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Group 2 metabotropic glutamate receptors induced long term depression in mouse striatal slices

Laetitia Kahn[a], Gérard Alonso[b], David Robbe[a], Joël Bockaert[a], Olivier J. Manzoni[a,*]

[a]CNRS UPR 9023, 141, Rue de la Cardonille, 34094 Montpellier Cedex 05, France
[b]CNRS UMR 5101, 141, Rue de la Cardonille, 34094 Montpellier Cedex 05, France

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Abstract

We studied the roles of mGlu2/3 receptors (mGlu2/3) in glutamatergic transmission at corticostriatal synapses in mice brain slices. Perfusion of the selective mGlu2/3 agonists LY354740 and L-CCG1 caused the long term depression (LTD) of evoked synaptic responses. Photonic and electronic microscopy showed mGlu2/3 on axonal fibers and glial processes but not on striatal dendrites. mGlu2/3-LTD was independent of synaptic activity and insensitive to specific antagonists of dopamine D1, D2, $GABA_B$, *N*-methyl-D-aspartate or adenosine A1 receptors. Manipulation of the cAMP/protein kinase A cascade had no effect on the mGlu2/3-LTD. In contrast, MEK1-2 inhibitors reduced both mGlu2/3 initial depression and LTD suggesting the involvement of the mitogen activated kinase pathway in mGlu2/3-LTD.

Keywords: Striatum; Glutamate; Metabotropic glutamate; Long term depression; Mitogen-activated protein kinase; Presynaptic inhibition

The physiological roles of glutamate (Glu) are mediated through ionotropic receptors and G-protein coupled metabotropic receptors (mGlu). mGlu are classified into three families [4]: group 1 mGlu (mGlu1/5) are coupled to phospholipase C whereas group 2 (mGlu2/3) and group 3 (mGlu4/6/7/8) mGlu inhibit adenylate cyclase (AC). mGlu2/3 are instrumental in both cAMP/PKA-dependent long term depression (LTD) [2,8], and mitogen-activated protein kinase (MAPK)-dependent LTD [10].

At the cortical excitatory projection pathways to the striatum, we found that activation of presynaptic group 2 mGlus causes a LTD dependent on the MAPK signaling cascade.

Mice brain slices were prepared as described before [1]. Double fluorescence immunostaining for light and confocal microscopy as well as peroxidase immunostaining procedures have been described previously [1]. The anti-mGlu2/3 antibody was from Chemicon (1:500), the peroxidase-labeled Fab fragment of goat IgG anti-rabbit IgG was from Biosys (Compiègne, France, 1:1000), the rabbit polyclonal antibodies against GABA were kindly provided by Dr M. Geffard (1:5000) and those against tyrosine hydroxylase were from J. Boy laboratories (Blois, France, 1:5000).

Parasagittal striatal slices (400 μm) were prepared from 4–6-week-old male C57-BL6 mice as previously described [13]. Picrotoxin (100 μM) was present in all experiments to block $GABA_A$ synaptic responses. To evoke synaptic potentials, stimuli (100 μs duration) were delivered at 0.33 Hz through bipolar stainless steel electrodes placed at the cortical-striatal border. Extracellular field potentials were recorded as described before [13]. Both EPSP slope and amplitude were measured and gave similar values (fEPSP amplitudes were plotted on the final figures). All values are given as the mean ± SEM. Statistical analyses were done with the Mann–Whitney *U*-test using Statview (Abacus Concepts, Inc., USA); statistical significance was set at $P < 0.05$, and is marked as * on the graphs.

The drugs used were: L-AP4, picrotoxin (Sigma); (R,S)-DHPG, 2-amino-2-(2-carboxycyclopropan-1-yl)-3-(dibenzopyran-4-yl) propanoic acid (LY341495), 2-(carboxycyclopropyl)glycine (L-CCG1) (Tocris Neuramin); sulpiride, (+)-SCH-23390 hydochloride, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (R.B.I.); (+)-2-aminobicyclo-[3.1.0]hexane-2,6-dicarboxylic acid (LY354740) was a gift of Drs A. Schoepp and Monn at Elli-Lilly (USA) and CGP-38345 was a gift of Dr A. Sedlaceck at Ciba-Geigy (Switzerland).

