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Slow Synaptic Inhibition Mediated by Metabotropic Glutamate Receptor Activation of GIRK Channels

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Dutar, Patrick, Jeffrey J. Petrozzino, Huan M. Vu, Marc F. Schmidt, and David J. Perkel. Slow synaptic inhibition mediated by metabotropic glutamate receptor activation of GIRK channels. *J Neurophysiol* 84: 2284–2290, 2000. Glutamate is the predominant excitatory neurotransmitter in the vertebrate CNS. Ionotropic glutamate receptors mediate fast excitatory actions whereas metabotropic glutamate receptors (mGluRs) mediate a variety of slower effects. For example, mGluRs can mediate presynaptic inhibition, postsynaptic excitation, or, more rarely, postsynaptic inhibition. We previously described an unusually slow form of postsynaptic inhibition in one class of projection neuron in the song-control nucleus HVC of the songbird forebrain. These neurons, which participate in a circuit that is essential for vocal learning, exhibit an inhibitory postsynaptic potential (IPSP) that lasts several seconds. Only a portion of this slow IPSP is mediated by GABA_B receptors. Since these cells are strongly hyperpolarized by agonists of mGluRs, we used intracellular recording from brain slices to investigate the mechanism of this hyperpolarization and to determine whether mGluRs contribute to the slow synaptic inhibition. We report that mGluRs hyperpolarize these HVC neurons by activating G protein-coupled, inwardly-rectifying potassium (GIRK) channels. mGluR antagonists blocked this response and the slow synaptic inhibition. Thus, glutamate can combine with GABA to mediate slow synaptic inhibition by activating GIRK channels in the CNS.

INTRODUCTION

Glutamate mediates the vast majority of excitatory synaptic transmission in the vertebrate CNS through two classes of receptors. Postsynaptic actions of ionotropic glutamate receptors are always excitatory. Metabotropic glutamate receptors (mGluRs), on the other hand, can have diverse effects, including modulation of second messenger levels, ion-channel activity, and synaptic efficacy (Conn and Pin 1997; Nakanishi 1994; Pin and Duvoisin 1995). Effects of mGluRs on membrane potential are almost always depolarizing, either through inhibition of potassium conductances (Charpak and Gahwiler 1991; Charpak et al. 1990) or activation of nonselective cation conductance (Glaum and Miller 1992; Staub et al. 1992). In all but two of the cases in which mGluR activation induces hyperpolarization (Knoflach and Kemp 1998; Slaughter and Miller 1981), the response is mediated by Ca²⁺-activated K⁺ channels (Fagni et al. 1991; Fiorillo and Williams 1998; Holmes et al. 1996; Rainnie et al. 1994; Shirasaki et al. 1994).

In songbirds, the forebrain nucleus HVC (used here as the

proper name; Brenowitz et al. 1997) is essential for producing learned song and provides input to a circuit that is essential for learning (Bottjer and Arnold 1997; Brenowitz et al. 1997; Doupe and Kuhl 1999; Margoliash 1997; Nottebohm et al. 1976). HVC has two populations of projection neurons (Dutar et al. 1998; Katz and Gurney 1981). Those projecting to nucleus robustus archistriatalis (RA) form a primary motor pathway for song production (Nottebohm et al. 1976). Those HVC neurons projecting to a basal ganglia-like region called area X provide input to a circuit that is essential for song learning but not for song production (Bottjer et al. 1984; Scharff and Nottebohm 1991; Sohrabji et al. 1990). The area X-projecting HVC neurons have physiological and pharmacological properties distinct from those of the RA-projecting neurons (Dutar et al. 1998; Kubota and Taniguchi 1998). In particular, area X-projecting HVC neurons exhibit an unusually slow synaptic inhibition which is only partially mediated by GABA_B receptors (Dutar et al. 1998; Schmidt and Perkel 1998). These cells are robustly hyperpolarized by the mGluR agonist aminocyclopentane dicarboxylic acid (ACPD) (Dutar et al. 1998) or by the GABA_B receptor agonist baclofen (Dutar et al. 1998, 1999; Schmidt and Perkel 1998). This hyperpolarization resembles the GABA_B receptor-mediated slow inhibition in mammalian hippocampus (Dutar and Nicoll 1988), which involves activation of a G-protein-coupled inwardly rectifying class of potassium (GIRK) channels (Lüscher et al. 1997). mGluRs share substantial sequence homology with GABA_B receptors (Kaupmann et al. 1997) and, in a heterologous expression system, can activate GIRK channels (Saugstad et al. 1996; Sharon et al. 1997). However, native coupling of activation of GIRK channels by mGluR agonists has been observed only in a subtype of cerebellar interneuron (Knoflach and Kemp 1998).

