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Depolarization induced slow current in cerebellar Purkinje cells does not require mGluR1

Jung Hoon Shin^{1,4}, Yu Shin Kim^{1,4}, Paul F. Worley^{1,2}, and David J. Linden^{1,3}

¹ Department of Neuroscience, The Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205 USA

² Department of Neurology, The Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205 USA

Abstract

Activation of cerebellar Purkinje cells by either brief depolarizing steps or bursts of climbing fiber synaptic activation evokes a slow inward current, which we have previously called depolarization-induced slow current or DISC. DISC is triggered by Ca influx via voltage-sensitive Ca channels and is attenuated by inhibitors of vacuolar ATPase or vesicle fusion. This led us to suggest that DISC required vesicular release of glutamate from the somatodendritic region of Purkinje cells. Furthermore, we found that DISC was attenuated by the mGluR1 antagonist CPCCOEt, indicating that DISC required autocrine activation of mGluR1. Here, we have revisited the role of mGluR1 and found that it is, in fact, not required for DISC. CPCCOEt, but not three other specific mGluR1 antagonists (JNJ16259685, 3-MATIDA, Bay 36-7620), attenuated DISC, even though all four of these drugs produced near-complete blockade of current evoked by puffs of the exogenous mGluR1/5 agonist DHPG. Cerebellar slices derived from mGluR1 null mice showed substantial DISC that was still attenuated by CPCCOEt. mGluR5 is functionally similar to mGluR1, but is not expressed at high levels in cerebellar Purkinje cells. MPEP, an mGluR5 antagonist, did not attenuate DISC, and DISC was still present in Purkinje cells derived from mGluR1/mGluR5 double null mice. Thus, neither mGluR1 nor mGluR5 are required for DISC in cerebellar Purkinje cells.

Keywords

CPCCOEt; DHPG; autocrine; mGluR5

Purkinje cells are an important element of cerebellar circuitry. They constitute the sole output of the cerebellar cortex and signal to their target structures such as the deep cerebellar and vestibular nuclei through the release of GABA from their presynaptic terminals. In recent years, several different lines of evidence have suggested that, in addition to axonal release of GABA, Purkinje cells can release glutamate from their soma or dendrites following strong depolarization.

³corresponding author: E-mail: dlinden@jhmi.edu, +1-410-614-1529 (voice), +1-410-614-7334 (fax).

⁴These authors contributed equally.

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Duguid and Smart (2004) reported that brief, strong depolarization of Purkinje cells produced an increase in the frequency of mIPSCs which lasted from 10–20 min, a phenomenon they called depolarization-induced potentiation of inhibition (DPI). DPI was blocked by postsynaptic application of a fast Ca chelator or compounds that interfered with fusion of vesicles such as GDP β S, N-ethylmaleimide and Botulinum toxin B (Duguid et al., 2007). Furthermore, DPI was blocked by bath application of an NMDA receptor antagonist, leading Duguid and coworkers to suggest that depolarization of Purkinje cells triggered somatodendritic glutamate release which then diffused in a retrograde fashion to activate NMDA receptors on interneuron terminals, thereby triggering DPI.

Depolarization-induced glutamate release from Purkinje cells was also suggested by experiments monitoring parallel fiber EPSCs. Using P18–20 rats, an endocannabinoid independent depolarization-induced brief suppression of excitatory parallel fiber PF EPSCs was observed (Crepel, 2007). This phenomenon was potentiated by bath application of a glutamate transporter blocker, TBOA. Furthermore it was reduced in the presence of a kainate receptor inhibitor, SYM 2081, and was absent in cerebellar slices derived from Glu6 null mice, suggesting that glutamate release from Purkinje cells can ligate parallel fiber kainate receptors to produce transient presynaptic suppression of EPSCs.

Complementary evidence for depolarization-evoked glutamate release from Purkinje cells also came from our own group (Shin et al., 2008). We reported that brief, strong depolarization or brief bursts of excitatory climbing fiber activation produced a slow inward current (DISC). DISC was completely abolished by blockers of voltage-sensitive Ca channels and was attenuated by postsynaptic application of bafilomycin A (an inhibitor of vacuolar ATPase) or Botulinum toxin D (an inhibitor of SNARE-dependent vesicular fusion). DISC was attenuated by the mGluR1 antagonist CPCCOEt but not by the NMDA receptor antagonists D-AP5 or (R)-CPP. Apart from the blockers of voltage-sensitive Ca channels, the drugs that attenuated DISC did not significantly reduce depolarization-evoked somatodendritic Ca transients. These findings led us to propose a model in which strong Purkinje cell depolarization evokes Ca influx and this Ca triggers the fusion of glutamate-containing vesicles in the somatodendritic region. The released glutamate could then activate mGluR1 on the same Purkinje cell in an autocrine fashion to produce DISC or diffuse in a retrograde manner to ligate interneuronal NMDA receptors thereby triggering DPI.

