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Antagonism of neuronal kainate receptors by lanthanum and gadolinium

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Abstract

The effects of lanthanum and gadolinium on currents evoked by excitatory amino acids were studied in cultured rat hippocampal and cortical neurons, in freshly dissociated dorsal root ganglion neurons, and in human embryonic kidney 293 cells expressing the GluR6 kainate receptor subunit. In all of these cells, currents mediated by kainate-preferring receptors were antagonized by low micromolar concentrations of the trivalent ions. At negative holding potentials, the IC_{50} values for inhibition in DRG cells were 2.8 μM for La and 2.3 μM for Gd. Kainate receptor-mediated currents in hippocampal neurons and in 293 cells expressing GluR6 were blocked by La with IC_{50} values of 2.1 and 4.4 μM, respectively. Steady-state inhibition by the lanthanides showed very slight dependence on membrane potential, however, we were not able to resolve any systematic variation with membrane potential in the kinetics of block onset or recovery. Inhibition was not use-dependent and was not overcome by increasing the concentration of agonist. These results indicate that lanthanides probably do not bind deep within the ion pore or directly compete for the agonist binding site. In contrast to neuronal AMPA receptors, which require more than 100 μM lanthanides for half-maximal blockade, the inhibition of neuronal and recombinant kainate receptors by these ions displays significantly higher potency.

Keywords: Hippocampus; Dorsal root ganglion; Glutamate receptors; GluR6; GYKI 53655; Concanavalin A

1. Introduction

The amino acid glutamate activates three different families of ion channels that are named for the agonists *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and kainate (Collingridge and Lester, 1989). Previous work has demonstrated that NMDA and AMPA receptors underlie the rapid depolarization of postsynaptic neurons at fast excitatory synapses throughout the central nervous system (Monaghan et al., 1989). More recently, kainate receptors have attracted wide attention as potential mediators of both presynaptic (Chittajallu et al., 1996; Clarke et al., 1997; Rodriguez-Moreno et al., 1997) and postsynaptic (Castillo et al., 1997; Vignes and Collingridge, 1997) responses in the rat hippocampus. In addition, all three receptor subtypes have been implicated in the neuronal damage associated with ischemia and other disorders (Monaghan et al., 1989).

Detailed analysis of neuronal kainate receptors was originally restricted to freshly dissociated dorsal root ganglion (DRG) neurons, which do not express functional NMDA or AMPA receptors (Huettner, 1990; Wong and Mayer, 1993). In particular, the fact that most agonists (Wong et al., 1994) and antagonists (Wilding and Huettner, 1996) of non-NMDA receptors display relatively little selectivity between AMPA and kainate-preferring receptors made it difficult to distinguish the relative contributions of these two subtypes in neurons that expressed both. More recently, the discovery of compounds such as GYKI 53655 (Donevan et al., 1994), which produce potent, selective, non-competitive block of AMPA receptors has made it possible to dissect out the currents that flow through kainate receptor channels in CNS neurons (Paternain et al., 1995; Wilding and Huettner, 1997). As expected from work on DRG cells (Huettner, 1990; Wong and Mayer, 1993) and recombinantly expressed kainate receptor subunits (Herb et al., 1992; Sommer et al., 1992), currents mediated by kainate receptors in hippocampal neurons

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(Lerma et al., 1993; Wilding and Huettner, 1997) undergo strong desensitization, recover from desensitization very slowly, and are activated by low micromolar concentrations of kainate.

In order to characterize better the pharmacological differences between native AMPA and kainate receptors, we have examined the action of divalent and trivalent cations on currents mediated by these receptors in cultured or freshly dissociated neurons. A number of previous studies have demonstrated that excitatory amino acid receptors exhibit differential sensitivity to inhibition by metal ions including magnesium (Mayer et al., 1984; Nowak et al., 1984), zinc (Peters et al., 1987; Westbrook and Mayer, 1987) and lanthanum (Reichling and MacDermott, 1991). Because much of this earlier work focused on NMDA and AMPA receptors, however, relatively little is known about the sensitivity of the kainate receptor subtype to inhibition by metal ions. In the present study, we have followed up on a preliminary observation that kainate receptors expressed by hippocampal (Wilding and Huettner, 1997) and primary sensory neurons (Huettner, 1991) are significantly more sensitive than AMPA receptors to blockade by lanthanum (La) and gadolinium (Gd).