To examine whether the coupling of mGluRs to GIRK channels occurs in area X-projecting HVC neurons and contributes to slow inhibition, we made intracellular recordings in brain slices. We report that mGluR agonists hyperpolarize HVC neurons by activating GIRK channels and that mGluR antagonists block slow synaptic inhibition. This action of mGluRs represents a novel synaptic signaling mechanism by which glutamate receptors can inhibit central neurons.

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METHODS

Preparation of slices and electrophysiological recording

Adult male zebra finches (*Taeniopygia guttata*) at least 120 days old were obtained from a local supplier or were bred in our colony. Slices were prepared as described by Schmidt and Perkel (1998) and the procedures were approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania. Briefly, a bird was anesthetized with halothane and decapitated. The brain was rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) that had been pre-gassed with 95% O₂-5% CO₂. The composition of the ACSF was (in mM) 119 NaCl, 2.5 KCl, 1 NaH₂PO₄, 26.2 NaHCO₃, 11 D-glucose, 2.5 CaCl₂, and 1.3 MgSO₄. Parasagittal slices (300–400 μm thick) were prepared with a vibrating microtome and stored submerged in bubbled ACSF. For recording, a slice was transferred to a chamber where it was submerged and superfused at 1–2 ml/min with pre-gassed ACSF.

Conventional intracellular recordings were obtained from neurons within HVC, whose borders could be seen in the unstained, living slice. We concentrated on those HVC neurons projecting to another song nucleus, area X, which are distinguished by a number of physiological and pharmacological properties (Dutar et al. 1998). The

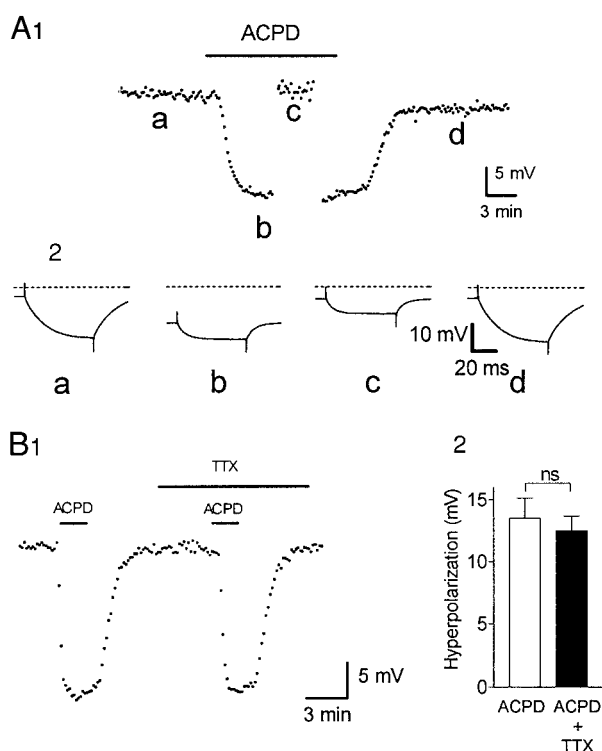


FIG. 1. Aminocyclopentane dicarboxylic acid (ACPD) causes a direct hyperpolarization in HVC neurons. *A*: hyperpolarizing effect of the metabotropic glutamate receptor (mGluR) agonist 1S,3R-ACPD. Bath application of ACPD (100 μM) induced a membrane hyperpolarization (*A1*). The hyperpolarization was associated with a decrease in input resistance, as measured by the voltage deflection caused by a hyperpolarizing current pulse (−0.1 nA) (*A2*). Traces represent responses to such current pulses at the times indicated (*a–d*). The input resistance was decreased during ACPD application (*A2b*), even when the membrane potential was depolarized back to the control value by injection of a steady depolarizing current (*A2c*). Resting membrane potential was −65 mV. *B*: ACPD-induced hyperpolarization in the presence of the Na⁺ channel blocker tetrodotoxin (TTX), demonstrating a direct action of ACPD on the cell recorded. *B1*: response to application of ACPD before and during the application of TTX. The hyperpolarization induced by ACPD in the two conditions was very similar. *B2*: summary data from 9 neurons showing that the ACPD response was −13.5 ± 1.6 mV in control conditions and −12.5 ± 1.2 mV in the presence of TTX.