Here, we have further explored the role of mGluR1 in DISC through the use of additional antagonist drugs and mGluR1 and mGluR5 null mice. To our surprise, we found that DISC is largely unaffected by these manipulations indicating that, in fact, mGluR1 is not required for DISC.

Experimental procedures

Slice preparation and electrophysiology

250 μ m-thick sagittal slices of the cerebellar vermis were prepared from postnatal day 13–18 C57Bl6 mice or postnatal day 15–19 rats using a Vibratome 3000 (Leica Biosystems St. Louis LLC, St. Louis, MO). The cerebellum was bathed in ice-cold cutting solution containing (in mM) 135 *N*-methyl-D-glucamine, 1 KCl, 1.5 MgCl₂, 0.5 CaCl₂, 1.2 KH₂PO₄, 24.2 choline bicarbonate, and 13 glucose bubbled with 95% O₂/5% CO₂ (pH 7.4). Slices were recovered for 30 minutes in a chamber with standard external saline containing (in mM) 124 NaCl, 2.5 KCl, 1.3 MgCl₂, 2.5 CaCl₂, 1 NaH₂PO₄, 26.2 NaHCO₃ and 20 glucose bubbled with 95% O₂/5% CO₂ (pH 7.4) at room temperature. They were then placed in a submerged chamber that was perfused at 2 ml/min with external saline at room temperature. 5 μ M SR 95531 hydrobromide (gabazine) was added to the external saline to block GABA_A receptors during recording. A visualized whole-cell patch-clamp recording was performed with a Zeiss

Axioskop equipped with gradient contrast infrared optics and an Axopatch 200A amplifier (Molecular Devices, Union City, CA). Recording electrodes, with a resistance between 1.5 and 2 M Ω , were filled with a solution containing (in mM) 135 Cs-methanesulfonate, 6 CsCl, 2 MgCl₂, 0.15 CaCl₂, 10 HEPES, 0.2 EGTA, 4 Na-ATP, and 0.4 Na-GTP (pH 7.2 – 7.3). Cells were voltage-clamped at –70 mV. (*S*)-3,5-DHPG (100 μ M) together with glutamate (10 μ M) was pressure-injected (10 psi, 50 msec) using a Picospritzer III (Parker Hannifin Corporation, Fairfield, NJ) in the outer one-third of the molecular layer to evoke an mGluR1-mediated slow current (I_{DHPG}).

Analysis

The DISC charge transfer was measured from a 1 sec-long segment centered upon the DISC peak. Traces were subjected to a digital high pass filter at 10 Hz to extract noise information. The noise standard deviation (SD) was calculated from a 1 sec-long segment centered at the DISC peak of the digitally high-pass filtered traces. Δ noise SD was calculated by subtracting the noise SD in a 1 sec long sample prior to depolarization (baseline noise SD) from that during DISC sampling period and normalized by baseline noise SD. For I_{DHPG} , charge transfer was measured from the entire inward current.

Materials

SR95531 hydrobromide, CPCCOEt, (*S*)-3,5-DHPG, and MPEP were purchased from Ascent Scientific (Princeton, NJ) and JNJ16259685, 3-MATIDA, and Bay 36-7620 from Tocris (Ellisville, MO). All other chemicals were purchased from Sigma (St. Louis, MO). mGluR1 null mice were obtained from Jackson Laboratories (Bar Harbor, ME) and mGluR5 null mice were a gift from the lab of Frank Margolis. mGluR1 null mice were created by breeding mGluR1 heterozygotes since mGluR1 homozygotes do not live to breeding age. mGluR1/mGluR5 double null mice were created by breeding mGluR5 homozygous nulls to mGluR1 heterozygotes to generate double heterozygotes. Double heterozygotes were mated to mGluR5 homozygous nulls to create breeding stock of mGluR5 homozygous null/mGluR1 heterozygotes. These animals generate double null mice at the predicted Mendelian ratio.

