mGluR1, But Not mGluR5, Mediates Depolarization of Spinal Cord Neurons by Blocking a Leak Current

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INTRODUCTION

Activation of metabotropic glutamate receptors (mGluRs) can have various cellular and synaptic effects ranging from actions on single ion channels and synaptic transmission, to the modulation of activity of a neural network (Anwyl 1999). Eight subtypes of mGluRs have been cloned in mammals and show a high sequence similarity to those found in invertebrates and fish (Bargmann 1998; Pang et al. 1994; Parmentier et al. 1996; Pin and Duvoisin 1995). They have been divided into three groups depending on their signal transduction pathways, pharmacology, and genetic sequence. The group I mGluRs consist of mGluR1 and mGluR5 and are located postsynaptically (Pin and Duvoisin 1995). mGluR1 and mGluR5 have been considered to have similar cellular effects, since they both activate phospholipase C (PLC), leading to a production of IP3 (Pin and Duvoisin 1995). Recently, however, these two group I mGluR subtypes have been shown to mediate different cellular responses. When expressed in oocytes, mGluR1 and mGluR5 produce different patterns of changes in the intracellular calcium concentration ([Ca2+]i). mGluR1 induced a single-peaked calcium response, while mGluR5 mediated persistent [Ca2+]i oscillations through a release from internal stores (Kawabata et al. 1996; Nakamichi et al. 1998). In lamprey spinal neurons, mGluR5, but not mGluR1, induced [Ca2+]i oscillations, and their endogenous activation modulates the locomotor rhythm (Kettunen et al. 2002).

A few recent studies have shown that mGluR1 and mGluR5 also have different effects on the membrane excitability of neurons. For example, mGluR1 depolarizes pyramidal neurons in the CA1 of hippocampus, while mGluR5 potentiates N-methyl-d-aspartate (NMDA) currents in the same cell (Mannion et al. 2001). Interestingly, there appears to be a wide range of underlying mechanisms for the same resulting response in different cell types and animal species, which makes generalization of the effects of mGluR activation difficult.

The modulatory roles of mGluRs in a neural network underlying behavior have been studied in the isolated lamprey spinal cord preparation (El Manira et al. 2002). Here, the two subtypes of group I mGluRs are both activated by endogenously released glutamate during locomotor activity but mediate opposing effects on the frequency of the fictive swimming rhythm induced by NMDA (El Manira et al. 2002). mGluR5 releases Ca2+ from intracellular stores and decreases the frequency of NMDA-induced swimming activity in the spinal cord (Kettunen et al. 2002). mGluR1 interacts with NMDA receptors and potentiates NMDA-induced calcium response and current (Krieger et al. 1998, 2000). This potentiation on the receptor level results in a modulation of NMDA-induced, TTX-resistant membrane potential oscillations and an increase in locomotor frequency (Krieger et al. 1998, 2000).

This study examines the effects of group I mGluRs on the membrane properties of spinal neurons to further determine the mechanisms by which they modulate neuronal excitability and...
locomotor network activity. Our results show that mGluR1, but not mGluR5, depolarizes the membrane potential by blocking a leak conductance, mediated by K\(^+\) and Na\(^+\), that contributes to the resting membrane potential. This effect requires PLC activation and release of Ca\(^2+\) from internal stores. The results further indicate that the two receptor subtypes possess distinct and separate roles in a locomotor network.