2. Materials and methods

2.1. Cell preparation

DRG neurons were dissociated by trituration following incubation with protease XXIII (Sigma, St. Louis, MO) as described by Wilding and Huettner (1995). Freshly dissociated cells were maintained overnight at room temperature in Earl's balanced salt solution (EBSS) until used the following day. Hippocampal and cortical neurons from 2- to 6-day-old Long–Evans rats were dissociated with papain (Huettner and Baughman, 1986; Wilding and Huettner, 1997). Hippocampi from two rat pups were cut into 500-μm slices with a McIlwain tissue chopper. Contaminating subiculum and entorhinal cortex were removed from each slice with fine forceps. The tissue was incubated with gentle stirring under 95% O_2/5% CO_2 in EBSS containing papain (20 units/ml; Worthington Biochemical, Freehold, NJ). After 90 min, slices were rinsed with EBSS containing ovomucoid and BSA, each at 1 mg/ml, and then triturated with a fire-polished Pasteur pipette. The resulting cell suspension was plated onto glass coverslips coated with poly-DL-ornithine. Cells were maintained at 37°C in Eagle's MEM containing 500 μM glutamine, 20 mM glucose, 100 units/ml penicillin, 0.1 mg/ml streptomycin, and supplemented with 5% rat serum.

cDNA encoding the GluR6 (Egebjerg et al., 1991) subunit in pBluescript was kindly provided by Dr Steven Heinemann (Salk Institute). For transfection of HEK 293 cells the receptor cDNA was excised with *Eco*RI and *Xba*I, and subcloned into the pcDNA vector (Invitrogen, San Diego, CA). HEK cells from the American Type Culture Collection were kindly provided by Dorothy Turetsky (Washington University). The cells were maintained at 37°C under 5% CO_2 in growth medium composed of MEM, α-formulation (Life Technologies, Grand Island, NY), 100 units/ml penicillin, 0.1 mg/ml streptomycin and supplemented with 10% fetal calf serum. HEK cells were cotransfected with GluR6 in pCDNA and with cDNA encoding the mouse L3T4 surface antigen (Tourvieille et al., 1986) in the pRBG4 vector (generously donated by Dr David Clapham, Harvard Medical School), which was used to target cells for recording. GluR6/pCDNA (1 μg) and L3T4/pRBG4 (0.5 μg) were incubated for 45 min with SuperFect reagent (15 μg; Qiagen, Santa Clarita, CA) in OptiMEM (100 μl; Life Technologies), then diluted 1:5 onto HEK cells at 50–70% confluence. After 3–4 h, three volumes of growth medium containing 5 mM kynurenic acid was added. For most experiments, transfected cells were incubated with protease XXIII (1 mg/ml; 10–15 min), dissociated into a single cell suspension, and replated at low density onto glass coverslips 12–24 h after transfection. Recordings were obtained 24 h after replating (48 h after the start of transfection). Before recording, the cells were incubated for 30 min with phycoerytherin-conjugated monoclonal anti-L3T4 (PharMingen, San Diego, CA). Isolated cells that were labeled with the fluorescent antibody were targeted for recording. Kainate applications elicited currents in more than 90% of the labeled cells but did not evoke currents in untransfected, or sham transfected cells.

2.2. Electrophysiology

Whole-cell electrodes pulled from boralex pipettes were filled with an internal solution consisting of 10 mM Hepes, 10 mM EGTA, 5 mM CsCl, and 140 mM $CsCH_3SO_3$ or CsF, titrated to pH 7.4 with CsOH. The open tip resistance ranged from 1 to 5 MΩ. The recording chamber was perfused with Tyrode's solution (in mM): 150 NaCl, 4 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 glucose, 10 Hepes, pH 7.4. An agar bridge containing 4 M KCl connected the recording chamber to a ground well filled with internal solution. Currents were recorded with an Axopatch 200 amplifier (Axon Instruments, Foster City, CA), filtered at 1 KHz (−3 dB, 4-pole Bessel) and digitized at 5–10 kHz.

2.3. Drug application

Metal ions were purchased as chloride salts in the highest purity grade available (Aldrich, Milwaukee, WI). The external solution for drug applications was

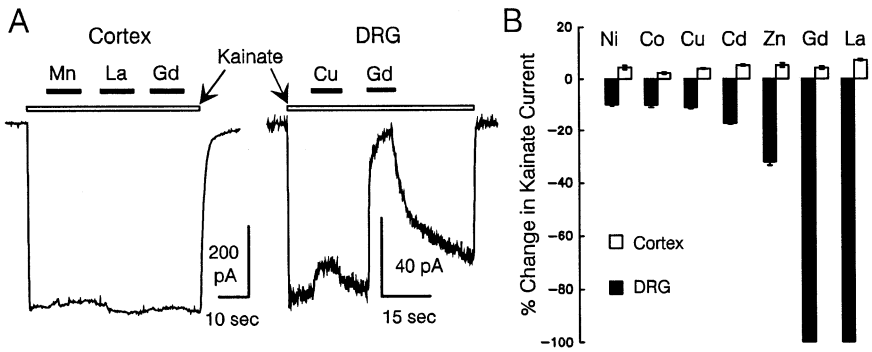

Fig. 1. Selective inhibition of kainate receptors by La and Gd. (A) Whole-cell currents evoked by 200 μM kainate in a cultured cortical neuron (left) and a freshly dissociated DRG neuron (right, after exposure to ConA). Kainate was applied as indicated by the open bars. Solid bars indicate periods of co-application of polyvalent cations at 100 μM. Holding potential, −70 mV. (B) Bars show the mean percentage change in currents evoked by kainate (±S.E.M.) during co-application of each polyvalent cation. A reduction in current relative to control is plotted as a negative percentage change, with −100% corresponding to complete block.