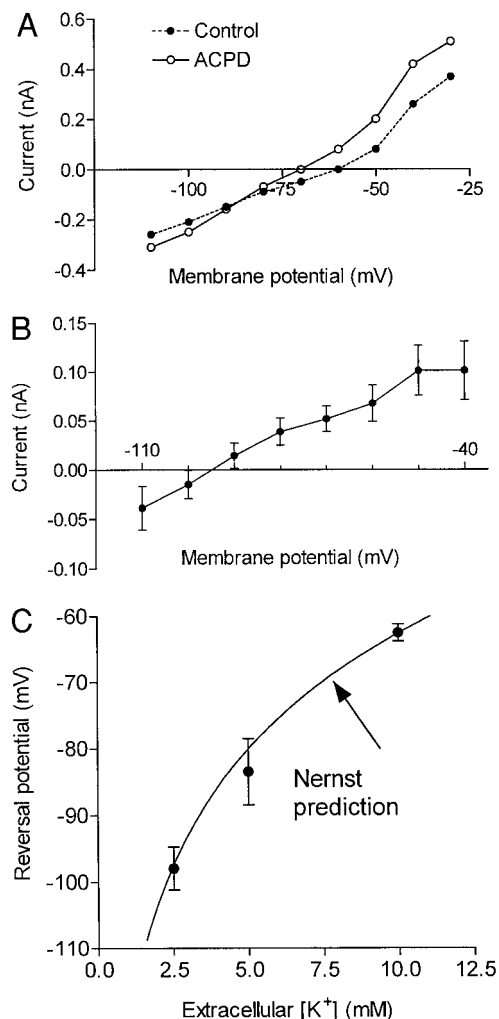


FIG. 2. ACPD increases inwardly rectifying conductance for K⁺ ions. *A*: current-voltage relation measured under current-clamp conditions in an HVC neuron in control conditions (filled circles) and in the presence of 100 μM ACPD (open circles). *B*: average ACPD-induced current ($n = 4$ cells) as a function of membrane potential. Inward rectification was observed with a shoulder potential near −50 mV. TTX (1 μM) was present to block action potentials. *C*: dependence of the reversal potential of the response to ACPD on the extracellular concentration of K⁺. The reversal potential (filled circles) was -97 ± 3.2 mV ($n = 9$) in control artificial cerebrospinal fluid (ACSF) containing 2.5 mM K⁺, -83.4 ± 4.9 mV ($n = 4$) in the presence of 5 mM K⁺, and -62.5 ± 1.3 mV ($n = 3$) in the presence of 10 mM K⁺. Continuous curve represents the Nernst potential for potassium, assuming an intracellular K⁺ concentration of 120 mM.

electrodes were filled with 4 M potassium acetate and 0–20 mM KCl and, when required, 1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (BAPTA), guanosine 5'-O-(3-thiotriphosphate) (GTP-γ-S), guanosine 5'-O-(2-thiodiphosphate) (GDP-β-S), or *N*-(2,6-dimethylphenylcarbonylmethyl)triethyl ammonium bromide (QX-314). Membrane potential was amplified using an Axoclamp 2B microelectrode amplifier (Axon Instruments, Foster City, CA). Signals were low-pass filtered at 1–5 kHz, digitized at twice the filter cutoff frequency, and stored on a computer hard disk. Acquisition and analysis were carried out using a program written in Labview (National Instruments, Austin, TX). To measure the current induced by the application of ACPD or baclofen, the membrane potential was manually clamped to the initial membrane potential. Average values are given as mean ± SE and the statistical test used was Student's *t*-test.

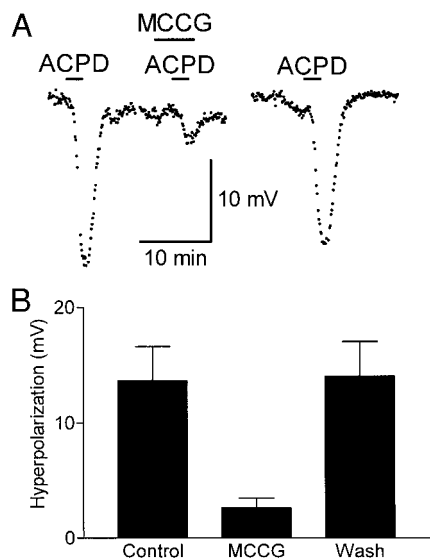


FIG. 3. Blockade of the ACPD-induced hyperpolarization by the group II mGluR antagonist (2S, 3S, 4S)-2-methyl-2-(carboxycyclopropyl)glycine (MCCG). *A*: membrane potential plotted against time in an Hvc neuron. Application of ACPD (100 μ M) at the times indicated by the horizontal bars caused hyperpolarization. In the presence of MCCG (500 μ M), the ACPD response was nearly blocked. Resting membrane potential was -61 mV. *B*: ACPD-induced hyperpolarization was 2.7 ± 0.9 mV in the presence of MCCG compared with 13.7 ± 2.9 mV in control conditions ($P = 0.016$; $n = 4$). The blockade by MCCG was reversible.