**METHODS**

**Dissection and cell culture**

Larval lampreys (*Petromyzon marinus*) were anesthetized with tricaine methane sulfonate (MS-222; 100 mg/l, Sigma, St. Louis, MO), and the spinal cord was isolated in cooled oxygenated physiological solution composed of (in mM) 138 NaCl, 2.1 KCl, 1.2 MgCl\(_2\), 1.8 CaCl\(_2\), 4 glucose, and 2 HEPES, with pH adjusted to 7.4. Motoneurons were prelabeled for subsequent identification after dissociation by injecting fluorescein-coupled dextran amine (Molecular Probes, Leiden, The Netherlands) into the muscle on both sides of the body to allow retrograde transport of dye along ventral root axons. The dorsal roots were lesioned to prevent transport of the dye in sensory neurons. After 18 h of incubation, the spinal cords were dissected from the notochord. They were cut in smaller pieces and treated with collagenase (1 mg/ml; 30 min, Sigma), both dissolved in Leibovitz L-15 culture medium (270 mOsm, Sigma), containing penicillin-streptomycin (2 \(\mu\)g/ml) (El Manira and Bussières 1997). The spinal cord was then washed and carefully triturated with a sterilized 5-ml glass pipette in Leibovitz’s solution. The solution with the fully dissociated tissue was then distributed into 15 culture dishes (35 mm, Corning, VWR International, Stockholm) and incubated at 10°C for 2–4 days.

**Electrophysiology**

An Axopatch 200A (Axon instruments, Foster City, CA) was used for whole cell patch-clamp recordings from isolated spinal cord neurons in culture. Dissociated cells were continuously perfused using a gravity-driven multibarreled perfusion system (ALÀ Scientific Instruments) with the tip positioned close to the recorded neuron. The extracellular control solution contained (in mM) 124 NaCl, 2 KCl, 1.2 MgCl\(_2\), 5 CaCl\(_2\), 10 glucose, and 10 HEPES, with pH adjusted to 7.6. Three modified extracellular solutions were used to determine the ionic nature of the conductance studied: a high [K\(^+\)] solution with a threefold increase of the KCl concentration to 6 mM, a K\(^+\)-free solution, and a low [Na\(^+\)] solution in which 50% of the NaCl was substituted with an equimolar concentration of choline chloride (Sigma). Recordings were made in solution cooled to around 10°C. Strychnine (5 \(\mu\)M) was added to all solutions throughout the experiments to avoid the effect of trace amounts of glycine in the (R,S)-3,5-dihydroxyphenylglycine (DHPG) preparation (Contractor et al. 1998). All solutions except those used in the experiments shown in Fig. 1A also contained tetrodotoxin (TTX, 150 nM) to block Na\(^+\) channels. The cells studied were both labeled motoneurons (MNs) and unlabeled neurons with a small diameter corresponding primarily to MNs and spinal interneurons. The larger, mechanosensory dorsal neurons were not included in this study. The electrode solution consisted of (in mM) 113 KCH\(_2\)SO\(_4\), 1.2 MgCl\(_2\), 10 glucose and 10 HEPES, with pH adjusted to 7.6 with KOH. The recorded neurons had a resting membrane potential between −50 and −65 mV, and only one cell per dish was studied.

Measurements of the membrane potential and currents were performed in gap-free mode, with the membrane potential held either at rest or at −40 mV. Input resistance measurements were done by measuring the induced currents by hyperpolarizing voltage steps at holding potentials of −60 and −40 mV. In voltage clamp, the net current was measured in gap-free mode at holding potentials of −60 and −40 mV. Steps from −60 to −40 mV for 5 s, in control and in DHPG with leak current subtraction, were also applied to test the effect of DHPG on voltage-gated currents. Linear leak currents and residual capacity transients were subtracted on-line using a P/-4 subtraction protocol. In this protocol, four hyperpolarizing presteps, each −1/4 of the main test pulse amplitude, were used to estimate the involvement of leak current. The holding potential during the presteps was −70 mV and was maintained for 500 ms before application of the four presteps to −75 mV. After this, the holding potential was set to −60 mV for 500 ms at which the depolarizing main test step to −40 mV was applied. The leak current from the four presteps was averaged, scaled, and subtracted on-line during each test pulse using Clampex software (Axon Instruments). Recordings were digitized using a Digidata 1320a interface, monitored, and stored with pClamp.
compensated to 75–85%. Currents and voltage signals were filtered through the amplifier at 1–5 kHz and digitized at 10–100 kHz.