160 mM NaCl, 1 mM $CaCl_2$, 10 mM Hepes, pH 7.4, and 500 nM tetrodotoxin. Control and test solutions were delivered to the cells through eight separate fused-silica tubes (320 μm I.D.; J.&W. Scientific, Folsom, CA), which were aligned within a local perfusion nozzle, made from 1.58 mm I.D. borosilicate glass. Solution flow in each of the eight lines was controlled by a computer-gated solenoid valve (General Valve Corp., Fairfield, NJ, or The Lee Company, Westbrook, CT). For most experiments the drug reservoirs were maintained under static air pressure of 5–10 psi. The rate of solution exchange for this apparatus was determined by measuring the change in holding current as a solution containing 10 mM KCl was applied. In the whole-cell mode, exposure to the test solution developed with a time constant of 5–15 ms. Some of the experiments to determine steady-state inhibition employed drug applications through a linear array of capillary tubes with manual valves and gravity-driven flow. Using this method, solution exchanges were complete within 100–200 ms.

To minimize the formation of insoluble hydroxides or lanthanide polymers, stock solutions were prepared each day in polypropylene containers and diluted to their final desired concentration immediately. In addition, teflon tubing was used to connect the polypropylene drug reservoirs to the teflon valves and then to the application pipette. In several cases, a spectrophotometric assay for lanthanum was performed on the test solutions at the end of the day's experiments. Lanthanum concentrations were determined from the absorbance of arsenazo III at 652 nm using a modification of the method described by Fernandez-Gavarron et al. (1987). Test solutions were centrifuged to remove insoluble hydroxides and the supernatant adjusted to pH 3.0 with 5 M nitric acid. Arsenazo III (Sigma) was initially prepared at 25 mM in DMSO and then diluted to a 2.5 mM stock in double-distilled water. Absorbance at 652 nm was determined for lanthanum solutions containing 25 μM arsenazo III and compared to a standard curve constructed from an atomic absorption standard solution (1 mg/ml La in 1% HNO_3; Aldrich). These tests showed no evidence for significant loss of lanthanum relative to the drug solutions as initially prepared. In additional control experiments, we observed that La solutions prepared from the atomic absorption standard displayed similar potency of block to those made from the chloride salt.

3. Results

3.1. Selective inhibition of kainate current in DRG cells

Whole-cell currents activated by 200 μM kainate were used to study the action of a series of metal cations on kainate and AMPA receptors. Kainate receptors were studied in freshly dissociated DRG neurons that had been exposed to concanavalin A in order to block desensitization (Huettner, 1990). Currents mediated by AMPA receptors were recorded in cultured rat cortical and hippocampal neurons. Numerous previous studies have demonstrated that kainate evokes large maintained currents through AMPA receptors in both cortical (Wilding and Huettner, 1995) and hippocampal (Patneau and Mayer, 1991) neurons.

Initially, we tested seven different cations at a concentration of 100 μM: nickel (Ni), cobalt (Co), copper (Cu), cadmium (Cd), zinc (Zn), lanthanum (La) and gadolinium (Gd). As shown in Fig. 1, each of these ion produced a slight potentiation, on average, of currents elicited by kainate in the cultured cortical neurons. Similar enhancement of AMPA receptor-mediated current by polyvalent cations has been reported by several groups (Peters et al., 1987; Westbrook and Mayer, 1987; Reichling and MacDermott, 1991). In contrast to