Drugs

Chemicals used in this study included baclofen (30 μ M), BAPTA (100–200 mM in the recording pipette), cesium chloride (10 mM), tetraethylammonium (TEA) (1 mM), TTX (1 μ M), bicuculline methiodide (BMI) (40 μ M), GTP- γ -S (20 mM in the pipette), and GDP- β -S (20 mM in the pipette), all obtained from Sigma Chemical Co., St. Louis, MO. ACPD (100 μ M), (2S, 3S, 4S)-2-methyl-2-(carboxycyclopropyl)glycine (MCCG) (500 μ M), and QX-314 (100 mM) were from Tocris Cookson, Ballwin, MO; CGP35348 (0.5 mM) was from Novartis, Basel, Switzerland; and LY307452 (100–200 μ M) was from Eli Lilly, Indianapolis, IN). All drugs were added to the superfusion medium by dilution of a stock solution made in water, except for BAPTA, GTP- γ -S, and QX-314, which were included in the recording electrolyte and delivered to neurons by diffusion and by passing current pulses.

RESULTS

Bath application of ACPD (100 μ M) caused a robust hyperpolarization (14.1 ± 0.6 mV, $n = 52$) (Fig. 1*A1*) in area X-projecting Hvc neurons, identified by their intrinsic properties (Dutar et al. 1998), accompanied by an increase in membrane conductance (Fig. 1*A2*). The response was not caused by an action potential-dependent release of inhibitory neurotransmitters by neighboring cells because the hyperpolarization was unaffected by the presence of the Na^+ channel blocker TTX (1 μ M, $n = 9$) (Fig. 1*B*). The current-voltage relation of the ACPD-induced response indicated that the conductance had a reversal potential near -90 mV (Fig. 2*A*) and was inwardly rectifying (Fig. 2*B*).

To investigate the ionic basis of the response, we measured the reversal potential of the hyperpolarization while the slice was bathed in different concentrations of K^+ . The reversal potential for the ACPD-induced response closely matched that

predicted by the Nernst equation, assuming an internal K^+ concentration of 120 mM. This match indicates that ACPD caused an increase in membrane conductance for K^+ ions. In addition, the nonspecific K^+ channel blocker Cs^+ (10 mM) reversibly reduced the ACPD response from -12.7 ± 3.6 mV to -2.5 ± 1.2 mV ($n = 4$, $P < 0.05$).

Use of selective agonists and antagonists indicated that group II or group III mGluR agonists could evoke this hyperpolarizing response, which is not caused by cross-reactivity with GABA_B receptors (Dutar et al. 1999). Application of an additional group II-selective mGluR antagonist, MCCG (Jane et al. 1994; Knopfel et al. 1995), blocked the ACPD response (Fig. 3).

To test whether mGluRs and GABA_B receptors might share effector pathways, we investigated whether the outward current evoked by ACPD and baclofen applied together was

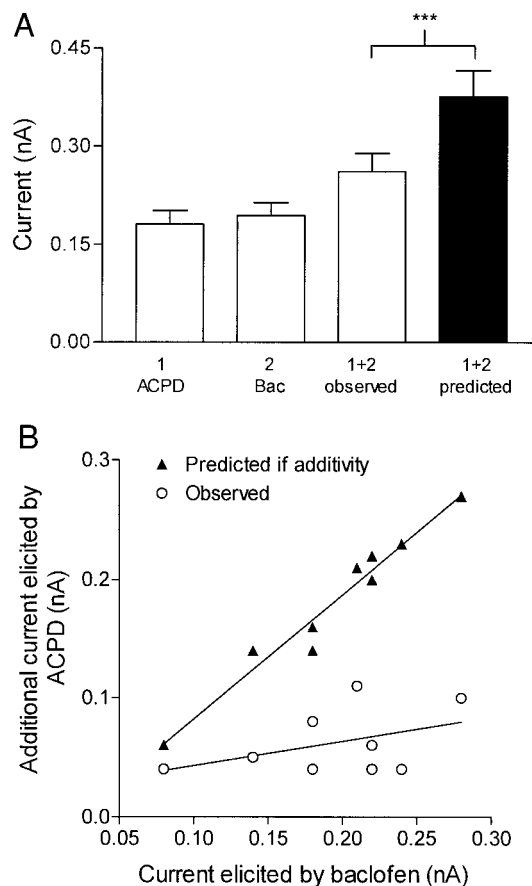


FIG. 4. The current elicited by baclofen occludes the current elicited by ACPD. *A*: the membrane potential of the neurons was manually clamped. The current necessary to maintain the membrane potential at the resting level was measured at the peak of the drug response. The currents applied during ACPD (1) (0.18 ± 0.02 nA; $n = 9$) and baclofen (2) (0.19 ± 0.02 nA; $n = 9$) were comparable. When ACPD was reapplied (1 + 2 observed) during the baclofen response, the resulting additional current was 0.26 ± 0.03 nA, substantially less than the algebraic sum of the currents evoked by each drug individually (0.37 ± 0.04 nA). The filled column (1 + 2 predicted) represents the predicted total current, assuming the currents were additive. $***, P < 0.0005$. *B*: currents measured in 9 individual neurons. The current induced by baclofen alone is plotted on the abscissa. The additional current induced by ACPD in the presence of baclofen is plotted on the ordinate (open circles). Also plotted is the expected additional current elicited by ACPD (filled triangles), assuming linear addition of the currents, i.e., if ACPD and baclofen acted entirely independently.

