997, 1999). As previously shown (Günzel et al., 1997), multibarrelled ion-selective microelectrodes give reliable recordings of the membrane potential and of intracellular ion concentrations in leech Retzius neurones. Since, in the present study, no significant differences were found between values recorded with double-, triple-, or four-barrelled microelectrodes and all resting values were comparable to those reported earlier in the same preparation (for review see Günzel and Schlue, 2000) it is unlikely that the multibarrelled microelectrodes damaged the cells.

The present results demonstrate that the application of AMPA/kainate receptor agonists induced small, but significant changes in the signal of the Mg2+-selective barrel of the microelectrodes, indicating increases in [Mg2+]i, (∆[Mg2+]i) in leech Retzius neurones. These changes were observed in all cells investigated; however, due to the variability in both the resting [Mg2+]i and in ∆[Mg2+]i, evaluation of these signals is debatable. Simple averaging of ∆[Mg2+]i values was not possible as the data lacked normal distribution. Averaging of the corresponding ∆pMg values is possible but rather less meaningful, considering that, for example, an increase from 0.2 mM [Mg2+]i to 0.3 mM (∆[Mg2+]i = 0.1 mM) corresponds to a ∆pMg of 0.17, while the same ∆[Mg2+]i, from 0.5 to 0.6 mM corresponds to a ∆pMg of 0.08. The relatively narrow 95% confidence limits of 0.1–0.14 mM for [Mg2+]i suggests that [Mg2+]i increases by 30% of its resting value.

None of the values are significantly different from 100% (kainate induced increase in the absence of La3+ or menthol).

### TABLE V

Relative Kainate-induced Changes in Em, [Mg2+]i, and [Na+]i, in the Presence of La3+ and Menthol

| ΔEm | ΔpMg | ΔpNa |
|-----|------|------|
| %   | %    | %    |
| 114 ± 47 | 112 ± 63 | 93 ± 41 |
| n = 7 | n = 7 | n = 3 |
| 130 ± 25 | 105 ± 35 | — |
| Kainate + 2 mM La3+ |
| Kainate + 2 mM menthol |

Effects of Magnesium-aspartate and Magnesium-aspartate Hydrochloride on [Mg2+]i

Magnesium aspartate (MgAsp2) and magnesium aspartate hydrochloride (MgAspHCl) have been used in in vivo studies to increase Mg2+ levels in the blood and both compounds are used to supplement Mg2+ in humans.

As aspartate has been shown to act as an agonist of glutamate receptors in various preparations (compare Cemerikic et al., 1988), the effects of 5 mM of both magnesium aspartate compounds on [Mg2+]i, [Na+]i, pHi, and Em in leech Retzius neurones were compared with the effect of 100 μM kainate and to the effect of 5 mM MgCl2. In addition, it was tested whether the effect of 5 mM MgAspHCl could be mimicked by 2.5 mM MgAsp2 plus 2.5 mM MgCl2. As summarized in Table VI, both magnesium aspartate compounds turned out to be considerably less potent agonists of AMPA/kainate-receptors in leech Retzius neurones than kainate. However, [Mg2+]i changes were significantly larger upon the application of magnesium aspartate compounds than upon the application of 5 mM MgCl2.

### TABLE VI

Effects of Mg-aspartate Compounds on Em, [Mg2+]i, [Na+]i, and pH, Relative to the Changes Induced by 100 μM Kainate in Standard Leech Saline

| 2.5 mM MgAsp2 |
| 5 mM MgAsp2, 5 mM MgAspHCl + 2.5 mM MgCl2, 5 mM MgCl2 |
| %       | %       | %       |
| 85 ± 51 | 41 ± 29 | 40 ± 18 | 20 ± 19 |
| n = 14  | n = 14  | n = 8   | n = 14  |
| 29 ± 14 | 24 ± 17 | 17 ± 10 | 6 ± 9   |
| n = 14  | n = 14  | n = 7   | n = 15  |
| 58 ± 47 | 33 ± 36 | 0 ± 0   | 7 N D   |
| n = 7   | n = 7   | n = 7   | —       |
| 83 ± 32 | 63 ± 35 | 61 ± 21 | 15 ± 27 |
| n = 16  | n = 16  | n = 8   | n = 16  |

None of the values are significantly different from 100% (kainate induced increase in the absence of La3+ or menthol).
Artifacts from Rapid Changes in $E_{Mg}$ and Interfering Ions

The $Mg^{2+}$ sensor ETH 5214 is not perfectly selective for $Mg^{2+}$ and under physiological conditions may also react to some extent to changes in $K^+$ or $Na^+$. In addition, $E_{Mg}$, the potential difference between the signal of the $Mg^{2+}$-selective barrel and the reference barrel, may be distorted by rapid changes in $E_{Mg}$ due to the differences in the response times of the microelectrode barrels.