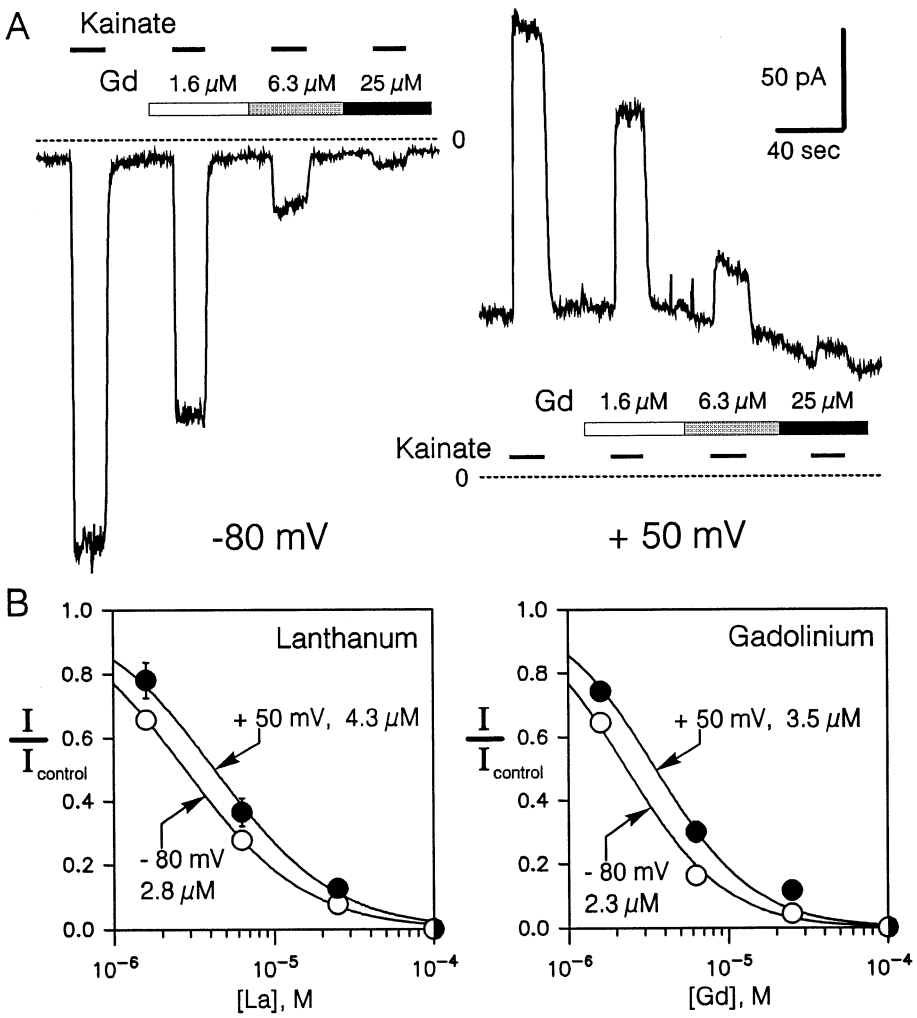

Fig. 2. Steady-state block by lanthanides shows only slight voltage dependence. (A) Whole-cell currents evoked in a DRG neuron by 200 μM kainate at −80 mV (left) and +50 mV (right). Thin bars indicate the periods of kainate application. The thick bar indicates co-application of increasing concentrations of Gd. (B) Currents recorded during co-application of La (left, five cells) or Gd (right, four cells) plotted as a function of the antagonist concentration. Points show the mean current ± S.E.M. as a fraction of the control response to kainate alone. Smooth curve: best fit of $I/I_{control} = 1/(1 + ([ion]/IC_{50})^n)$, where IC_{50} is the concentration that produced half-maximal block and n is the slope factor. For La, $IC_{50} = 2.8$ μM at −80 mV (2.5–3.2 μM, 95% confidence interval) and 4.3 μM (3.6–5.2 μM) at +50 mV (difference is significant at $P < 0.01$). Both curves were constrained to have the same slope factor, $n = 1.2$; individual fits with separate slope factors were not significantly better as judged by the ratio of residual variance. For Gd, $IC_{50} = 2.3$ μM (2.1–2.5 μM) at −80 mV and 3.5 μM (3.2–3.9 μM) at +50 mV (significant at $P < 0.01$); $n = 1.4$ for both curves.

this slight increase in current amplitude at AMPA receptors, all seven of the cations produced inhibition of currents evoked by kainate in DRG neurons. Inhibition by the divalent cations, at 100 μM, averaged between 10 and 35%, whereas the trivalent ions La and Gd produced complete blockade at this concentration (Fig. 1B). Significant inhibition of AMPA receptor-mediated currents was only achieved with lanthanum concentrations greater than 100 μM (data not shown).

3.2. Concentration–response relation: steady-state inhibition and membrane potential

To determine the potency of inhibition by La and Gd we examined the blockade of current as a function of concentration (Fig. 2). If La and Gd were inhibiting kainate receptors by binding within the conducting pore of the channel, then the potency of antagonism would be expected to vary with changes in the membrane potential. Specifically, when cells were held at positive membrane potentials both the transmembrane field and the outward movement of internal cations would help to speed the exit of a blocker from the pore (Armstrong, 1971). To test for this possibility, we studied the inhibition of kainate current produced by increasing concentrations of lanthanum in DRG cells that were held steadily at −80 or at +50 mV. As shown in Fig. 2 the concentration-dependence of inhibition by La