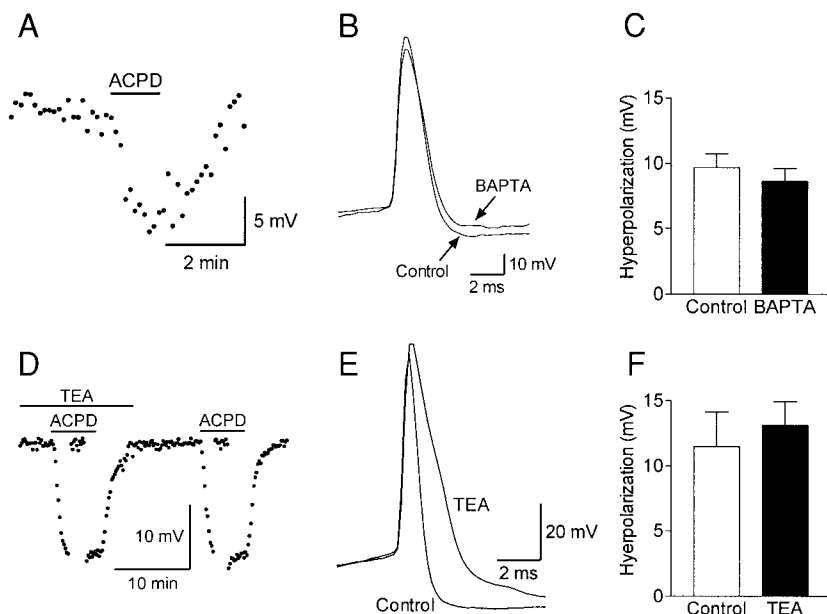


FIG. 5. The ACPD response is not mediated by increased intracellular calcium or Ca^{2+} -activated K^+ channels. *A*: in cells recorded with the calcium chelator 1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (BAPTA) (100–200 mM) included in the recording electrode, ACPD caused normal hyperpolarization. *B*: as a test for entry of the buffer into the cell, we monitored the duration of the action potential, which was increased in the presence of BAPTA. *C*: the mean amplitude of the ACPD-induced hyperpolarization in control cells (-9.6 ± 1.0 mV, $n = 15$) was not significantly different from that in the population of neurons recorded with electrodes filled with 100–200 mM BAPTA (-8.6 ± 1.0 mV; $n = 16$; $P > 0.05$). The control cells were recorded interleaved with those filled with BAPTA. *D*: ACPD caused a similar hyperpolarization in the presence and absence of extracellular tetraethylammonium (TEA). During application of ACPD, the cell was depolarized to the original membrane potential for several sweeps to measure the input resistance, accounting for the discontinuous appearance of the responses to ACPD. *E*: the presence of TEA was confirmed by its action on the duration of the action potential. ACPD was applied only after action potentials were broadened by TEA. *F*: on average, the ACPD-induced hyperpolarization was 13.1 ± 2.6 mV in the presence of TEA, compared with 12.0 ± 1.8 mV in the same cells after washing out TEA ($n = 4$). The difference was not statistically significant ($P > 0.05$).

different from the sum of the currents evoked by the individual agonists applied separately. In nine cells, ACPD and baclofen induced similar currents. Coapplication of ACPD and baclofen to the same cells induced a current that was significantly less than the current predicted assuming linear addition ($P < 0.0005$) (Fig. 4). Thus, the currents partially occluded one another, which is consistent with convergence of the two receptors onto the same G protein or channel population.

MGLuRs can activate potassium conductance by raising in-

tracellular Ca^{2+} (Rainnie et al. 1994). We tested for this mechanism in HVC neurons by including the Ca^{2+} chelator BAPTA (100–200 mM) in the pipette solution. In the presence and absence of intracellular BAPTA, ACPD caused a compa-