* Corresponding author. Tel.: +33-4-67142983; fax: +33-4-67542432.
E-mail address: manzoni@ccipe.montp.inserm.fr (O.J. Manzoni).

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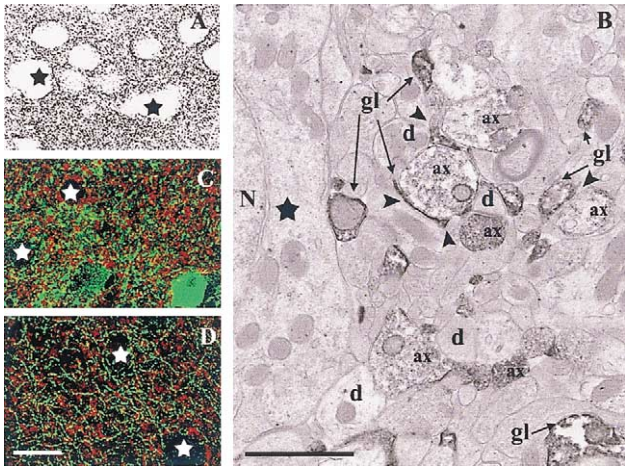

Fig. 1. Immunolocalization of mGlu2/3 in the striatum. (A) Light microscope observation of single peroxidase immunostained sections: mGlu2/3 immunostaining is associated with dot structures. (B) At the electron microscope level, mGlu2/3 immunostaining is associated with both presynaptic axon (ax) and glial processes (gl) and is frequently closely apposed to labeled axonal profiles (arrowheads). Both neuronal cell body (asterisk) and dendritic profiles (d) are devoid of mGlu2/3 immunostaining. Confocal microscope observation of double fluorescence immunostained sections: mGlu2/3-immunostained fibers (red in C,D) are distinct from fibers immunostained for tyrosine hydroxylase (green in C) or for GABA (green in D). Scale bars: (A–D) 25 μm; (B) 1 μm; N, nucleus.

Light microscope examination of single peroxidase immunostained sections indicated that organization of mGlu2/3-immunostained structures conformed to previous descriptions in the rat [11]. Within the striatum, mGlu2/3 labeling was associated with numerous dot structures dispersed between unlabeled neuronal cell bodies (Fig. 1A). At the electron microscope level, striatal mGlu2/3 immunostaining was associated with presynaptic axonal terminals and glial processes (Fig. 1B). Postsynaptic dendrites and neuronal cell bodies were unstained. In axon terminals, immunostaining was associated with the plasma membrane facing the synaptic contacts and some of the synaptic-like vesicles. Confocal microscope examination of double fluorescence immunostained sections indicated that striatal mGlu2/3 immunostaining was never associated with axonal fibers immunostained for GABA or tyrosine hydroxylase (Fig. 1C,D). Fig. 2A shows that there were two components to the LY354740 (100 nM, 15 min)-induced inhibition. The depression reached its maximum during agonist perfusion (60% inhibition) and after wash-out of the agonist, LTD was always observed (about 20% inhibition, up to 90 min after agonist perfusion). The plateau phase of the LTD was not affected by the group 2 mGlu antagonist LY341495 [3] (Fig. 2A). Thus, mGlu2/3-LTD was not due to permanent agonist binding to the receptor. L-CCG1 (10 μM, 5 min), a group 2 mGlu agonist structurally unrelated to LY354740, also caused LTD (Fig. 2A). Pre-incubation with LY341495 (200 nM) prevented both mGlu2/3-induced acute inhibition and LTD

