Suppression of Kainate-Evoked AMPA Receptor Mediated Responses by Lanthanum in Rat Sacral Dorsal Commissural Neurons

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Key Words

Lanthanum · Kainate · α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor · Sacral dorsal commissural nucleus · Nystatin-perforated patch-clamp technique

Abstract

The effect of lanthanum (La) on kainate (KA) responses in neurons acutely dissociated from the rat sacral dorsal commissural nucleus (SDCN) was investigated using the nystatin-perforated patch-recording configuration under voltage-clamp conditions. The responses to KA were mediated by activation of the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in SDCN neurons. La^{3+} reversibly inhibited KA (100 μM) activated currents (I_{KA}) in a concentration-dependent manner over the range from 30 μM to 30 mM, with IC_{50} values of 0.64 ± 0.06 mM at a holding potential (V_{H}) of -40 mV. Our further study indicated that the effects of La^{3+} on I_{KA} were voltage independent. Moreover, the inhibition was not use dependent and was not overcome by increasing the concentration of agonist. These findings indicate that La^{3+} is an efficacious inhibitor of AMPA receptor mediated responses which may contribute to its cytotoxic effect.

Introduction

Lanthanum (La) is a trivalent rare earth element that is of increasing interest to toxicologists. It is not only a by-product of the nuclear industry, but also increasingly used in the manufacture of metals with special properties. Greater use of La increases the chances of accidental exposure, both acute and chronic. Early studies have found reductions in the number of successful pregnancies in mice injected with La [1] and failure of cephalic neural tube closure in rat embryos exposed to the ion [2]. La is also a potential behavioral teratogen [3]. The above facts, in addition to its complicated pharmacological properties [4], justify attempts to characterize the effects of La^{3+} on neurotransmitter receptors and synaptic responses.

La has been reported to influence neuronal transmitter systems. Most studied properties are the effects of La^{3+} on the γ -aminobutyric acid type A receptor function [5–7], and it has also been shown that La^{3+} could modulate glycine currents in rat septal cholinergic neurons in culture [8] as well as acetylcholine-induced currents in guinea pig ileal smooth muscle cells [9]. Glutamate receptor induced responses can also be affected by La in various preparations, including rat dorsal root ganglia (DRG), cultured rat hippocampal and cortical neurons [10, 11], and rat dorsal horn neurons [12]. In these studies, La^{3+} displayed a biphasic effect on α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor

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mediated responses, i.e., low concentrations of La^{3+} (1–100 μM) potentiated AMPA receptor mediated currents, while the currents were blocked by $\text{La}^{3+} > 100 \mu\text{M}$.

The sacral dorsal commissural nucleus (SDCN) represents the dorsal gray matter of the central canal in the lower lumbar and sacral spinal cord. Our previous work [13] has shown some distinctive characteristics of AMPA receptors in the SDCN: the lack of the glutamate receptor 2 subunit and the presence of flip forms of receptors. These raise the possibility that the effects of La^{3+} on AMPA receptors are different between SDCN and other brain regions, and the distinctness may add further diversity to the pharmacological properties of La^{3+} . Therefore, the effect of this metal on AMPA receptor mediated responses evoked by kainate (KA) was investigated using the nystatin-perforated patch-recording configuration in SDCN neurons.

Materials and Methods

Preparation

The SDCN neurons were acutely dissociated as described elsewhere [14]. Two-week-old Sprague-Dawley rats were anesthetized with sodium pentobarbitone (50 mg/kg i.p.), and laminectomy was performed to expose the lower lumbar and sacral spinal cord. The lumbosacral ($\text{L}_6\text{--S}_2$) segment (approximately 10–15 mm) was dissected out from the spinal cord and immersed in freezing incubation solution, followed by animal decapitation. After removing the attached dorsal rootlets and the pia mater, the spinal segment was affixed with cyanoacrylic glue to a $10 \times 15\text{-mm}$ agar base with the latter supporting the spinal block. The tissue block together with the agar base was positioned onto the bottom of the cutting chamber of a vibratome tissue slicer (DTK-1000; Dosaka, Kyoto, Japan), with the dorsal surface of the cord facing the vibrating blade. Cold incubation solution (approximately 5°C ; see below) aerated with 95% O_2 and 5% CO_2 was subsequently poured into the chamber to immerse the spinal block. The spinal segment was sectioned to yield several transverse slices (400 μm thick) containing the SDCN region. The slices were preincubated in oxygenated incubation solution (see below) for 50 min at room temperature ($22\text{--}25^\circ\text{C}$). Thereafter, the slices were treated enzymatically in oxygenated incubation solution containing 1 mg (6–8 ml) pronase for 20 min at 31°C , followed by exposure to 1 mg (6–8 ml) thermolysin for 15 min under identical conditions. After enzymatic treatment, the slices were kept in enzyme-free incubation solution for 1 h. Then a portion of SDCN region was micropunched out by using an electrolytically polished injection needle and transferred into a culture dish filled with standard external solution (see below). Neurons were mechanically dissociated with fire-polished Pasteur pipettes under visual guidance by means of a phase-contrast microscope (model IX70; Olympus, Tokyo, Japan). The dissociated neurons adhered to the bottom of the dish within 20 min, allowing electrophysiological studies to be conducted. Neurons that retained their original morphological features, such as the dendritic processes, were then used for the experiments. The experimental procedures have been ap-