Pharmacology

Experiments were performed using the extracellular solution as control and drugs prepared as stock solutions and then dissolved in the control solution, which was corrected for possible changes in pH and osmolarity. The group I mGluR agonist DHPG and the mGluR1 and mGluR5 antagonists LY 367385 (100 μM) and 2-methyl-6-(phenylethynyl)pyridine (MPEP;100 μM), respectively, were purchased from Tocris Cookson (Bristol, UK). Thapsigargin (1 M) was purchased from Calbiochem (Stockholm).

DHPG was applied for approximately 10 s during voltage-clamp and current-clamp experiments and continuously during the input resistance measurements. The mGluR antagonists LY 367385 and MPEP as well as the PLC blocker U-73122 (1 μM) was purchased from Calbiochem (Stockholm).

Statistical analysis

Software used for data analysis and statistical calculations included Clampfit (Axon Instruments), Origin (Microcal software, Northampton, MA), and Graphpad (Prism, GraphPad Software Inc.). n = number of cells studied. Unless otherwise stated, the results are expressed as mean ± SE. Means were compared using Student’s t-test or one-way ANOVA (Graphpad).

RESULTS

Activation of group I mGluRs induces firing in depolarized neurons

To study the effect of group I mGluR activation on neuronal excitability, whole cell patch-clamp recordings were performed on isolated lamprey spinal cord neurons. At resting membrane potentials, i.e., between −50 and −65 mV, a 10-s application of the group I mGluR agonist DHPG (100 μM) only resulted in a slight depolarization of the membrane potential of 2.5 ± 0.5 nV (n = 4; Fig. 1A). However, when the same cell was held at a membrane potential close to the firing threshold (around −40 mV) by current injection, application of DHPG further depolarized the membrane and induced repetitive firing with bursts of action potentials (n = 4; Fig. 1A). In the presence of TTX, which blocks Na+ channels, DHPG application at resting membrane potential resulted in a small depolarization (Fig. 1B) but induced yet a larger depolarization when the membrane potential was held at −40 mV (Fig. 1B). In total, at resting membrane potentials DHPG slightly depolarized the neurons by 2.3 ± 0.2 mV (n = 20), whereas at depolarized membrane potentials, DHPG induced a significantly larger depolarization of 7.4 ± 0.5 mV (n = 27; P < 0.0001; unpaired t-test; Fig. 1C).

DHPG induces an inward current by decreasing the membrane conductance

The nature of the current underlying the DHPG-induced depolarization of spinal cord neurons was examined in voltage-clamp experiments. At a holding potential of −40 mV, an application of DHPG induced an inward current with an amplitude of 36.1 ± 2.9 pA that was associated with a decrease in the membrane conductance (n = 27; Fig. 2A). When the membrane potential was held at −60 mV, DHPG decreased the membrane conductance without inducing any significant inward current (Fig. 2A). Similar effects were seen in labeled motoneurons in which the amplitude of the inward current induced by DHPG was 33.1 ± 9.6 pA (n = 5). The inward current could be induced by repetitive application of DHPG (n = 5; Fig. 2B), indicating that the receptors did not desensitize during this brief application. The effect of DHPG always recovered on washout.
The reversal potential of the DHPG-induced current was measured using a voltage ramp (from −120 to −40 mV, 2 s) in control and during the application of DHPG (Fig. 2C). The current intersected at −61.2 ± 1.2 mV (n = 7; Fig. 2C), which corresponds to the reversal potential of the current affected by DHPG. Subtraction of the current induced in control from that induced in DHPG revealed that the current blocked by DHPG varied in a linear manner with membrane potential (Fig. 2D). This suggests that the conductance underlying the current blocked by DHPG is voltage independent, and it may correspond to a leak conductance.

