Metabotropic Glutamate Receptor-Mediated Currents at the Climbing Fiber to Purkinje Cell Synapse

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Different forms of synaptic plasticity in the cerebellum expressed at the synapses onto Purkinje cells (PCs) are mediated by membrane metabotropic glutamate receptors (mGluRs). There are three main mGluR groups with a total of 8 subtypes. Although mGluRs are also found at the climbing fiber (CF) to PC synapses, the distribution and biological activity of their types is not well-known. Using whole cell patch-clamp recordings from PCs in rat cerebellar slices with inhibitors of ionotropic receptors and glutamate uptake blockers we demonstrate a complex pharmacology of currents obtained by CF stimulation. The mGluR1 specific antagonist CPCCOEt in a group of cells suppressed this response, but in a similar number of other cells it induced a potentiating effect. It was found that a switch between these two biopharmaceutical effects might occur with age.

INTRODUCTION

Different forms of synaptic plasticity in the cerebellum are mediated by metabotropic glutamate receptors (mGluRs). Understanding the biopharmaceutical chemistry of these receptors is thus important for the regulation of sinaptogenesis in such processes as learning, development, and regeneration. Metabotropic glutamate receptors are G-protein coupled receptors that have been subdivided into three groups, based on sequence similarity, pharmacology, and intracellular signaling mechanisms. There are three main mGluR groups with a total of 8 subtypes: mGluRs 1 and 5 (group I), mGluRs 2 and 3 (group II), and mGluRs 4, 6, 7, and 8 (group III). Group I receptors are coupled to phospholipase C and intracellular calcium signaling, while group II and group III receptors are negatively coupled to adenylyl cyclase.

In the cerebellar cortex two different excitatory inputs project to separate domains of the Purkinje cell (PC) dendrites. The distal one, rich in spines, is innervated by hundreds of thousands of parallel fibers (PFs), while the proximal one has only a few spines, however, consisting of hundreds of release sites,[1] originating from a single climbing fiber (CF). In these synapses glutamatergic transmission involves ionotropic (iGluRs) and metabotropic glutamate receptors (mGluRs). At PF to PC synapses activation of mGluR gives rise to a well-known slow synaptic current inhibited by antagonists of mGluR1.[2−4] The distribution of mGluR types in the CF to PC synapses is not well-known. In PCs in addition to the postsynaptic mGluR1[5] there is also some evidence for the expression of GluR3, 4 and 7.[6,7] An mGluR1α-mediated all-or-none postsynaptic current (mGluR PSC) was also demonstrated at the CF-PC synapse.[8,9]

The aim of the present study was to shed more light on the complex pharmacology of the CF-mGluR PSC, especially regarding the dual effect of the mGluR1 antagonist CPCCOEt. It was shown that the antagonist augments the current in older experimental rats. In addition to mGluR group I the role of groups II and III was confirmed.

MATERIALS AND METHODS

Experimental animals were P22-76 Wistar rats. Cerebella were isolated in ice-cold extracellular medium (in mM: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$, 20 glucose) gassed with 95% O_2/5% CO_2 mix. Parasagittal slices (200 μm thin) were cut and used in experiments with constant perfusion with O_2/CO_2-bubbled extracellular saline (22−26 °C).

Whole-cell patch-clamp was performed with an EPC 7 patch-clamp amplifier (Heka, Germany) with the ITC-16 (Instrutech, U.S.A.) A/D converter and a stimulus pulse generator (stimulus pulse width was 20−100 μs) with an isolator unit (WPI, U.S.A.). Recordings were filtered at 3 kHz, and the sampling rate was 5−40 kHz. Pipets were pulled from borosilicate glass (2−3 MΩ when filled) and contained in mM: 130 CsCl, 20 TEA, 10 HEPES, 4 Na_2-ATP, 0.4 Na_2GTP, 0.1 Ca_2Cl, 2 $MgCl_2$, 1 EGTA, pH 7.3 adjusted with CsOH (all Sigma, Italy). Stimulation of CFs was performed with a soda lime glass pipet (tip diameter ∼10 μm) filled with extracellular solution and placed in the white matter or the molecular layer several cell diameters away from the patched PC. Recordings were performed in 20 μM bicuculline (Sigma) and 50 μM APV (Tocris Cookson Ltd., U.K.) to block $GABA_A$ and NMDA responses, respectively.