Results

Whole-cell voltage clamp recordings were made from the somata of Purkinje cells in sagittal cerebellar slices derived from P13–18 mice. Initially, we sought to confirm the activity and define the time course of action of a panel of mGluR1 antagonist drugs in our preparation. A holding potential of –70 mV was imposed and Purkinje cells were activated by a puff of solution containing the mGluR1/5 agonist DHPG (100 μ M) and glutamate (10 μ M). This evoked a slow inward current (mean time to peak = 3.95 ± 1.30 sec; $n=20$) that has been previously shown to require activation of mGluR1 in Purkinje cells (Takechi et al., 1998; Tempia et al., 1998; Kim et al., 2003). The panel of mGluR1 antagonist drugs consisted of CPCCOEt (100 μ M), a non-competitive antagonist, JNJ16259685 (0.1 μ M), another non-competitive antagonist, 3-MATIDA (150 μ M), a competitive antagonist, and Bay 36-7620 (10 μ M), a non-competitive antagonist. Recent reports have indicated that both mGluR1 and mGluR5 have a form of agonist independent activity (Joly et al., 1995; Prezeau et al., 1996). Bay 36-7620, but not CPCCOEt, blocks the agonist-independent activity of mGluR1 (Ango et al., 2001; Carroll et al., 2001). To our knowledge, the effects of 3-MATIDA and JNJ16259685 on agonist-independent activity of mGluR1 have yet to be reported.

DHPG/glutamate puffs were repeated at 1 min intervals and stable baseline responses were measured (Figure 1). After 5 min of baseline recording, the perfusion line was switched to introduce external saline supplemented with various mGluR1 antagonists. While there was some small variation in the time course of mGluR1-mediated slow current blockade, all of

these drugs, at the concentrations indicated, produced a near-complete blockade within 10 min of application ($-0.09 \pm 0.64\%$ of baseline at 10 min of application of CPCCOEt; $7.4 \pm 6.8\%$, JNJ16259685; $0.1 \pm 1.8\%$, 3-MATIDA; $5.3 \pm 2.2\%$, 36-7620; $n=5$ for all groups).

We then applied these validated mGluR1 antagonist drugs to determine their action upon DISC (Figure 2). DISC was evoked by a train of five 10 msec-long depolarizing command pulses from -70 to 0 mV, delivered at 10 Hz. As previously described in rat Purkinje cells, this protocol evoked a biphasic inward current in which the fast component is mediated by Cl^- efflux and the slow component by cation influx (Shin et al., 2008). The mean time to peak of the slow component, DISC, was 2.26 ± 0.55 sec ($n=20$). Also as previously described, DISC was associated with a transient increase in current noise that is illustrated by the high pass filtered exemplar traces. The main finding is that while CPCCOEt produced a slow attenuation of DISC, the other three mGluR1 antagonists did not. We quantified this effect with mGluR1 antagonists applied for 20 min, well beyond that at which blockade of DHPG-evoked mGluR1-mediated current was complete. At this time point the mean DISC charge transfer, with values normalized to a baseline of 0–5 min, was $49.3 \pm 7.2\%$ for CPCCOEt ($n=8$), $108.4 \pm 9.0\%$ for JNJ16259685 ($n=5$), $96.4 \pm 8.5\%$ for 3-MATIDA ($n=5$), and $84.8 \pm 1.6\%$ for Bay 36-7620 ($n=5$). These findings indicate that the attenuation of DISC by CPCCOEt is unlikely to be related to mGluR1 antagonism.

Another way to test the hypothesis that mGluR1 is required for DISC is to use mGluR1 null mice. Recordings were made from Purkinje cells in slices derived from mGluR1 null mice at P16–20 (Figure 3). The main finding is that DISC was present in these Purkinje cells. Moreover, the DISC amplitude in an age-matched population of mGluR1 null Purkinje cells and C57Bl6 wild type Purkinje cells was similar (1254.1 ± 454.1 pA for C57Bl6, postnatal day 17.0 ± 0.8 , $n=20$; 1318.7 ± 553.2 pA for mGluR1 null, postnatal day 18.5 ± 2.0 , $n=16$). In order to further address the concern that CPCCOEt attenuated DISC through actions unrelated to mGluR1 antagonism, CPCCOEt was applied to mGluR1 null Purkinje cells and DISC was recorded. This revealed that CPCCOEt strongly attenuated DISC even in the absence of mGluR1 ($28.9 \pm 4.9\%$ of baseline at $t = 20$ min after drug application, $n=5$). In fact, this attenuation was somewhat stronger than that recorded in wild type Purkinje cells. Taken together, the results with multiple mGluR1 antagonist drugs (Figure 2) and mGluR1 null mice (Figure 3) indicate that mGluR1 activation is not required for DISC.