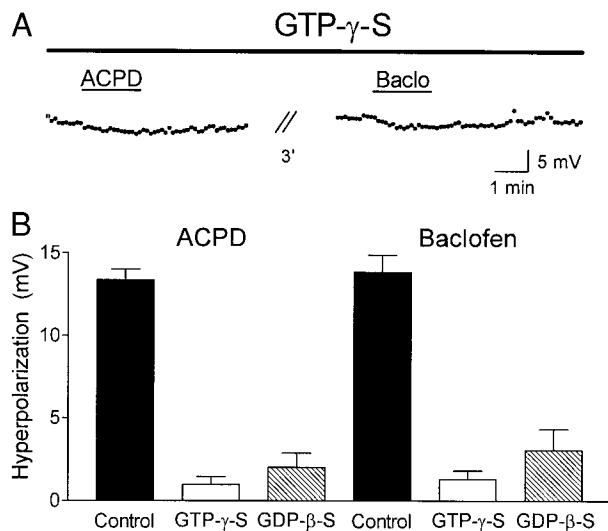


FIG. 6. The ACPD response is G-protein dependent. *A*: example responses to bath application of ACPD and baclofen in a neuron recorded with an electrode filled with the nonhydrolyzable GTP analogue guanosine 5'-O-(3-thiotriphosphate) (GTP- γ -S). In these conditions, the response to ACPD, as well as the response to baclofen, was greatly reduced. The membrane potential was returned to the initial resting potential prior to application of baclofen. *B*: on average, ACPD-induced hyperpolarization was 13.2 ± 0.9 mV in control neurons ($n = 7$), 1.0 ± 0.5 mV in cells recorded with electrodes containing GTP- γ -S ($n = 4$), and 2.0 ± 0.9 mV in cells recorded with electrodes containing guanosine 5'-O-(2-thiodiphosphate) (GDP- β -S) ($n = 3$). Baclofen-induced hyperpolarization was 13.0 ± 0.7 mV in control neurons ($n = 7$), 1.3 ± 0.5 mV in cells recorded with electrodes containing GTP- γ -S ($n = 4$), and 3.1 ± 1.3 mV in cells recorded with electrodes containing GDP- β -S ($n = 3$). $P < 0.0001$.

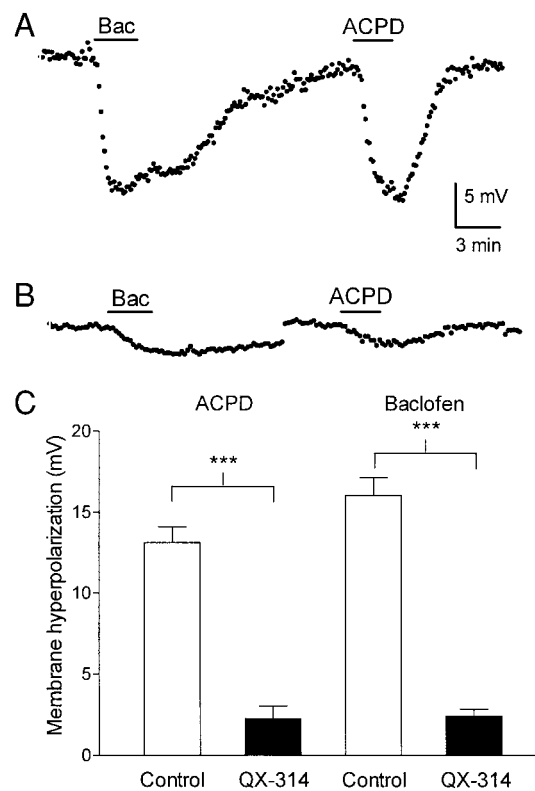


FIG. 7. The ACPD response is blocked by *N*-(2,6-dimethylphenylcarbamoyl-methyl)triethyl ammonium bromide (QX-314). *A*: example of a response to baclofen (60 μM) and ACPD (100 μM) in a control neuron. *B*: in a neuron loaded with the inwardly rectifying potassium (GIRK) channel blocker QX-314, the responses to baclofen or ACPD were strongly reduced. Same scale bars as in *A*. *C*: mean hyperpolarization amplitude induced by ACPD (13.1 ± 0.9 mV; $n = 16$) and baclofen (16.0 ± 1.1 mV; $n = 13$) in control neurons (open bars) and in neurons recorded with electrodes filled with 100 mM QX-314 (response to ACPD, 2.3 ± 0.1 mV; $n = 5$; response to baclofen, 2.4 ± 0.4 mV; $n = 6$; filled bars). $***, P < 0.0001$.

rable hyperpolarization (8.9 ± 1.0 mV, $n = 15$ with BAPTA; 9.7 ± 1.1 mV, $n = 15$ control; $P = 0.6$) (Fig. 5, A–C). We verified BAPTA entry into cells by observing broadening of the action potential, the duration of which is governed by Ca^{2+} -dependent K^+ channels (Fig. 5B) (Lancaster and Nicoll 1987).