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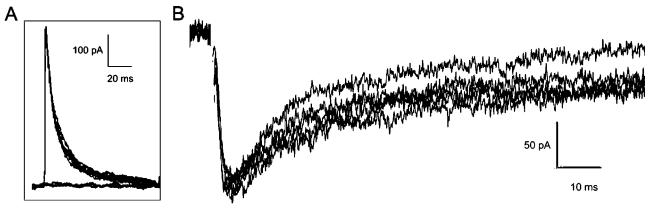

Figure 1. Purkinje cell PSCs evoked by CF stimulation. (A) Several superimposed traces of CF-EPSCs with sub- and suprathreshold stimulation (only $GABA_A$ receptors were blocked with bicuculline), $V_h = 30$ mV; (B) Superimposed traces of CF-mGluR PSCs evoked in bicuculline, NBQX, GYKI53655, and TBOA with suprathreshold stimuli (160−1000 μA) at $V_h = -70$ mV. Stimulus artifacts were abolished.

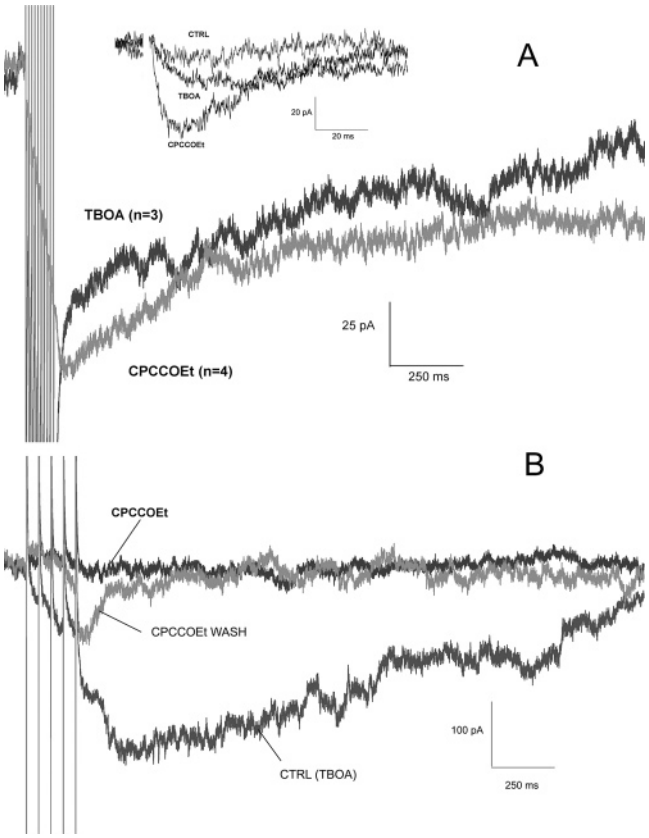

Figure 2. The dual effect of CPCCOEt on the mGluR PSC. (A) Potentiating effect: average superimposed recordings of currents in response to CF stimulation of 10 pulses at 100 Hz, $V_h = -50$ mV in TBOA (with GABA- and AMPA-receptors blocked; average of 3 traces), and after CPCCOEt addition (average of 4 traces). Inset*:* single stimulation with ionotropic receptors blocked ("CTRL"), after additional block of glutamate transport ("TBOA") and after supplementing mGluR1 antagonist ("CPCCOEt"); $V_h = -50$ mV, superimposed single traces; stimulus artifacts were abolished. (B) Inhibiting effect: superimposed single traces of currents in response to CF stimulation of 5 pulses at 20 Hz, $V_h = -60$ mV in control with TBOA ("CTRL (TBOA)"), after the antagonist ("CPCCOEt") and after its partial wash ("CPCCOEt WASH").