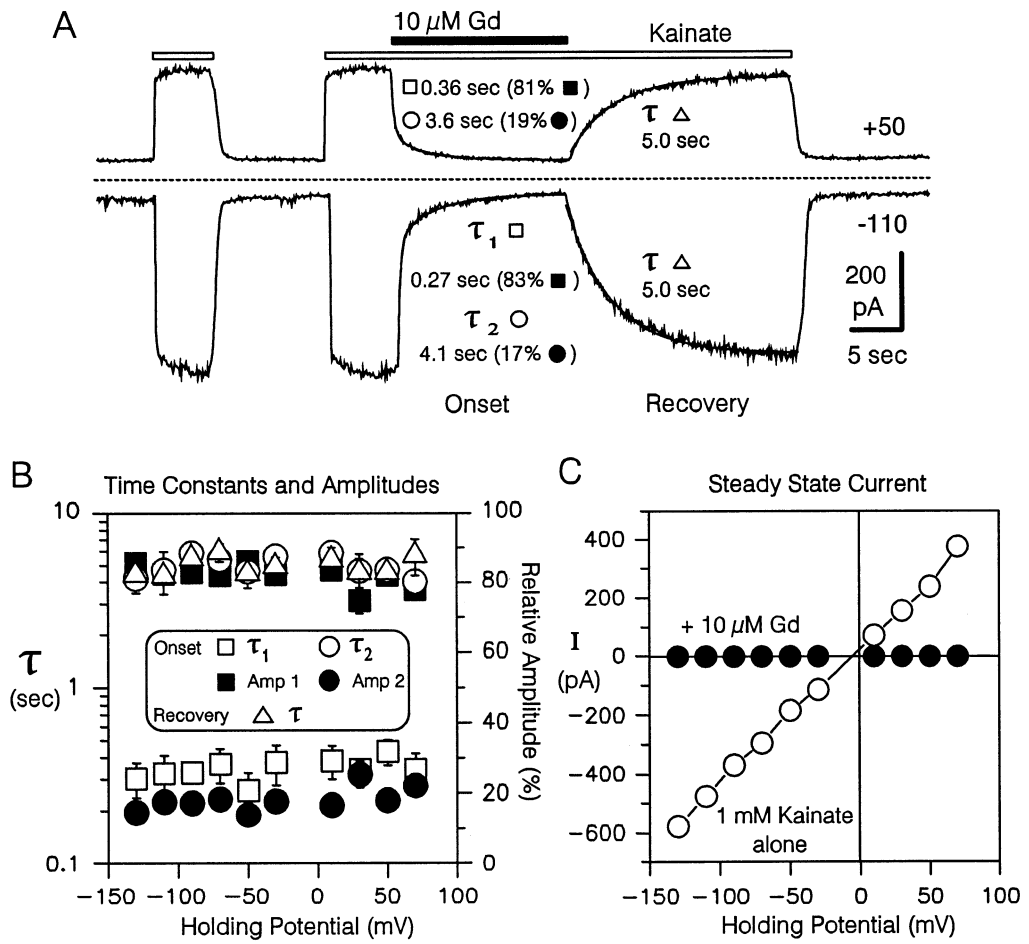

Fig. 3. Block and recovery kinetics are voltage-independent. (A) Currents evoked by kainate in a DRG neuron at a holding potential of −110 mV (bottom trace), and subsequently at +50 mV (top trace). Open bars indicate periods of exposure to 1 mM kainate; filled bar indicates co-application of 10 μM Gd. Onset of block was fit with a sum of two exponential functions, which is shown superimposed on the experimental traces. In this cell, the recovery from block was well described by single exponential functions. Symbols refer to the graph shown on the left below. (B) Time constants and relative amplitudes of the two components of onset of block and the time constant of the recovery are plotted as a function of the holding potential. Points show the mean ± S.E.M. of data from five cells. (C) Steady-state currents evoked by kainate alone (○) or in the presence of 10 μM Gd (●) for the cell shown in panel (A).

or Gd was similar at these two holding potentials. Both La and Gd produced half-maximal inhibition of current at a concentration of 2–4 μM. For both ions, the potency of block was slightly weaker when cells were held at +50 mV than for currents evoked at −80 mV; however, in both cases, there was less than a 2-fold difference in IC_{50} values at the two potentials.

3.3. Kinetics of blockade and recovery

In an attempt to define better the apparent voltage-dependence of inhibition by Gd, we examined the kinetics of blockade and recovery from inhibition as a function of holding potential. In contrast to our results with steady-state applications of kainate and Gd, however, we saw little or no change in the time course of either block or unblock when DRG cells were held at potentials ranging from −130 to +70 mV. The experiment in Fig. 3A is a particularly striking example. In this cell we were able to make two or three applications of Gd at 10 different holding potentials. Fig. 3A shows two sample traces, one recorded at +50 mV and the other at −110 mV. In all of the cells that we studied the onset of blockade by 5–100 μM Gd or La was described by a sum of two exponentials. In some cells, such as the one shown in Fig. 3A, recovery from block could be fit by a single exponential function, but in other cases there was an additional slow component of recovery that accounted for 5–20% of the total. The plots in Fig. 3B show that in the five cells tested over a range of voltages there was no consistent trend as a function of voltage in either the time constants or the relative amplitudes of the exponential curves that described the onset of block. In addition, the time constant of recovery from block (Fig. 3B) and the