As a second test for a role of internal Ca^{2+} , we bath applied TEA, which blocks one class of Ca^{2+} -dependent K^+ channels and broadens the action potential in hippocampal neurons (Lancaster and Nicoll 1987). TEA (1 mM), despite dramatically prolonging action potential duration (Fig. 5E), had no effect on the hyperpolarization induced by ACPD (11.5 ± 2.6 mV control; 13.1 ± 1.8 mV in the presence of TEA; $n = 4$; $P > 0.05$) (Fig. 5, D and F). These results indicate that ACPD hyperpolarizes HVC neurons via a mechanism independent of Ca^{2+} -activated K^+ channels.

We hypothesized, based on the results in the preceding paragraphs and on the high degree of sequence similarity between GABA_B receptors and mGluRs (Kaupmann et al. 1997), that ACPD activates GIRK channels. We first investigated the possible role of G proteins by recording HVC neurons with electrodes containing the hydrolysis-resistant analogues GTP- γ -S and GDP- β -S, which irreversibly activate and block G protein-coupled responses, respectively (Andrade et al. 1986). In the presence of GTP- γ -S or GDP- β -S, the response to either ACPD or baclofen was almost completely blocked (Fig. 6). These data indicate that the effect of ACPD is mediated by a G protein-dependent mechanism.

Next, we recorded HVC neurons with electrodes containing the lidocaine derivative QX-314, which blocks GIRK channels (Alreja and Aghajanian 1994; Andrade 1991; Nathan et al. 1990) as well as Na^+ channels (Strichartz 1973). In neurons filled with QX-314, the responses to application of ACPD or baclofen were reduced by 82% ($n = 5$) and 85% ($n = 6$), respectively, compared with those in control cells (Fig. 7,

A–C). We concluded that ACPD hyperpolarizes HVC neurons by activating GIRK channels.

Finally, we asked whether synaptically released neurotransmitters can activate this mechanism. Brief tetanic stimulation of afferents to HVC neurons causes a slow inhibitory postsynaptic potential (sIPSP) that is partially mediated by GABA_B receptors (Schmidt and Perkel 1998). We isolated the GABA_B receptor-independent component of the sIPSP by recording in the presence of a cocktail of antagonists to ionotropic glutamate receptors and GABA_A and GABA_B receptors. The group II mGluR antagonist MCGG reduced the peak sIPSP amplitude by $60 \pm 11\%$ ($n = 6$; $P < 0.005$) (Fig. 8). In four cases tested, the sIPSP recovered, following washout of MCGG, to $71 \pm 13\%$ of control values. In addition, the group II mGluR antagonist LY307452 (Wermuth et al. 1996) reduced the sIPSP measured in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 2-amino-5-phosphonovaleric acid (APV), and BMI (but not CGP35348) by $25 \pm 6\%$ ($n = 8$). Thus, group II mGluRs contribute to the generation of the sIPSP.

DISCUSSION

The main results of the present study are that in X-projecting cells of nucleus HVC of the adult male zebra finch, group II/III mGluR-induced hyperpolarization (Dutar et al. 1999) is mediated by activation of GIRK channels, and that this process has a physiological role in contributing to the slow inhibition observed after brief tetanic stimulation of afferents. Our conclusion that mGluRs activate GIRK channels in HVC neurons is based on the following findings. ACPD, an agonist at mGluRs, activates an inwardly rectifying K^+ conductance. The response requires activation of G proteins and is independent of intracellular Ca^{2+} . Occlusion of the ACPD response with that of baclofen, which is known to involve GIRK channels (Lüscher et al. 1997), indicates a shared mechanism. Responses to ACPD and to baclofen are both blocked by intracellular QX-314, which blocks GIRK channels (Alreja and Aghajanian 1994; Andrade 1991; Nathan et al. 1990). Since the GABA_B receptor-independent component of the sIPSP is sensitive to two different group II mGluR antagonists, this mechanism contributes to slow inhibition.

Presynaptic inhibitory actions of mGluR agonists have been extensively described in the hippocampus, spinal cord, amygdala, striatum, neocortex, and cerebellum (Pin and Duvoisin 1995; Stefani et al. 1996). In these structures, mGluR agonists depress the release of neurotransmitters by inhibiting ω -conotoxin-sensitive or dihydropyridine-sensitive calcium channels. Therefore, mGluRs serve as autoreceptors at glutamatergic synapses or heteroreceptors at GABAergic synapses and contribute to the regulation of synaptic transmission (Conn and Pin 1997).