proved by the Animal Care and Use Committee of the Fourth Military Medical University.

Solutions

The incubation solution (containing in mM: NaCl 124, NaHCO_3 24, KCl 5, KH_2PO_4 1.2, CaCl_2 2.4, MgSO_4 1.3, and glucose 10) was aerated with 95% O_2 and 5% CO_2 to a final pH of 7.4. The normal external standard solution (containing in mM: NaCl 150, KCl 5, CaCl_2 2, MgCl_2 1, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid – Hepes – 10, and glucose 10) was adjusted to pH 7.4 with tris-(hydroxymethyl)-aminomethane (Tris base), while the patch pipette solution (containing 150 mM CsCl and 10 mM Hepes, adjusted with Tris base to pH 7.2) was used for nystatin-perforated patch recordings. A final nystatin concentration of 400 $\mu\text{g/ml}$ dissolved in acidified methanol was added to the patch pipette solution just before use. When the current-voltage ($I\text{--}V$) relationship for I_{KA} was examined, 0.3 μM tetrodotoxin and 10 μM CdCl_2 were added to the standard external solution. CdCl_2 had no noticeable effect on the I_{KA} at the concentrations used.

Perforated Patch Recordings

Electrical measurements were carried out using a nystatin-perforated patch-recording configuration, which maintains the intracellular levels of Ca^{2+} and other second messengers [15], under voltage-clamp conditions at room temperature ($22\text{--}25^\circ\text{C}$). Patch pipettes were pulled from glass capillaries with an outer diameter of 1.5 mm (Narishige, Tokyo, Japan) on a two-stage puller (PB-7; Narishige). The resistance between the recording electrode filled with pipette solution and the reference electrode was 4–6 $\text{M}\Omega$. The patch pipette was positioned onto a neuronal body using a hydraulic micromanipulator (WR-3; Narishige). The electrode was connected to a patch-clamp amplifier (Axopatch 200A; Axon Instruments, Union City, Calif., USA). Both current and voltage were monitored with a pen recorder (Omniace RT 3100; San-ei, Osaka, Japan), filtered at 1 kHz, and sampled and analyzed using a DigiData 1200A interface and a computer running with the pCLAMP 6.0.2 program (Axon Instruments). The morphological and electrophysiological features of the isolated SDCN neurons were similar to those reported previously [16, 17]. The resting membrane potential was in the range from -45 to -60 mV. The series resistance, estimated from the optimal cancellation of the capacity transient, was 10–30 $\text{M}\Omega$. In most experiments, 70–90% series resistance compensation was applied. The membrane potential was held at -40 mV throughout the experiment, except when examining the $I\text{--}V$ relationship. All measurements were started after stabilization of the KA responses (15–25 min after cell attachment).

Drugs and Statistics

Pronase was purchased from Calbiochem (La Jolla, Calif., USA), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) was from Tocris Neuramin (Bristol, UK), and NS-102 and GYKI 52466 were from Research Biochemicals International (Natick, Mass., USA). The other drugs were from Sigma Chemical (St. Louis, Mo., USA). La was purchased as chloride salt of the highest purity grade available. The drugs were applied via a 'Y tube' [15]. This system allows complete exchange of an external solution surrounding a neuron within 20 ms. All data are expressed as mean values $\pm$ SEM. The data were fitted to a modified Michaelis-Menten equation by use of a least-square fitting for EC_{50} or IC_{50} and the Hill coefficient [14].

Fig. 1. Effects of CNQX, GYKI 52466, and NS-102 on KA-induced whole-cell currents. Inward currents induced by 100 μ M of KA were inhibited by coadministration of 10 μ M CNQX (**A**), 300 μ M GYKI 52466 (**B**), but not by 3 μ M NS-102 (**C**). All antagonists were perfused for 30 s before simultaneous application of KA. Data in **A–C** were from different neurons. Similar results were obtained from another 5 neurons.