CFs were identified by a double pulse protocol at holding voltage, $V_h = +30$ mV, showing characteristic paired pulse depression (PPD).[10] The stimulating electrode was adjusted so that the subthreshold response of the PFs (otherwise showing paired pulse facilitation) is minimal or absent (Figure 1A). After the AMPA receptor block (with 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[*f*]quinoxaline-7-sulfonamide, NBQX, 25 μM and GYKI53655, 25 μM), DL-*threo*-β-benzyloxyaspartic acid, TBOA (100 μM), was used to

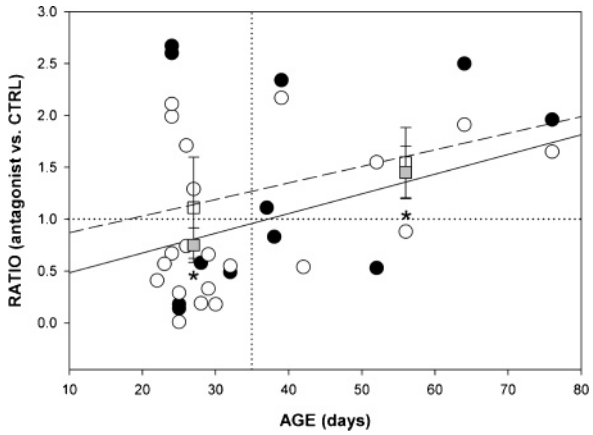

Figure 3. Correlation of mGluR-PSC amplitude in CPCCOEt ("antagonist") relative to the control ("CTRL") with animal age. Relative CF-mGluR PSC amplitude in CPCCOEt rises with animal age. *Closed circles* − single pulse stimulation, with regression line (*interrupted*; $r = 0.2701$). *Open circles* − train stimulation, with regression line (*continuous*; $r = 0.3964$). *Open squares* − mean ratio for single pulse stimulation below and above 35 days of age. *Grey squares* − mean ratio for train stimulation below and above 35 days of age. *Asterisks* - significant difference ($p < 0.05$, *t*-test) between mean ratios. *Dotted vertical line* − 35-day limit.

block glutamate transporters and augment the effect of the released transmitter on mGluRs in CF-PC synapses. Additionally, a stimulus train of 10, 20, or 100 Hz (5 or 10 pulses) was delivered to further induce larger glutamate release. The pharmacology of the remaining CF-induced PSC response was checked at negative holding voltage with mGluR inhibitors CPCCOEt, 7-(hydroxyimino) cyclopropa-[*b*]chromen-1a-carboxylate ethyl ester (100 μM), that blocks mGluR1, the member of group I, and MSPG, (*R*,*S*)-alpha-2-methyl-4sulfonophenylglycine (100 μM), that targets groups II and III (both antagonists from Tocris Cookson Ltd.).

Reported values are mean ± SEM.

RESULTS AND DISCUSSION

With AMPA receptors blocked and $GABA_A$ and NMDA receptors inhibited (with bicuculline and APV, respectively), the CF origin of the response in TBOA was evidenced by its all-or-none behavior (Figure 1).

Responses to train stimulation showed a recovery tail current immediately after the last pulse (see Figure 5), which in some cases formed an inward current peak (see Figure 4). With train stimulation a slow late current phase was also observed (see Figure 2B).

The mGluR1 antagonist CPCCOEt showed a dual effect. In some cells it augmented the mGluR PSC amplitude by factors of 2.20 ± 0.24 ($n = 6$) and 1.80 ± 0.11 ($n = 8$) for single and train stimulation, respectively (see example in Figure 2A with inset), but in others caused its suppression by factors of 0.46 ± 0.11 ($n = 6$) and 0.53 ± 0.07 ($n = 13$), for single and train stimulation, respectively (see an example for the latter in Figure 2B).