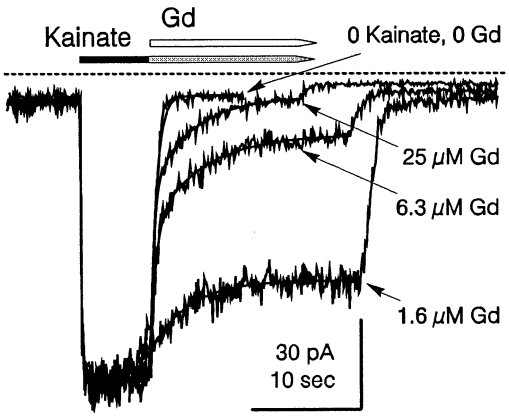

Fig. 4. Kinetics of block depends on Gd concentration. Currents evoked by four different kainate (200 μM) applications to a DRG neuron are shown superimposed. Smooth curves plot the best fit of a single exponential function to deactivation ($\tau = 0.62$ s) and the onset of block by 1.6 μM Gd ($\tau = 3.4$ s). Block by higher concentrations of Gd was better described by a sum of two exponentials, also shown as smooth curves. For 6.3 μM Gd, $\tau_1 = 0.47$ s (62%) and $\tau_2 = 4.6$ s (38%). For 25 μM Gd, $\tau_1 = 0.35$ s (77%) and $\tau_2 = 4.0$ s (23%). Holding potential −70 mV.

steady-state level of block (Fig. 3C) were virtually the same at all potentials studied.

The experiment in Fig. 4 examines the onset of block as a function of Gd concentration. The figure shows four individual traces superimposed. In one trace, the cell was returned from kainate to control solution in order to determine the time course of deactivation. In the other three traces the cell was switched from kainate alone to kainate plus Gd. As shown by the time constants and relative amplitudes provided in Table 1, the main effect of lowering the concentration of Gd from 25 to 6.3 μM and then 1.6 μM was to reduce the proportion of rapid block; the time constants for block onset were much less dependent on Gd concentration than were the relative amplitudes. For La or Gd concentrations less than 1 μM we found it difficult to measure the onset of block; however, in none of the cells did we observe potentiation of kainate receptor-mediated currents, as is seen for the action of La on AMPA receptors (Reichling and MacDermott, 1991).

Table 1
Time constants for onset of current blockade

[Gd] (μM)	τ (s)	Amp. (%)	τ_2 (s)
25	0.42 ± 0.10^a	86 ± 4	4.90 ± 0.36^b
6.3	0.50 ± 0.02^a	65 ± 2	8.20 ± 1.67^b
1.6	6.51 ± 3.31^b	100	
0, deactivation	0.59 ± 0.10	100	

All values are mean ± S.E.M. ($n = 4$ cells). Blockade by 6.3 and 25 μM Gd was best described by a sum of two exponentials. Deactivation and block by 1.6 μM Gd were well fit by a single exponental function.
[a,b] Differences between the means are not significant, $P < 0.5$.

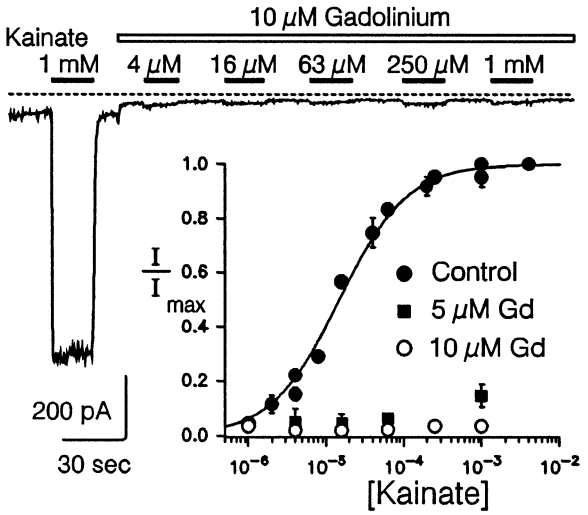

Fig. 5. Inhibition by lanthanides is non-competitive. The trace shows currents in a DRG neuron evoked by 1 mM kainate alone and then currents elicited by 4 μM to 1 mM kainate in the continuous presence of 10 μM gadolinium. The inset graph plots steady-state current evoked by kainate alone (●, data from five cells, taken from Huettner, 1990), or in the presence of either 5 μM (■, four cells) or 10 μM (○, six cells) gadolinium, as a function of the kainate concentration. Points show the mean ± S.E.M. as a fraction of the maximal current evoked by kainate alone. Smooth curve: best fit of $I/I_{max} = 1/(1 + (EC_{50}/[\text{kainate}]))$, where EC_{50} (15 μM) is the half-maximal dose of kainate.

3.4. Inhibition is not competitive

If La or Gd were acting at the receptor's binding site for agonists, then increasing the concentration of kainate would be expected to circumvent the inhibition. As the trace shown in Fig. 5 demonstrates, the inhibition produced by continuous exposure to 10 μM Gd was not overcome by increasing the kainate concentration up to 1 mM. In a series of recordings from DRG cells that had been exposed to Con A, exposure to 5 or 10 μM Gd reduced the maximal amplitude of whole-cell current evoked by all concentrations of kainate from 1 μM to 1 mM (Fig. 5 inset).