Fig. 2. **A** Suppression of the I_{KA} by La^{3+} in a concentration-dependent manner. **a** Currents induced by 100 μ M of KA in the presence of 1 and 10 mM La^{3+} . **b** Test of the use dependence of the La block. Currents induced by 100 μ M of KA in the presence of 1 mM La^{3+} did not decline. The fully developed inhibition of the I_{KA} observed in the absence of receptor activation indicates that open channels are not required for the La effect. Data shown in **a** and **b** were from the same neuron. **B** Concentration-response curve of La^{3+} -induced suppression of the I_{KA} . Each point is the average of the values from 5–7 cells. Here and in the subsequent figures, the vertical bars show the mean $\pm$ SEM.

Results

The KA Responses Were Mediated by AMPA Receptors

The application of KA evoked inward currents in all SDCN neurons tested. The inward currents induced by KA did not exhibit noticeable desensitization at the concentrations evaluated. The KA (100 μ M) induced current was significantly depressed by the selective non-N-methyl-D-aspartate (non-NMDA) receptor antagonist CNQX (fig. 1A). Previous work has demonstrated that the non-NMDA receptors include AMPA and KA receptors which are distinct receptor complexes, although both of them can be activated by KA. To examine which subfamily of non-NMDA receptors is present in the acutely dissociated SDCN neurons, the effects of GYKI 52466, the AMPA-receptor-selective noncompetitive antagonist [18], and NS-102, the selective antagonist of KA receptors [19], were investigated. GYKI 52466 (300 μ M) reversibly blocked $95 \pm 2\%$ of the current induced by 100 μ M KA (fig. 1B). In contrast, NS-102 produced no detectable effect on the I_{KA} (fig. 1C). These data indicated that the responses to KA were mediated by AMPA receptors in SDCN neurons.

Suppression of I_{KA} by La^{3+}

Under the present experimental conditions for recording I_{KA} , application of La^{3+} alone induced no noticeable current at levels of up to 30 mM, and with La^{3+} at $<30 \mu$ M, there was not any change in the I_{KA} detected in SDCN neurons. However, previous reports suggested a modest augmentation of AMPA receptor currents by low concentrations of La^{3+} (about 15 μ M) [10–12]. To determine whether there was a small enhancement of current by $<30 \mu$ M of La^{3+} , we next compared the I_{KA} in the presence and absence of La^{3+} in CA1 pyramidal neurons of the rat hippocampus. In contrast, in CA1 pyramidal neurons, the I_{KA} , which was mediated by AMPA receptors as confirmed by their significant depression by CNQX and GYKI 52466, was potentiated by about 12% with 10 μ M of La^{3+} (data not shown).

In SDCN neurons, the I_{KA} was inhibited by extracellular coapplication of $La^{3+} >30 \mu$ M. As shown in figure 2A (see a), the amplitudes of I_{KA} activated by 100 μ M KA decreased with an increase of the La^{3+} concentration. The suppressive effect of La^{3+} was rapidly and completely reversed by washing out La^{3+} in the bathing medium. We further characterized the mechanism of La^{3+} block by testing whether or not the effect was use dependent,

Fig. 3. Effects of KA on the block by La^{3+} . **A** Responses to $100\ \mu\text{M}$ of KA (**a**) and $1\ \text{mM}$ of KA (**b**) under the control condition and in the presence of $1\ \text{mM}$ La^{3+} in the same neuron. **B** Plot of the percentage of block by $1\ \text{mM}$ La^{3+} as a function of the KA concentration. **C** Plot from the same data as in **B**. The concentration-response curves for KA in the control solution (■) and in the presence of $1\ \text{mM}$ La^{3+} (●). All responses were normalized to the peak current induced by $100\ \mu\text{M}$ KA alone (*). Each point is the average of the values from 4–7 neurons.

an effect interpreted as being consistent with an open channel blocking mechanism [20]. The magnitude of the inhibitory action of La^{3+} was not changed by repeated application of $100\ \mu\text{M}$ KA in the continued presence of $1\ \text{mM}$ La^{3+} (fig. 2A; see b), indicating that the full development of La^{3+} inhibition does not require AMPA receptor channel activation. Thus, the inhibition by La^{3+} was clearly not dependent on the presence of open channels.

In addition, as shown in figure 2B, the inhibitory effect of La^{3+} on the I_{KA} was concentration dependent from $30\ \mu\text{M}$ to $30\ \text{mM}$, and significant inhibition of the I_{KA} was achieved with $\text{La}^{3+} > 0.1\ \text{mM}$. With $\text{La}^{3+} > 30\ \text{mM}$, the I_{KA} was completely eliminated. The IC_{50} value obtained from the inhibition curve was $0.64 \pm 0.06\ \text{mM}$.