A priori, it is unlikely that interference from $K^+$ or $Na^+$ could modify the $E_{Mg}$ signal. The selectivity coefficients for these ions determined in vitro are such that the changes in $[K^+]_i$ and $[Na^+]$ measured in the present study should not interfere with $E_{Mg}$. However, selectivity coefficients could be different in vivo, but this can be ruled out by the following considerations.

Since $[K^+]_i$ decreased during the application of kainate, any interference from $K^+$ ions would be expected to cause an apparent decrease, not an increase in $[Mg^{2+}]_i$. An interference from $Na^+$ ions can be ruled out by the observation that the kainate-induced changes in $E_{Mg}$ were not affected by the nominal absence of extracellular $Na^+$. Furthermore, increases in $[Na^+]_i$, caused by an inhibition of the $Na^+/K^+$ ATPase by saline with a reduced $K^+$ content did not induce comparable changes in $E_{Mg}$ although the increases in $[Na^+]_i$ were not significantly different from the kainate-induced increases.

$E_{Mg}$ is the difference between the potential of the $Mg^{2+}$-selective barrel and the reference barrel of the microelectrode. Since the response of the reference barrel to a depolarization is more rapid than that of the $Mg^{2+}$-selective barrel, a depolarization could cause a spurious increase in $[Mg^{2+}]_i$. However, this increase in $[Mg^{2+}]_i$ would be transient and, moreover, the changes in $E_{Mg}$ could not be mimicked by depolarization caused by $30 \text{ mM}[K^+]_o$.

Finally, kainate-induced changes in $E_{Mg}$ were dependent on $[Mg^{2+}]_o$. These changes were greatly reduced in the nominal absence of extracellular $Mg^{2+}$ and completely abolished after a removal of extracellular $Mg^{2+}$ by exposure of the neurones to EDTA-buffered saline. This treatment did not affect kainate-induced membrane depolarization.

Taken together, these results rule out the possibility that the observed kainate-induced changes in $E_{Mg}$ were due to rapid changes in $E_{m}$ or to interference of $K^+$ or $Na^+$ at the $Mg^{2+}$-selective barrel of the microelectrodes. It is concluded that the kainate-induced changes in $E_{Mg}$ in leech Retzius neurones truly reflect increases in $[Mg^{2+}]_i$.

Changes in Cell Volume

The application of $100 \mu \text{M}$ kainate induced an average increase in the volume of leech Retzius neurones of $11.4\%$. This implies that the concentrations of all ions that are neither buffered nor crossing the cell membrane should decrease by this percentage. This is almost ideally the case for $[K^+]_o$, which decreased by $13.6\%$. In contrast, both $[Na^+]_i$ and $[Cl^-]$ approximately doubled and have been reported to be the major cause of the kainate-induced volume increase (Dierkes et al., 2002). $[Mg^{2+}]_i$ increased by $0.10-0.14 \text{ mM}$, corresponding to an increase of $\sim 30-40\%$. Due to the volume increase and to intracellular $Mg^{2+}$ buffering (as quantified by Günzel et al., 2001), an uptake of $\sim 1 \text{ mM} Mg^{2+}$ would be necessary to bring about the observed increase in $[Mg^{2+}]_i$.

In the absence of extracellular $Na^+$ the kainate-induced volume increase is abolished (Trosiner, 2003). As the kainate-induced $[Mg^{2+}]_i$ increase under these conditions is not significantly altered, it can be excluded that $Mg^{2+}$ enters the cells through a volume-activated transport mechanism.