To reveal the possible origin of the dual effect of CPCCOEt we studied the influence of animal age on the ratio of mGluR-PSC amplitude in the antagonist relative to the one in the control (Figure 3). If P35 was taken as the arbitrary limit of two age groups it was shown that with train stimulation in younger animals (<P35) CPCCOEt had a predominantly blocking effect (agonist vs control ratio was

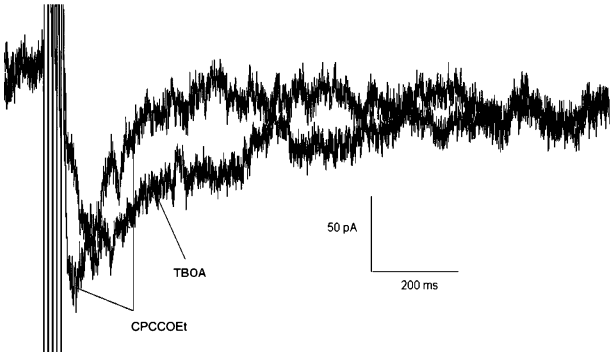

Figure 4. Different effect of CPCCOEt on the early and late phases of mGluR PSC. Superimposed traces of currents evoked by CF train stimulation (5 pulses, 100 Hz; $V_h = -60$ mV) in control with blocked glutamate transport ("TBOA") and after addition of antagonist ("CPCCOEt").

0.75 ± 0.17), while in older animals the effect was significantly ($p < 0.05$) different and predominantly potentiating (ratio was 1.45 ± 0.25). The difference was also apparent for single pulse stimulation, but it was not statistically significant (ratio 1.11 ± 0.48 vs 1.54 ± 0.34).

In some cells the dual effect was observed on the same mGluR PSC recording of a single cell. Namely, in the same cell CPCCOEt could potentiate the early current phase, while the late slow component was abolished (Figure 4). This may prove that the two components are of different origin acting differently to the same mGluR1 antagonist. These two processes could be based on the phospholipase C transduction pathway and some, yet unknown, Ca^{2+}-dependent conductance vs direct coupling as recently described for the TRPC1 cation channel.[11] The transduction mechanism of the early component may be prevalent in older animals, thus giving rise to the potentiating effect of CPCCOEt as opposed to younger animals having a more pronounced coupling pathway for the late current component that can be inhibited by the antagonist. Furthermore, earlier data[9] on the complex nature of the CF-mGluR PSC were further confirmed by the additional block with MSPG, the antagonist of mGluR groups II and III. Namely, as illustrated in Figure 5, MSPG inhibited the CF-mGluR PSC in CPCCOEt by 0.54 ± 0.09 ($n = 5$).

Thus, in some cells the postsynaptic inhibition of mGluR1 may cause the expected decrease of its current,[8] but in others, and more likely in older animals, this effect may be overcome by a rise of PSCs. Since these currents are recorded with iGluRs blocked, the latter PSC rise may originate from other mGluR types, the action of which can be suppressed by mGluR1-modulated activity (see Andjus and co-workers, 2005, and above). The plausible hypothesis that the two different effects of mGluR1 antagonist originate in two different and age-related mGluR PSC components needs further experimental justification.

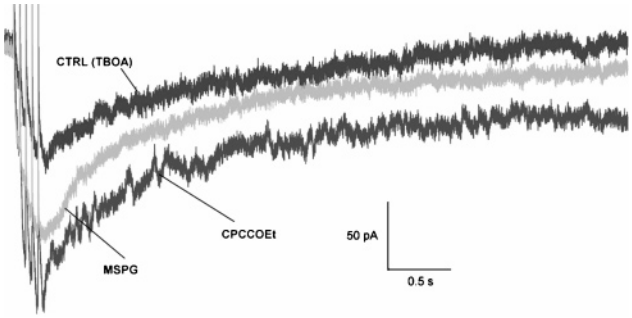

Figure 5. Composite origin of mGluR PSC. Superimposed currents evoked by CF train stimulation (5 pulses, 20 Hz; $V_h = -30$ mV): in control with TBOA ("CTRL *(*TBOA)"), after the potentiating effect of the antagonist ("CPCCOEt") and upon supplementing with mGluR II/III antagonist ("MSPG").

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