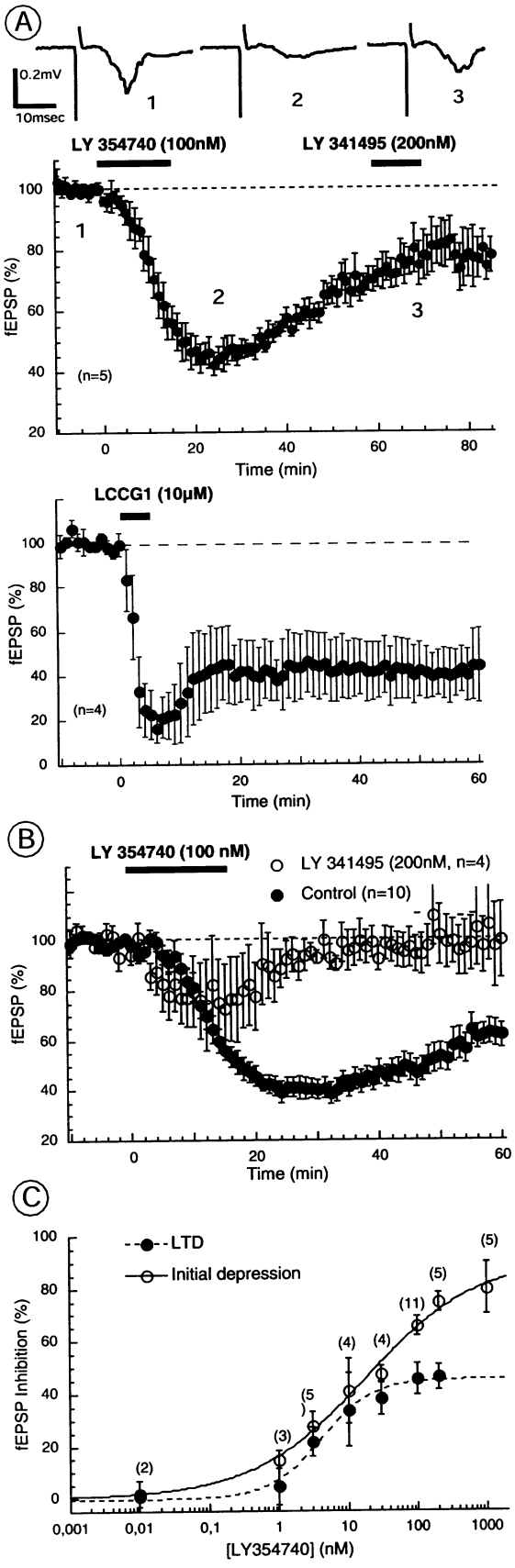

Fig. 2 cont'd

(Fig. 2B). The EC50 for LY354740 peak inhibition was 4.0 ± 1.1 μM in agreement with its reported affinity for mGlu2/3 [5]. The EC50 for inducing LTD (17.4 ± 7.3 μM) was comparable (Fig. 2C). This confirmed the implication of mGlu2/3 in corticostriatal LTD. The possibility that mGlu2/3 activation causes the release of other neurotransmitter(s) that would in turn induce LTD was tested. mGlu2/3-LTD was successfully induced after treatment (20 min) with an antagonists cocktail of D1 (SCH-23390, 20 μM), D2 (sulpiride, 20 μM), $GABA_B$ (CGP-35348, 200 μM), NMDA (D-AP5, 50 μM) and A1 adenosine (DPCPX, 200 nM) receptor (Fig. 3). By contrast with hippocampus [2], dentate gyrus [2] and amygdala [8] where synaptic activity is necessary for mGlu2/3-LTD, pharmacological activation of mGlu2/3 is sufficient to induce mGlu2/3-LTD in mice striatum (Fig. 3B).

In order to determine the importance of the cAMP/PKA pathway in striatal mGlu2/3-LTD, we used forskolin (FSK), an activator of ACs, which indirectly stimulates A1 receptors and complicate experiments [2]. Thus, prior to FSK, A1 adenosine receptors were blocked with DPCPX (200 nM). Alone, 20 min of DPCPX treatment had no detectable effect on striatal synaptic transmission (fEPSP $= 101 \pm 8\%$ of basal transmission, $n = 6$). Twenty minutes of bath-application of FSK (10 μM) increased synaptic transmission showing the sensitivity of these synapses to cAMP elevation (fEPSP $= 156 \pm 14\%$ of basal transmission, $n = 6$). Bath-application of mGlu2/3 agonist during and after the FSK treatment induced levels of LTD similar to controls (Fig. 4A). As expected from the lack of effect of the cAMP elevation, the mGlu2/3-LTD was not altered by blockade of PKA activity with either H89 (10 μM, 20 min slice treatment) or KT5720 (1 μM, 2 h slice treatment, Fig. 4B,C). Thus, the cAMP/PKA cascade does not play a role in striatal mGlu2/3-LTD.