mGluR1 and mGluR5 are structurally and functionally similar: both exert their effects, at least in part, through activation of a G-protein/phospholipase C cascade. While mGluR1 is strongly expressed in Purkinje cells, mGluR5 is not (Grandes et al., 1994; Negyessy et al., 1997). Nonetheless, we have sought to address the concern that trace amounts of mGluR5 may play a role in DISC. To do this, we have made use of MPEP, a non-competitive mGluR5 antagonist that also blocks agonist-independent activity of mGluR5 (Pagano et al., 2000). Bath application of MPEP ($10 \mu\text{M}$) failed to significantly attenuate DISC ($89.6 \pm 8.3\%$ of baseline, $n=5$).

One formal possibility is that mGluR5, while not strongly expressed in wild type Purkinje cells, is upregulated in mGluR1 null Purkinje cells. To address this, we have recorded DISC in Purkinje cells derived from mGluR1/mGluR5 double null mice. DISC is present in these cells. The amplitude of DISC current is smaller than that recorded in approximately age-matched C57Bl6 control cells (mGluR1/5 double null: 674.4 ± 210.3 pA, postnatal day 18.0 ± 0.0 , $n=10$; C57Bl6 wild type: 1254.1 ± 454.1 pA, postnatal day 17.0 ± 0.8 , $n=20$). However, it should be noted that mGluR1/mGluR5 double null mice are severely compromised. They are ataxic, have lower body weight at P18 and are generally weak. Thus, the reduced amplitude of DISC in Purkinje cells from these mice is likely to reflect an overall developmental disturbance or delay rather than a specific action on DISC mechanisms.

Discussion

The main finding here is that among four different validated mGluR1 antagonists, only CPCCOEt blocked DISC. Furthermore, DISC was intact in Purkinje cells derived from mGluR1 null mice and CPCCOEt still attenuated DISC in these cells. The mGluR5 antagonist MPEP had no effect on DISC and DISC was still present in mGluR1/mGluR5 double null Purkinje cells. Taken together, these results indicate that neither mGluR1 nor mGluR5 are required for DISC and that the attenuation of DISC by CPCCOEt is through some side effect unrelated to mGluR1 antagonism.

Previous work has shown electrophysiological side effects of CPCCOEt. Fukunaga et al. (2007) showed that CPCCOEt (100 μ M) produced a transient potentiation of climbing fiber evoked complex spike responses in rat Purkinje cells. In particular, recruitment of an additional late spikelet was often observed. This effect was not mimicked or occluded by other mGluR1 antagonists. While the target of CPCCOEt that produced this effect was not determined, it appeared to be a postsynaptic voltage-sensitive Ca-independent conductance other than the hyper-polarization-activated cation current, I_h . It is not clear if the side-effect of CPCCOEt described by Fukunaga et al. (2007) is the same one that underlies the attenuation of DISC seen here.

In our previous report (Shin et al., 2008) CPCCOEt was not the only drug that led us to the erroneous conclusion that mGluR1 was required for DISC: we also had found that JNJ16259685 (50 μ M) attenuated DISC. Unfortunately, that concentration of JNJ16259685 was much too high. JNJ16259685 has an IC_{50} for mGluR1 inhibition of 0.55 nM (Mabire et al., 2005). When 50 μ M JNJ16259685 was applied in the present conditions (mouse versus rat, 1 min versus 30 sec test pulse interval), it also produced a slow attenuation of DISC ($73.9 \pm 3.4\%$ of baseline at $t = 20$ min after drug application, $n=7$). However, when 0.1 μ M JNJ16259685 was used, a concentration which abolishes I_{DHPG} (Figure 1), no attenuation of DISC was observed (Figure 2). Similar pharmacological effects were also observed in age-matched rat Purkinje cells with the same test pulse interval (1 min) used in this manuscript (Supplementary Figure 1).

In both rat (Shin et al., 2008) and mouse Purkinje cells, DISC is associated with an increase in current noise above baseline values. This characteristic has been previously reported for mGluR1-mediated slow inward currents in Purkinje cells in response to either exogenous mGluR1 agonist or burst stimulation of parallel fiber synapses (Canepari et al., 2001; Canepari et al., 2004). The present findings indicate that this noise signature is not unique to mGluR1-triggered conductances in Purkinje cells. Recent work has indicated that the mGluR1-mediated slow current requires cation channels containing the TRPC3 subunit (Hartmann et al., 2008).