Postsynaptic actions of mGluRs are mainly excitatory in the CNS. For instance, in hippocampal pyramidal neurons, mGluR activation leads to neuron depolarization associated with an increase in input resistance and a decrease in the accommodation of action potential discharge (Davies et al. 1995). Postsynaptic excitatory effects of mGluR activation also occur in other central structures, many of which are mediated by group I mGluRs, and are caused by the inhibition of various potassium currents, such as I_M , I_{AHP} , and I_{Leak} (Pin and Duvoisin 1995; Schoepp and Conn 1993). However, mGluR-induced depolar-

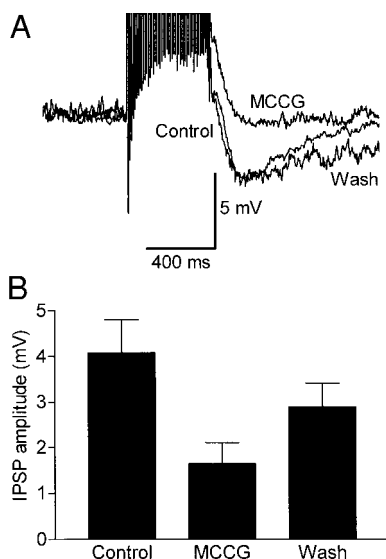


FIG. 8. Synaptic activation of mGluRs contributes to a slow inhibitory postsynaptic potential (sIPSP). A: control sIPSP recorded in the presence of 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 50 μM 2-amino-5-phosphonovaleric acid (APV), bicuculline methiodide (BMI), and CGP 35348. The sIPSP was reversibly blocked by application of the group II mGluR antagonist MCGG. B: summary data showing that MCGG blocked the sIPSP by 60% on average ($n = 6$).

ization can also occur through a variety of other mechanisms, including increased cation conductance or electrogenic Na/Ca exchange current (Conn and Pin 1997).

Activation of mGluRs by exogenous agonists induces postsynaptic inhibitory actions in some cases (Fagni et al. 1991; Fiorillo and Williams 1998; Hirano and MacLeish 1991; Holmes et al. 1996; Knoflach and Kemp 1998; Rainnie et al. 1994; Shirasaki et al. 1994; Vranesic et al. 1993). When the mechanism is investigated, these effects almost always involve Ca^{2+} mobilization and the opening of Ca^{2+} -activated K^+ channels (Fiorillo and Williams 1998; Holmes et al. 1996; Rainnie et al. 1994) and may be mediated by group I or group II mGluRs. Application of exogenous group II mGluR agonists to cerebellar interneurons activates GIRK channels (Knoflach and Kemp 1998), but it is not known whether synaptic activity can evoke this process. Thus, our finding that the group II/III response observed here is unaffected by manipulations that prevent activation of Ca^{2+} -activated K^+ channels makes this mechanism in HVC neurons relatively unusual.

A variety of inhibitory neurotransmitter receptors converge to activate GIRK channels (Nicoll 1988; North 1989), probably via direct activation by G proteins (Clapham 1994). One of the prototypical receptors known to activate GIRK channels is the GABA_B receptor, the postsynaptic inhibitory action of which is not observed in knock-out mice lacking the GIRK2 protein (Lüscher et al. 1997). Our results confirm the coupling of GABA_B receptors to GIRKs in HVC neurons. In addition, we found that QX-314, which blocks GIRK channel activation, blocks the action of mGluRs, thus adding this receptor type to the family of receptors that activate GIRKs.

GABA_B receptors and mGluRs share a high degree of sequence homology (Kaupmann et al. 1997). In *Xenopus* oocytes injected with mRNA coding for mGluRs and GIRKs, receptor activation causes increased potassium conductance (Saugstad et al. 1996; Sharon et al. 1997), indicating that such coupling is feasible in an artificial system. But to date, synaptic coupling of these widely expressed receptors and channels has not been observed.

In some mammalian neurons, such as those of the hippocampus, GABA_B receptors activate GIRK channels to mediate an sIPSP lasting a few hundred milliseconds (Dutar and Nicoll 1988; Nicoll 1988). Inhibitory processes of similar or longer duration may help shape song-selective auditory responses in songbird nucleus HVC (Lewicki 1996; Margoliash 1997). Moreover, inhibitory processes may alter or suppress auditory responses during singing or for several seconds afterward (Lewicki 1996; Margoliash 1986; McCasland and Konishi 1981). Our results suggest that mGluRs, which activate GIRK channels, contribute to such slow inhibition in HVC.

More generally, our findings add a new mechanism by which glutamate receptors can play an inhibitory role in the CNS. An sIPSP mediated by mGluR activation was observed in dopamine neurons of the rat ventral tegmental area (Fiorillo and Williams 1998). As in previously described examples of mGluR-induced hyperpolarization in mammals, this effect is mediated by the release of Ca^{2+} from internal stores and the activation of Ca^{2+} -activated K^+ channels. Thus, the mGluR action on HVC neurons, not requiring a rise in internal Ca^{2+} and depending on GIRK channel activation, is quite distinct. It will be interesting to determine whether this mode of synaptic inhibition is specific to avian systems or whether it has been

overlooked or masked in mammals by the wide variety of additional excitatory actions of mGluRs.

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