3.5. Inhibition of kainate current in hippocampal neurons

In order to study the action of lanthanides on kainate receptors in cultured hippocampal neurons, we used the selective antagonists GYKI 53655 and MK-801 to block currents mediated by AMPA and NMDA receptors, respectively. Previous work (Paternain et al., 1995; Wilding and Huettner, 1995) has shown that noncompetitive inhibition by GYKI 53655 is more than 200-fold more potent against AMPA receptors in cortical and hippocampal neurons than against native or recombinant kainate receptors. During continuous exposure to GYKI 53655, rapid applications of kainate elicit

a desensitizing current in postnatal hippocampal neurons that represents approximately 10–15% of the current evoked in the absence of the inhibitor (Wilding and Huettner, 1997). Both the kinetics and the pharmacology of the current elicited in the presence GYKI 53655 are consistent with it being mediated by native kainate receptors (Paternain et al., 1995; Wilding and Huettner, 1997). As shown in Fig. 6A,B, lanthanum inhibited nearly all of the current that remained in the presence of 100 μM GYKI 53655. The half-maximal concentration for inhibition was 2.1 μM. About 5–10% of the kainate current recorded in the presence of GYKI 53655 was more resistant to inhibition by lanthanum. This current may reflect residual activation of AMPA receptors (less than 1% of control activation in GYKI's absence), because it was blocked by higher doses of lanthanum and by 5 μM NBQX (data not shown).

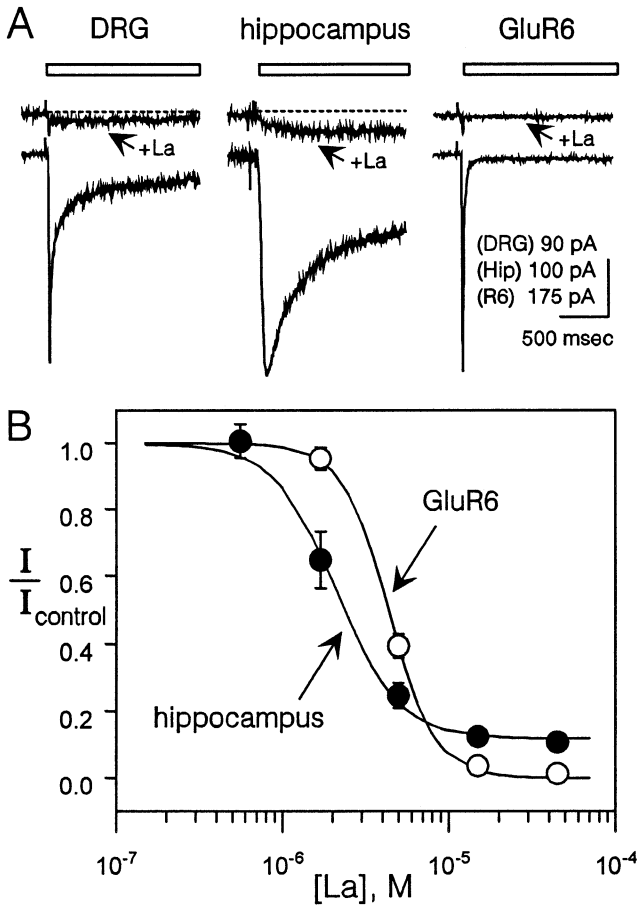

Fig. 6. Inhibition of GluR6, hippocampal and DRG cell kainate receptors. (A) Currents evoked by 300 μM kainate in a freshly dissociated DRG neuron (left), cultured hippocampal neuron (center) and in a HEK 293 cell transfected with GluR6 (right). For each cell type, currents are shown in the absence and presence of 15 μM La. Traces have been offset for clarity. Kainate was applied as indicated by the open bars. Holding potential, −70 mV. (B) Currents evoked in the presence of La as a function of La concentration were recorded in five hippocampal neurons (●) and in 11 HEK cells expressing GluR6 (○). Points show the mean current ± S.E.M. as a fraction of the control response to kainate alone. Smooth curve: best fit of $I/I_{control} = 1/(1 + ([La]/IC_{50})^n)$. For GluR6, $IC_{50} = 4.4$ μM (4.0–4.7 μM, 95% confidence interval) and $n = 3.1 \pm 0.45$. For hippocampal neurons, $IC_{50} = 2.1$ μM (1.7–2.7 μM), $n = 2.3 \pm 0.52$, and an additional parameter was included to account for lack of complete block. The residual current estimated from the best fit was 11 ± 4% of the peak control response.

3.6. Inhibition of GluR6 in HEK 293 cells

In addition to native receptors in hippocampal and DRG neurons, we also tested for inhibition of recombinant receptors produced by transient transfection of HEK cells with cDNA for the fully edited GluR6(*V*,*C*;*R*) subunit (Egebjerg et al., 1991; Köhler et al., 1993). As shown in Fig. 6A,B, currents mediated by GluR6 were blocked by La with similar potency as native receptors, although the slope of the concentration–inhibition curve was much steeper for recombinant receptors (Fig. 6B). La concentrations of 2 μM or lower produced virtually no inhibition, whereas 15 μM La caused nearly complete blockade. The curve for GluR6 in Fig. 6B indicates half-maximal inhibition at a concentration of 4.4 μM La with a slope factor of 3.1.