**DHPG induces an inward current by blocking a leak conductance**

The voltage independence of the current blocked by DHPG was further examined by testing the effect of DHPG without and with leak current subtracted (Fig. 3A). In cells held at −40 mV with passive leak current present, DHPG induced an inward current with an amplitude of 45.9 ± 12.9 pA (n = 9; Fig. 3, A and C). When leak current was subtracted (see METHODS), DHPG did not induce any significant inward current (n = 9; Fig. 3, B and C). These results together with those of experiments using voltage ramps indicate that the current blocked by activation of group I mGluRs is not carried by voltage-dependent channels, but rather corresponds to leak conductance.

To determine the ionic nature of the current underlying the leak conductance blocked by DHPG, extracellular solution with modified K⁺ and Na⁺ concentrations were used while the membrane potential of the neurons was held at −40 mV. The amplitude of the inward current induced by DHPG increased when the concentration of K⁺ and Na⁺ the extracellular solution was reduced. The amplitude of the inward current induced by DHPG was 28.9 ± 3.6 pA in control and increased significantly to 41.0 ± 5.4 pA (n = 10; P < 0.005; paired t-test; Fig. 3D) in a K⁺-free solution. Similarly, when the extracellular Na⁺ concentration was lowered to 50%, the amplitude of the inward current increased to 20.0 ± 3.8 versus 17.2 ± 3.1 pA in control (n = 5; P < 0.05; paired t-test; Fig. 3E). Conversely, when extracellular K⁺ concentration was increased threefold, the DHPG-induced inward current amplitude decreased to 26.6 ± 3.3 versus 31.8 ± 3.7 pA in control (n = 10; P < 0.05; paired t-test; Fig. 3F). These results indicate that the leak current blocked by DHPG is mediated by flux of both K⁺ and Na⁺ ions. Lowering the extracellular concentration of K⁺ (and also Na⁺) resulted in an increased leak current and thereby a large current blocked by DHPG. The opposite effect was seen in high K⁺ solution as the leak current was decreased, demonstrated as a decrease of the DHPG-induced current.

**mGluR1 is responsible for reducing the leak current**

The agonist DHPG acts on both group I mGluR subtypes, i.e., mGluR1 and mGluR5. Specific antagonists for mGluR1 and mGluR5 were therefore used to examine the receptor

![Fig. 3](http://jn.physiology.org/)

**FIG. 3.** Current blocked by DHPG corresponds to a leak conductance mediated by K⁺ and Na⁺. A: when leak currents were present, application of DHPG always induced an inward current. B: when leak currents were subtracted, a voltage step from −60 to −40 mV induced an outward voltage-dependent current. In these conditions, DHPG did not induce any inward current. The traces are averages of 5 sweeps. C: current amplitude induced by DHPG with leak current present and with leak current subtracted (n = 9). D: in K⁺-free solution, DHPG-induced inward current was larger than in control (n = 10; holding potential = −40 mV). E: DHPG-induced inward current was also increased in a low [Na⁺] solution compared with control (n = 5; holding potential = −40 mV). F: increasing the extracellular K⁺ concentration resulted in a decrease of the amplitude of the current induced by DHPG (n = 10; holding potential = −40 mV).
The effect of DHPG on the holding current was examined in control conditions and then in the presence of mGluR1 and mGluR5 antagonists (Fig. 4). In control, DHPG induced an inward current with the mean amplitude of 39.2 ± 5.0 pA (n = 8). This effect was counteracted by the mGluR1-specific antagonist LY 367385 (100 μM; 5–7 min), and the current induced by DHPG had an amplitude of only 2.1 ± 1.7 pA (Fig. 4, A and B). A partial recovery of the effect of DHPG was obtained after washout. In experiments in which a short application of LY 367385 was used (n = 6; 2–3 min), the amplitude of DHPG-induced current was decreased from 34.7 ± 10.9 to 15.7 ± 4.6 pA. After washout of LY 367385, the amplitude of the current recovered to 36.1 ± 8.7 pA. To test if mGluR5 was also involved, the specific antagonist MPEP (100 μM; approximately 5 min; Fig. 4, C and D) was used. Blockade of mGluR5 had no significant effect on the amplitude of the DHPG-induced inward current. The current induced by DHPG was 38.8 ± 5.7 pA in control and 38.5 ± 6.4 pA in the presence of MPEP (Fig. 4, C and D). These results thus indicate that activation of mGluR1 is responsible for blocking the leak current, resulting in a depolarization and firing of spinal neurons.