What is the identity of the conductance underlying DISC and how is it triggered? The present data do not speak to this question other than to rule out mGluR1 as a required activator. One formal possibility is that both mGluR1 and some other receptor activate a TRPC3-containing ion channel underlying DISC. Future work will be required to address this issue experimentally.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Comprehensive list of abbreviations

mGluR	metabotropic glutamate receptor
DISC	depolarization-induced slow current
CPCCOEt	7-(Hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester
MPEP	2-Methyl-6-(phenylethynyl)pyridine hydrochloride
DHPG	(S)-3,5-Dihydroxyphenylglycine
GABA	γ -Aminobutyric acid
DPI	depolarization-induced potentiation of inhibition
mIPSC	miniature inhibitory postsynaptic current
NMDA	N-Methyl-D-aspartic acid
PF	parallel fiber
EPSC	excitatory postsynaptic current
SNARE	SNAP and NSF attachment receptors
D-AP5	D-(-)-2-Amino-5-phosphonopentanoic acid
(R)-CPP	3-((R)-2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid
3-MATIDA	α -amino-5-carboxy-3-methyl-2-thiopheneacetic acid

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Figure 1.

DHPG-evoked slow current was rapidly blocked by various mGluR1 antagonists. DHPG (100 μ M) together with glutamate (10 μ M) was pressure-injected (10 psi, 50 msec) in the outer one-third of the molecular layer to induce an mGluR1-mediated slow current (I_{DHPG}). I_{DHPG} traces before (black) and after (gray) the bath-application of (a) CPCCOEt (100 μ M), (b) JNJ 16259685 (0.1 μ M), (c) 3-MATIDA (150 μ M), and (d) Bay 36-7620 (10 μ M). These traces are single, unaveraged exemplar responses. Scale bars: 200 pA, 5 sec. (e) A time course graph showing the mean I_{DHPG} charge transfer, with values normalized to a baseline of 0 – 5 min ($n=5$ for all groups). Error bars show the standard error. The gray bar represents the bath-application of mGluR1 antagonists.

Figure 2.

Among mGluR1 antagonists, only CPCCOEt significantly attenuated DISC. Exemplar current traces are accompanied by their corresponding high-pass filtered responses, the latter to show the noise envelope. Traces were plotted before (*left*) and 20 min after application of various mGluR1 antagonists (*middle*), (a) CPCCOEt (100 μ M), (b) JNJ 16259685 (0.1 μ M), (c) 3-MATIDA (150 μ M), and (d) Bay 36-7620 (10 μ M). Scale bars: 200 pA, 2 sec. The high-pass-filtered traces are displayed by using a range of -25 to 25 pA in (a) and (c), and -50 to 50 pA in (b) and (d). *Right*, Time course graphs showing the mean DISC charge transfer, with values normalized to a baseline of 0 – 5 min ($n=8$ for CPCCOEt and $n=5$ for all other groups).

Normalized mean I_{DHPG} time courses from Figure 1 are overlaid for comparison. The gray bar represents the bath-application of mGluR1 antagonists.

Figure 3.

DISC is present in Purkinje cells derived from mGluR1 $-/-$ mice and this DISC is CPCCOEt-sensitive. Exemplar current traces are accompanied by their corresponding high-pass filtered responses. Traces were plotted before (*left*) and 20 min after application of CPCCOEt (100 μ M). Scale bars: 200 pA, 2 sec. The high-pass-filtered traces are displayed by using a range of -25 to 25 pA. (b) DISC charge transfer was plotted as a function of time as CPCCOEt was bath-applied (gray bar). (c) Time course graphs showing the mean DISC charge transfer, with values normalized to a baseline of 0 – 5 min ($n=5$).

Figure 4.

DISC was not attenuated by an mGluR5 antagonist. Exemplar current traces are accompanied by their corresponding high-pass filtered responses. Traces were plotted before (*left*) and 20 min after application of MPEP (10 μ M). Scale bars: 200 pA, 2 sec. The high-pass-filtered traces are displayed by using a range of -50 to 50 pA. *Right*, Time course graphs showing the mean DISC charge transfer, with values normalized to a baseline of 0 – 5 min ($n=5$). The gray bar represents the bath-application of MPEP.