Considering the role of MAPK in cortical-LTD [10] and that activation of mGlu2 stimulates the MAPK cascade [7], we tested the involvement of the MAPK pathway in striatal mGlu2/3-LTD by pre-incubating slices, for at least 2 h, with two selective inhibitors of MEK1-2, U0 126 (50 μM) and PD 98059 (10 μM) [6]. U0 126 reduced both mGlu2/3-induced initial depression and LTD (at 60 min, LTD $= 20.4 \pm 5.5\%$, $n = 11$; in control, LTD $= 35.4 \pm 5.0\%$, $n = 10$, Fig. 4D). Similar results were obtained with PD 98059 (at 60 min, LTD $= 18.6 \pm 4.4\%$, $n = 17$). This suggests that MEK1 or MEK2 activity is important in the establishment of that form of LTD.

Our main finding is that activation of mGlu2/3 activation induces LTD at corticostriatal synapses. Electron microscopy revealed the association of striatal mGlu2/3 with presynaptic axonal terminals and glial processes, but not with dendritic processes or neuronal cell bodies. mGlu2/3 immunostaining was not colocalized with striatal GABAergic axonal fibers (Fig. 1D) or tyrosine hydroxylase containing (i.e. dopaminergic) axonal fibers suggesting that mGlu2/3 immunoreactive terminals are glutamatergic. Bath perfusion with selective group 2 mGlu agonists induced LTD of corticostriatal glutamatergic synapses. A previous study did not mention mGlu2/3-LTD [9] and contrary to this report we observed that the group 3 mGlu agonist L-AP4 (100 μM) reliably caused a reversible depression of corticostriatal fEPSP, in accord with Pisani et al. [12].

By contrast with the hippocampus [2], we show that in the absence of evoked release, direct stimulation of mGlu2/3 is sufficient for corticostriatal mGlu2/3-LTD suggesting that mGlu2/3 agonists perfectly mimic endogenous Glu actions. Upon stimulation of mGlu2/3 other neurotransmitters could be released from neighboring cells and could participate in the induction of LTD. Blocking NMDA, A1, GABA-B, D1

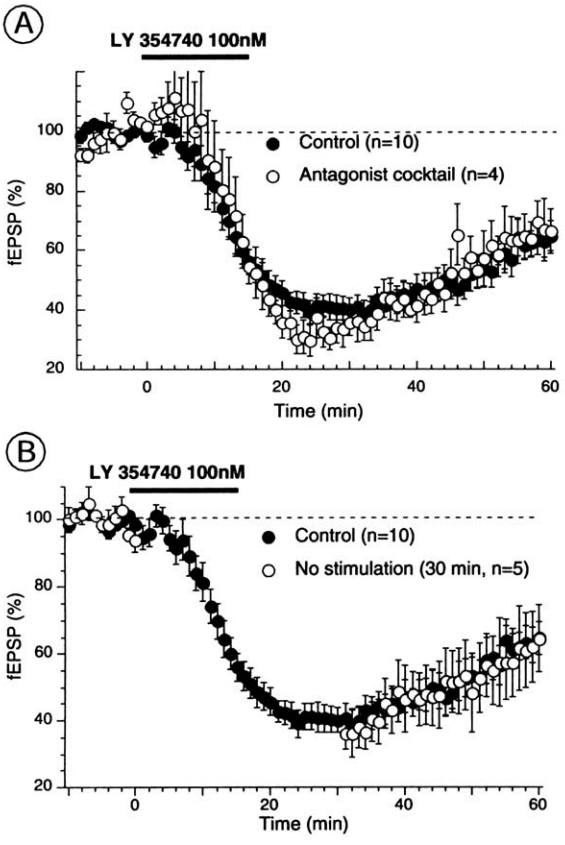

Fig. 3. Pharmacological activation of mGlu2/3 is sufficient to induce mGlu2/3-LTD. (A) Blocking NMDA (50 μM D-AP5), A1 adenosine (200 nM DPCPX), D1 dopamine (20 μM SCH-23390), D2 dopamine (20 μM sulpiride) and GABA-B receptors (200 μM CGP-35348) did not inhibit LY354740-induced LTD. (B) mGlu2/3-LTD was not blocked in the absence of presynaptic activity.