mGluR1-induced inhibition of the leak current requires PLC activation and Ca²⁺ release from internal stores

The intracellular signal transduction pathways involved in group I mGluR-mediated depolarization have so far not been described in detail in any model system. However, both PLC activation and Ca²⁺ release from internal stores have been shown to be involved in mGluR5-mediated [Ca²⁺], oscillations in lamprey spinal neurons (Kettunen et al. 2002). To investigate whether PLC is involved in the pathway leading to the reduction of the leak current, neurons were perfused with the PLC blocker U-73122 (1 μM) for approximately 3 min before application of DHPG. Blockage of PLC counteracted the DHPG-induced inward current from 30.1 ± 6.7 pA in control to 4.1 ± 1.5 pA in U-73122 (n = 5; P = 0.187; paired t-test; Fig. 5, A and B). A prolonged application (approximately 5–8 min) of U-73122 completely blocked the effect of DHPG.

Activation of PLC leads to production of IP3 that in turn activates release of Ca²⁺ from intracellular stores. Involvement of intracellular Ca²⁺ release in the DHPG-induced inhibition of the outward current was examined by using the fast Ca²⁺ chelator BAPTA (10 mM) in the patch pipette. In control cells, DHPG induced an inward current of 30.5 ± 6.3 pA (n = 9; Fig. 5, C and D). In cells from the same dissociation loaded with BAPTA, the DHPG-induced current had an amplitude of only 0.8 ± 0.5 pA (n = 8; Fig. 5, C and D). The holding current itself was not changed in BAPTA. DHPG did not induce any depolarization in BAPTA-treated cells held at −40 mV in current-clamp mode (n = 10, data not shown).

Thapsigargin, which blocks the re-uptake of Ca²⁺ into intracellular compartments, was used to investigate if the required intracellular Ca²⁺ indeed originated from these stores. In dishes preincubated with thapsigargin (1 μM for 45 min preincubation; Fig. 5, E and F), DHPG did not induce any inward current (n = 6), but in control cells from the same dissociation, the DHPG-induced current had an amplitude of 38.8 ± 12.6 pA (n = 4). These results clearly indicate an involvement of PLC and a release of intracellular Ca²⁺. This effect was counteracted by the PLC blocker U-73122 (1 μM). Blockage of PLC counteracted the DHPG-induced inward current from 30.1 ± 6.7 pA in control to 4.1 ± 1.5 pA in U-73122 (n = 5; P = 0.187; paired t-test; Fig. 5, A and B). A prolonged application (approximately 5–8 min) of U-73122 completely blocked the effect of DHPG.
lar Ca\(^{2+}\) in the modulation of the leak conductances by mGluR1 activation.

Ca\(^{2+}\) influx through L-type calcium channels have been shown to be involved in mGluR5-mediated [Ca\(^{2+}\)]\(_i\) oscillations (Kettunen et al. 2002). However, the L-type channel blocker nimodipine (10 \(\mu\)M) had no effect on the DHPG-mediated response, suggesting that an influx of extracellular Ca\(^{2+}\) through these channels is not involved in the signaling pathway (n = 4, data not shown).

**DISCUSSION**

In this study, we have shown that activation of group I mGluRs by the specific agonist DHPG depolarizes neurons dissociated from the lamprey spinal cord. The depolarization induced by DHPG was mediated by mGluR1 and not by mGluR5. When neurons were held at the resting membrane potential, no significant depolarization was induced. A strong depolarization was produced by DHPG only when the membrane potential was set at a more positive value, which led to firing of the neurons. Correspondingly, no change in the holding current was seen at the resting membrane potential, but an inward current was induced by DHPG when the neurons were held at a depolarized membrane potential. The depolarization of the membrane potential was associated with an increase in input resistance.