Effect of La^{3+} on the Concentration-Response Curve of I_{KA}

The concentration-response curve of I_{KA} was examined with or without coapplication of $1\ \text{mM}$ of La^{3+} to elucidate the mechanism of the La^{3+} -induced inhibition of I_{KA} . As shown in figure 3A, the degree of inhibition by $1\ \text{mM}$ of La^{3+} was independent of the KA concentrations. Thus La^{3+} inhibited the I_{KA} in a noncompetitive manner. The noncompetitive nature of La^{3+} was also re-

vealed by plotting the current amplitude in the presence of $1\ \text{mM}$ La^{3+} as the ratio of control responses evoked by different concentrations of KA shown in figure 3B. La^{3+} reduced the amplitude of KA currents to about 41% of control values which is independent of the KA concentrations.

Figure 3C shows the dose-response curves for KA under the control condition and after bath application of La^{3+} . La^{3+} decreased the maximum value of the concentration response for KA without affecting the threshold concentration. The following values were obtained: $\text{EC}_{50} = 108.1 \pm 8.7\ \mu\text{M}$ and Hill coefficient = 1.12 ± 0.10 in the control solution and $\text{EC}_{50} = 107.6 \pm 12.8\ \mu\text{M}$ and Hill coefficient = 1.09 ± 0.12 in the La^{3+} solution. EC_{50} and Hill coefficient for KA were not altered significantly in the presence of La^{3+} , indicating that La^{3+} did not change the affinity of KA for AMPA receptors.

I-V Relationship of I_{KA}

To further characterize the La^{3+} suppression of I_{KA} , the voltage dependence of KA ($100\ \mu\text{M}$) induced current was examined in the absence and presence of $1\ \text{mM}$ of La^{3+} (fig. 4). Representative traces of I_{KA} inhibited by La^{3+} at membrane V_{H} of -40 and $+40\ \text{mV}$ are shown in

Fig. 4. Effects of La^{3+} on the I - V relationship for KA. **A** Responses to 100 μ M of KA at V_H of +40 and -40 mV under the control condition and in the presence of 1 mM La^{3+} . **B** The I - V relationships of KA responses induced by 100 μ M of KA in the absence (■) and presence (●) of 1 mM of La^{3+} . The inhibitory effect of La^{3+} is similar at all V_H values. The membrane potential was held at each value for at least 1 min before application of KA. Currents were normalized to the peak current induced by 100 μ M of KA alone at a V_H of -40 mV (*). Each point is the mean of the values from 4–8 neurons.

figure 4A. The I - V curves for I_{KA} with or without 1 mM of La^{3+} are plotted in figure 4B. In the presence of La^{3+} , the linear voltage dependence of I_{KA} persisted at V_H over -60 mV to +60 mV, thus the suppression by La^{3+} was voltage independent. The reversal potentials of KA responses (E_{KA}) were -2.25 mV under the control condition and -3.90 mV with 1 mM of La^{3+} . Both of these E_{KA} values were close to the equilibrium potential for nonspecific cation channels calculated from the given extra- and intracellular cation concentrations, indicating that KA activated nonspecific cation channels in the acutely dissociated SDCN neurons and that La^{3+} decreased the I_{KA} without changing the ion selectivity of the AMPA receptor channel.

Discussion

In the present study, we have demonstrated an inhibitory effect of La^{3+} on KA-evoked AMPA receptor mediated responses in acutely isolated rat SDCN neurons. The

application of La^{3+} to SDCN neurons suppressed the I_{KA} in a concentration-dependent manner and reduced the efficacy of KA at AMPA receptors, without affecting both the reversal potential of I_{KA} and the apparent affinity of KA to AMPA receptors.

Block of AMPA Receptors by La^{3+}

In all of the cells that we examined, the whole-cell currents evoked by KA displayed no desensitization. Previous studies have demonstrated that KA evokes nondesensitizing currents through AMPA receptors [10, 13, 21], but faster and more complete desensitization through KA receptors [22, 23]. In addition, the present pharmacological data demonstrate the antagonism of non-NMDA receptor mediated responses by the AMPA receptor selective, noncompetitive antagonist, GYKI 52466, and the lack of antagonism by the selective antagonist of KA receptor, NS102. Collectively, these results indicate that the KA-evoked currents in SDCN neurons are mediated by activation of AMPA receptors.