Estimate of $Mg^{2+}$ Influx into Leech Retzius Neurones

The rate of $Mg^{2+}$ influx under resting conditions in Retzius neurones was estimated by extrapolating the relationship between $[Mg^{2+}]_i$ and the corresponding $[Mg^{2+}]_o$ (5, 10, 20, and 30 mM) to a $[Mg^{2+}]_o$ of 1 mM. Assuming the average cell diameter of 83.2 $\mu \text{m}$ and the intracellular $Mg^{2+}$ buffering reported by Günzel et al. (2001), this estimate yielded a value of $\sim 0.34 \text{ pmol/cm}^2/\text{s}$ that compares well to the $Mg^{2+}$ influx of $0.21 \text{ pmol/cm}^2/\text{s}$ reported by Page and Polimeni (1972) for rat ventricle and to the rate of $Mg^{2+}$ efflux from isolated rat ventricular myocytes in $Mg^{2+}$-free solutions of 0.15-0.61 pmol/cm$^2$/s observed by Handy et al. (1996). In contrast, the uptake of $\sim 1 \text{ mM} Mg^{2+}$ during the application of kainate would require an increase in membrane flux in the order of 10 pmol/cm$^2$/s. Due to the more favorable surface/volume ratio of dendrites compared with the cell soma, $[Mg^{2+}]_i$ increases occurring locally in the vicinity of the cell membrane or in the cell periphery can be expected to be of physiological relevance. Furthermore, as the stimulation of leech Retzius neurones with both kainate and carbachol caused membrane depolarizations, $[Na^+]_i$, and $[Ca^{2+}]_i$ increases, and intracellular acidifications, but only stimulation with kainate additionally increased $[Mg^{2+}]_i$, it may be speculated that $Mg^{2+}$ acts locally as an intracellular signal to discriminate between glutamatergic and cholinergic stimulation.

Changes in Intracellular $Mg^{2+}$ Buffering

In theory, part of the kainate-induced $[Mg^{2+}]_i$ increase could be brought about by changes in the intracellular buffering capacity. Intracellular buffering in Retzius neurones is known to be pH dependent (Günzel et al., 1997, 2001; Lüthi et al., 1999). However, Günzel et al. (2001) showed that pH would have to decrease to values of about pH 6.5 to account for the observed
[Mg\textsuperscript{2+}] increases. Thus, the kainate-induced pH\textsubscript{i} changes are too small to cause any detectable release of Mg\textsuperscript{2+} from intracellular buffers.

Increases in [Ca\textsuperscript{2+}] have also been reported to decrease the intracellular buffering capacity of Mg\textsuperscript{2+} (Brocard et al., 1993; Koss et al., 1993). However, kainate-induced [Mg\textsuperscript{2+}]\textsubscript{i} increases were not affected by Ca\textsuperscript{2+} channel blockers and even enhanced in Ca\textsuperscript{2+}-free solutions, so that effects of Ca\textsuperscript{2+} on intracellular Mg\textsuperscript{2+} buffering can also be ruled out.

**Reversal of Na\textsuperscript{+}/Mg\textsuperscript{2+} Antiport**

Stout et al. (1996) suggest that glutamate-induced [Na\textsuperscript{+}], increases in cultured rat cortical neurones may inhibit or even reverse Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport and thus induce increases in [Mg\textsuperscript{2+}]. Although a Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport does exist in leech Retzius neurones (Günzel and Schlue, 1996), this appears not to be the cause of the kainate-induced [Mg\textsuperscript{2+}]\textsubscript{i} increase, as comparable [Na\textsuperscript{+}], increases induced by a reduction of [K\textsuperscript{+}], had no effect on [Mg\textsuperscript{2+}].

**Mg\textsuperscript{2+} Influx through Voltage-activated Ca\textsuperscript{2+} Channels**

From the above considerations and from the dependence of the kainate-induced [Mg\textsuperscript{2+}]\textsubscript{i} increase on [Mg\textsuperscript{2+}], it has to be concluded that kainate triggers an influx of Mg\textsuperscript{2+} from the extracellular space. This influx could occur through the AMPA/kainate receptor–coupled cation channels or through the voltage-activated Ca\textsuperscript{2+} channels. The latter, however, is unlikely, as the Ca\textsuperscript{2+} channel blockers menthol and La\textsuperscript{3+} completely block the kainate-induced [Ca\textsuperscript{2+}]\textsubscript{i} increase (Dierkes et al., 1997b) but did not inhibit the [Mg\textsuperscript{2+}]\textsubscript{i} increase. Furthermore, depolarizations caused by an increase in [K\textsuperscript{+}], to 30 mM were comparable to kainate-induced depolarizations but did not cause comparable [Mg\textsuperscript{2+}]\textsubscript{i} increases. This observation also indicates that there are no separate, voltage-activated Mg\textsuperscript{2+} channels.