The conductance affected by DHPG may correspond to a leak current, contributing to the maintenance of the resting membrane potential. This is suggested by the fact that no change in the membrane potential or current was seen when DHPG was applied at rest, which corresponds to the equilibrium potential of this conductance. Interestingly, these findings differ from those previously described in other systems where the depolarization mediated by DHPG occurs in neurons when held at the resting potential and the conductance affected had an equilibrium potential more hyperpolarized than the resting potential (Awad et al. 2000; Guérineau et al. 1994; Mannaioni et al. 2001). The effect of mGluR1-activation on the leak conductance is dependent on release of Ca\(^{2+}\) from intracellular stores but does not require Ca\(^{2+}\) influx through L-type channels. It is not known if the intracellular Ca\(^{2+}\) acts directly to block the leak conductance or if it activates specific intracellular signaling pathways.

**FIG. 5.** mGluR1-induced inhibition of the leak current requires PLC activation and Ca\(^{2+}\) release from internal stores. A: DHPG-induced inward current was blocked with the PLC blocker U-73122 (1 \(\mu\)M). B: mean current amplitude in control and in the presence of U-73122 (n = 5). C: current induced by DHPG was eliminated when the intracellular free Ca\(^{2+}\) was chelated by BAPTA (10 mM). D: lack of an effect of DHPG on the current in the presence of intracellular BAPTA (n = 8). E: effect of DHPG could be blocked when the internal Ca\(^{2+}\) stores were depleted with thapsigargin (1 \(\mu\)M, 45-min preincubation). F: mean current amplitude induced by DHPG in control (n = 4) and in neurons preincubated with thapsigargin (n = 6).
Activation of group I mGluRs has been shown to depolarize the membrane potential in most cell types investigated. This increase in the excitability can be caused by either a decrease of a leak K⁺ conductance (Awad et al. 2000; Gue´rineau et al. 1994; Mannaioni et al. 2001), activation of a Na⁺/Ca²⁺ exchange (Hirono et al. 1998; Lee and Boden 1997), or an increase in a nonspecific cationic conductance (Chuang et al. 2000).

Depending on cell types, group I mGluRs can affect the excitability of neurons by modulating different types of conductances. This makes it inappropriate to generalize the effects found in some neurons to other regions of the CNS or to different species. Our results show that mGluR1 depolarizes spinal cord neurones by blocking a voltage-independent conductance responsible for maintaining the resting membrane potential.

The reversal potential of the current affected by DHPG was found to be around –60 mV, which is more depolarized than the equilibrium potential for K⁺ as predicted by the Nernst equation (around –90 mV). To further study the ionic nature of the leak current blocked by DHPG, extracellular solutions with modified K⁺ and Na⁺ concentrations were used. The results indicate that the leak current blocked by DHPG in lamprey spinal neurones is mediated by fluxes of both K⁺ and Na⁺. Interestingly, activation of an inward nonsel ective cationic current by DHPG has previously been described in the hippocampus (Congar et al. 1997; Cre´pel et al. 1994; Gu´erineau et al. 1995). However, in these neurones, the inward current was associated with an increased conductance, indicating opening of a channel. This is in contrast to our results, which show that the current induced by DHPG is associated with a decreased conductance due to the blockage of leak. In addition, the reversal potential of the current affected by DHPG in our study differs from that of the nonsel ective cationic currents described previously in the hippocampus.