Our experiments demonstrate that millimolar La^{3+} is an efficacious inhibitor of AMPA receptor mediated responses evoked by KA in acutely dissociated SDCN neurons. In contrast, the currents evoked by both native [11] and recombinant [10] KA receptors are strongly inhibited by micromolar La^{3+} . These studies raise the possibility that AMPA- and KA-preferring receptors show differential sensitivity to the inhibition by La^{3+} and that the much lower potency of antagonism at AMPA receptors makes blockade by the ion a useful diagnostic tool for distinguishing currents mediated by the AMPA and KA subtypes.

In addition, the action of La^{3+} on neural AMPA receptors in SDCN neurons described here seems to be different from the dual action of this ion on AMPA receptors which was reported previously in rat DRG, cultured rat hippocampal and cortical neurons [10, 11], and rat dorsal horn neurons [12]. Previous work showed that currents mediated by AMPA receptors were blocked with La^{3+} levels >100 μ M, but were potentiated by micromolar La^{3+} [10–12]. However, we only observed the inhibitory action of La^{3+} on AMPA receptors in SDCN cells. In addition, the modest augmentation of AMPA receptor currents by low concentrations of La^{3+} was observed in CA1 pyramidal neurons of the rat hippocampus under the same experimental conditions. The results suggest that AMPA receptors in SDCN neurons are pharmacologically distinct from those in other neurons, and the conspicuous difference seen between the distinct cell types also sug-

gests that the lack of enhancement of current by $<30 \mu\text{M}$ of La^{3+} is not a result of our recording or analysis techniques. However, the reason for this discrepancy remains unclear. One possible explanation is that the difference may reflect the heterogeneity of AMPA receptors expressed in different preparations. Future work should focus on the identification of subunit domains specific to AMPA receptors that underlie the inhibitory effect of La^{3+} .

Mechanism of Block

Our experiments demonstrate that the degree of inhibition of the I_{KA} by La^{3+} does not depend on the KA concentration. Similar findings have been discussed for the block of glutamate receptors in DRG neurons by La^{3+} [11] and for the inhibition of γ -aminobutyric acid type A receptors in DRG neurons by copper [24]. The failure of increasing KA concentrations to overcome the inhibition by La^{3+} suggests that the inhibition does not involve either competition for the agonist-binding site or a reduction in the effective concentration of KA which might occur with direct interaction between KA and La^{3+} . In addition, in the present study, La^{3+} produced a nearly 59% decrease in the maximal and half-maximal currents with the same EC_{50} in comparison with that under La^{3+} -free conditions, suggesting that La^{3+} changes the number of AMPA receptors available or decreases the open frequency of the AMPA receptors. Collectively, the properties of the La^{3+} -induced block are more consistent with an allosteric model of action, in which La^{3+} binds to a distinct site on the AMPA receptor and reduces the potency of KA.

To further characterize the mechanism of La^{3+} inhibition of I_{KA} , we observed the relationship between the KA current and the membrane potential in the absence and presence of La^{3+} . Our data indicated that the inhibitory

effect of La^{3+} is similar, as the V_{H} varied from -60 to $+60$ mV. La^{3+} did not change the linear dependence of KA current on the membrane potential. Open-channel block by cations is often voltage dependent [25], so the lack of voltage dependence of La^{3+} block on the I_{KA} makes it unlikely that La^{3+} exerts a blockade of the open channel by entering the pore. Furthermore, the time course of current inhibition gave little indication that the channels had to be open in order for the ions to bind or unbind from their site of action [20, 26]. Hence, the ion may bind to a superficial site near or at external orifices of the channel. Not only glutamate receptors, but also γ -aminobutyric acid type A [5–7], glycine [8], and acetylcholine [9] receptors can be modulated by La^{3+} . The multiplicity of the La^{3+} action also suggests that more than one mechanism is likely to be involved in the modulation of synaptic currents by La^{3+} . Further work will be needed to understand the structural and mechanistic bases of the multiplicity.

In summary, our results demonstrate that La^{3+} is an efficacious inhibitor of AMPA receptor mediated responses evoked by KA in SDCN neurons. In addition, the use of La has expanded, and the greater use of it increased the chances of exposure. Calculations made by Briner et al. [3] suggest that the concentration of La^{3+} in the CNS after exposure to La in food or drinking water may reach 0.7 mM . Thus, it seems that the IC_{50} values of La^{3+} determined here are within the limits that may be expected to occur in vivo by La exposure. The data in the present work add further diversity to the mechanisms of the cytotoxic effects of La^{3+} .

Acknowledgments

This work was supported by the National Natural Science Foundation of China (grant No. 30300077) and a grant from the Fourth Military Medical University to Dian-Shi Wang. We are very grateful to Drs. Zhi-Ming Wang and Hui Li for their technical assistance.

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