**Divalent Cation Influx through AMPA/kainate Receptor-coupled Cation Channels but Not through ACh Receptor-coupled Cation Channels**

In summary, the results presented here indicate that kainate triggers Mg\textsuperscript{2+} entry into leech Retzius neurones through AMPA/kainate receptor–coupled cation channels that have previously been reported to be impermeable to Ca\textsuperscript{2+} and Ni\textsuperscript{2+} but permeable to Co\textsuperscript{2+} (Dierkes et al., 1996, 1997b). Thus, our findings are only in partial agreement with the findings of Burnashev et al. (1992), who report that certain subunits of the AMPA/kainate receptor from rat and mouse brain show a high divalent cation permeability, as none of the channels investigated by Burnashev et al. (1992) showed a preference for Mg\textsuperscript{2+} over Ca\textsuperscript{2+}.

Transition metal hexamines are chemically stable analogs of the hydrated Mg\textsuperscript{2+} (Hampel and Cowan, 1997; Cowan, 1998; Kucharski et al., 2000). For that reason, various transition metal hexamines have previously been used to discriminate between Mg\textsuperscript{2+} binding sites that interact with the hydrated Mg\textsuperscript{2+} ion, e.g., nucleases and polymerases (for review see Cowan, 1998), the bacterial Mg\textsuperscript{2+} channel CorA (Kucharski et al., 2000), or the Mg\textsuperscript{2+} transport across fish intestinal epithelium cells (Bijvelds et al., 2001) and those that only accept the unhydrated Mg\textsuperscript{2+} ion, such as the bacterial Mg\textsuperscript{2+} transporters MgtA and MgtB (Kucharski et al., 2000). In this study, AMPA/kainate receptor–coupled cation channels appeared to be slightly permeable to Co(NH\textsubscript{3})\textsubscript{6}\textsuperscript{3+}, as kainate induced a small but rapid increase in the intracellular Co(NH\textsubscript{3})\textsubscript{6}\textsuperscript{3+} concentration. The major Mg\textsuperscript{2+} extrusion system in Retzius neurones, a Na\textsuperscript{+}/Mg\textsuperscript{2+} antiport (Günzel and Schlue, 1996), did not seem to transport Co(NH\textsubscript{3})\textsubscript{6}\textsuperscript{3+}, as the intracellular Co(NH\textsubscript{3})\textsubscript{6}\textsuperscript{3+} concentration remained high after the removal of extracellular Co(NH\textsubscript{3})\textsubscript{6}\textsuperscript{3+}.

In contrast to AMPA/kainate receptor–coupled cation channels, ACh receptor–coupled cation channels in leech Retzius neurones did not exhibit any permeability to divalent cations. Paul W. Dierkes (personal communication) did not find any indication for Ca\textsuperscript{2+}, Co\textsuperscript{2+}, or Ni\textsuperscript{2+} influx through ACh receptor–coupled cation channels and in the present study neither Mg\textsuperscript{2+} nor Co(NH\textsubscript{3})\textsubscript{6}\textsuperscript{3+} influx could be detected.

**Relevance of Kainate-induced [Mg\textsuperscript{2+}], Increase for Clinical Use of Mg\textsuperscript{2+} Aspartate Compounds**

Involvement of glutamate receptor stimulation in Mg\textsuperscript{2+} uptake may have to be considered, if magnesium aspartate compounds are used under experimental or clinical conditions. Intravenous Mg\textsuperscript{2+} administration of magnesium aspartate compounds, rather than MgSO\textsubscript{4}, may trigger additional Mg\textsuperscript{2+} uptake into tissues such as heart muscle cells that have recently been demonstrated to possess ionotropic glutamate receptors (Gill et al., 1998, 1999).

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