In addition to characterizing the group I mGluR subtype underlying the increase in the excitability of spinal neurones, we also investigated the signalling pathways involved. Our results show that the block of the leak conductance by mGluR1 requires activation of PLC and release of Ca²⁺ from internal stores but does not require Ca²⁺ influx through L-type voltage-dependent Ca²⁺ channels. Like mGluR5, mGluR1 appears to release Ca²⁺ from internal stores. However, unlike mGluR5, mGluR1 does not mediate Ca²⁺ oscillations in lamprey spinal neurones (see Kettunen et al. 2002). This can be explained by the fact that mGluR1 are coupled less efficiently than mGluR5 to phosphatidylinositol (PPI) hydrolysis (Casabona et al. 1997) and may thus mediate a small release of Ca²⁺ from internal stores, which does not result in oscillations. The mechanisms by which Ca²⁺ contributes to the modulation of the leak conductance are not yet clear. Most downstream signalling effects due to group I mGluR activation are thought to be mediated via G-proteins and an activation of PLC, leading to production of IP₃ and a release of intracellular Ca²⁺ (Pin and Duvoisien 1995). In addition, there are reports of some cell types in which modulation of ion channels can be done independently of either G-protein activation or PLC coupling. In mouse cerebellar Purkinje cells, mGluR1-induced depolarization is mediated through G-proteins but is independent of PLC activation and release of Ca²⁺ from internal stores (Hirono et al. 1998). In rat CA3 hippocampus pyramidal cells, mGluR1 inhibits the afterhyperpolarization through a G-protein–mediated mechanism, while mGluR1 in the same cells also gives rise to slow excitatory postsynaptic potentials (EPSPs), through activation of Src-family protein tyrosine kinases and independently of G-protein activation (Heuss et al. 1999). In the rat midbrain, however, G-proteins and not Src-family protein tyrosine kinases are involved in mediating an mGluR1-induced inward current (Tozzi et al. 2001). In these neurons, mGluR1 also activates an inward Na⁺ current through mechanisms independent of intracellular Ca²⁺ release (Guatteo et al. 1999). There are also suggestions that, in some cell types, mGluR1 and mGluR5 can be coupled to different G-proteins, which also could contribute to the diversity of signalling between cell types (Abe et al. 1992). Interestingly, we found both similarities and differences in the mechanisms of action between mGluR1 and mGluR5 in isolated lamprey neurones. While both subtypes require PLC activation and Ca²⁺ release from internal stores, the effect of mGluR1 on leak channels is independent of Ca²⁺ influx through L-type channels, which is not the case with mGluR5-induced Ca²⁺ oscillations.

In this study, we present evidence that, in spinal cord neurons as in the hippocampus (see Mannaioni et al. 2001), mGluR1 and mGluR5 have different signalling mechanisms and physiological roles. In the lamprey spinal cord, the two subtypes of group I mGluRs are activated by endogenously released glutamate during fictive locomotion and contribute to setting the level of activity of the locomotor network (Kettunen et al. 2002; Krieger et al. 1998, 2000). This study, together with our previous reports, clearly indicates that mGluR1 and mGluR5 use distinct and separate intracellular pathways to mediate different cellular effects and thus modulate the excitability of spinal neurones involved in the generation of locomotor pattern. We have previously shown that mGluR1 interacts with NMDA receptors and potentiates their responses (Krieger et al. 2000), while mGluR5 induces Ca²⁺ oscillations by releasing Ca²⁺ from internal stores (Kettunen et al. 2002). Through activation of these two mGluR subtypes, glutamate not only acts as a fast transmitter, but also as an intrinsic modulator to mediate fine-tuning of the locomotor rhythm. mGluR1-mediated inhibition of a leak current contributing to setting the resting membrane potential provides an additional mechanism for modulating the excitability of locomotor network neurones. The fact that the change in the excitability was seen only when neurones were depolarized suggests that mGluR1 may play an important role only during the excitatory phase of the locomotor cycle. This results in a boosting of the depolarization and an early onset of ventral root burst. This effect of mGluR1 can be added to its potentiation of NMDA receptors, resulting in an increase in the locomotor frequency. Whether these two cellular effects of mGluR1 are mediated by the same or separate intracellular transduction pathways remains to be determined.

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DISCLOSURES

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