4. Discussion

4.1. Relative potency

This study has demonstrated the potent inhibition by trivalent lanthanides, La and Gd, of whole-cell currents mediated by kainate receptors. Inhibition was half-maximal at 2–4 μM in freshly dissociated DRG cells, in cultured hippocampal neurons and in HEK 293 cells transfected with the GluR6 subunit. A very similar IC_{50} (2 μM) was obtained by Reichling and MacDermott (1991) for blockade of NMDA receptor-mediated currents in dorsal horn neurons. In contrast, inhibition by lanthanides is somewhat more potent against voltage-gated calcium currents ($IC_{50} = 163$ nM) and significantly less potent at neuronal AMPA receptors ($IC_{50} > 100$ μM; Reichling and MacDermott, 1991; Wilding and Huettner, 1997). Although inhibition by lanthanides clearly is not specific to kainate receptors, the much lower potency of antagonism at AMPA receptors makes blockade by these ions a useful diagnostic tool for distinguishing currents mediated by the AMPA and kainate subtypes (Wilding and Huettner, 1997).

4.2. Mechanism of block

As has previously been described for inhibition of NMDA receptors by La (Reichling and MacDermott, 1991), and by certain divalent ions(Mayer and West-

brook, 1987; Peters et al., 1987; Cristine and Choi, 1990; Legendre and Westbrook, 1990), we observed relatively weak voltage dependence to the blockade of kainate receptor-mediated currents. Steady-state IC_{50} values for La and Gd were approximately 2-fold higher at +50 mV than at −80 mV. We were unsuccessful, however, in our attempt to resolve the kinetic step responsible for this modest voltage dependence (e.g. binding vs. unbinding), because the time course of block onset and recovery did not change significantly as the holding potential was varied from −130 to +70 mV. Furthermore, the time course of current inhibition gave little indication that channels had to be open in order for the ions to bind or unbind from their site of action (see Huettner and Bean, 1988). Collectively, these results indicate that block is unlikely to result from binding deep within the channel pore. Instead the ions may bind to a superficial site near the mouth of the channel (cf. Mayer and Westbrook, 1987), or to an external site that experiences a modest allosteric reduction in affinity at positive membrane potentials.

The bi-exponential kinetics of block onset, and in some cases recovery for block, suggest that there may be two different binding sites responsible for inhibition (cf. Mayer and Westbrook, 1987; Cristine and Choi, 1990; Legendre and Westbrook, 1990), a rapidly equilibrating site with low micromolar affinity and a site with higher affinity that equilibrates more slowly, and which may produce only partial inhibition. In contrast to block by lanthanides, receptor deactivation upon wash out of kainate was well described by a single, relatively rapid ($\tau = 200$–600 ms), exponential decay (Fig. 4, Table 1), suggesting that the slow components of inhibition do not result from inadequate exchange of the extracellular solutions.

During exposure to low concentrations of La or Gd (≤ 2 μM), predominantly the slow component of inhibition was observed (Fig. 4). Application of higher concentrations increased the more rapid component of block onset and recovery. For block mediated by binding to a single site, the apparent affinity of inhibition can be calculated from the time constants of block onset and recovery. Using the time constants in Fig. 3 for rapid block (0.3 s) and recovery (5 s), we obtain an estimated K_d of approximately 0.6 μM, which is in reasonable agreement with the IC_{50} values observed for steady-state inhibition (Figs. 2 and 6).

The failure of increasing kainate concentrations to overcome the block by La or Gd suggests that inhibition does not involve either competition for the agonist binding site or a reduction in the effective concentration of kainate, as might occur with direct interaction between kainate and the metal ions. For either of these mechanisms, the simple competitive model of inhibition predicts that increasing agonist concentrations will eventually overcome the blockade produced by exposure to a fixed dose of inhibitor. The maximal current should be identical to control, although higher concentrations of agonist will be needed to achieve the maximal, and the half-maximal, response levels. Using the equations that define simple competitive inhibition (Arunlakshana and Schild, 1959; Leff and Dougall, 1993), we can estimate the shift in apparent affinity that would be expected for kainate during exposure to a fixed concentration of a purely competitive inhibitor. For our IC_{50} value of 2 μM, we calculate that the EC_{50} for kainate would be 390 μM in the presence of 5 μM inhibitor and 765 μM in 10 μM inhibitor. In contrast, our results shown in Fig. 5 indicate that steady-state block is not significantly alleviated even at 1 mM kainate.

4.3. Future questions

Recent studies with chimeric receptors that combine portions of the GluR6 and GluR3 subunits have helped to define the subunit domains that are responsible for agonist binding (Stern-Bach et al., 1994). In addition, point mutations in the proposed extracellular domain between the third and fourth hydrophobic segments of GluR6 and GluR1 have revealed residues that are involved in the selective modulation of AMPA receptors channels by cyclothiazide (Partin et al., 1995). Future work of this kind should allow the identification of subunit domains that are essential for the potent inhibition of kainate receptors by La and Gd, as well as domains specific to AMPA receptors that underlie the potentiation of this subtype by micromolar doses of lanthanides.

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