Fig. 2. Pharmacological characterization of the mGlu2/3-induced LTD of corticostriatal fEPSP. (A) Left: time-course of LY354740 actions. The group 2 mGlu antagonist LY341495 was perfused during the LTD plateau phase and did not affect LTD. Sample traces are from a typical experiment included in the averaged data. Right: time-course of the LTD induced by application of L-CCG1. (B) Inhibitory effects of LY341495 on mGlu2/3-induced LTD. (C) Dose response curves for LY354740-induced initial depression (solid line) and LTD (dashed line).

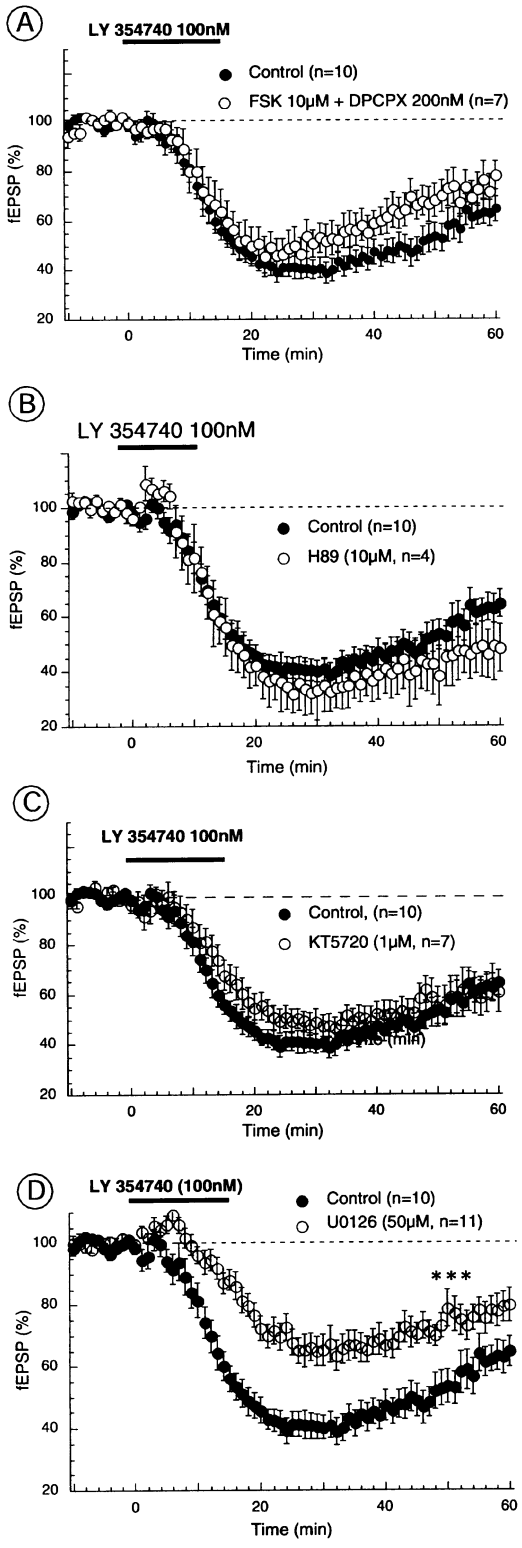

Fig. 4. mGlu2/3-LTD is independent of the cAMP/PKA pathway but is reduced by a MEK1/2 inhibitor. (A) The LY354740-induced LTD was not affected when cAMP levels were augmented by bath-applied FSK, either by the PKA inhibitors (B) H89 or (C) KT5720. (D) U0 126, a MEK1/2 inhibitor, markedly reduced LY354740-induced LTD.

and D2 receptors was without effect on mGlu2/3-LTD, reinforcing the idea that this LTD was solely due to presynaptic mGlu2/3 stimulation.

Observing that corticostriatal mGlu2/3-LTD was unaffected by manipulation of the cAMP/PKA pathway incited us to test the implication of the MAPK pathway [7,10]. Inhibition of MEK1 and MEK2 by the selective non-competitive inhibitors PD 98059 and U0 126 reduced mGlu2/3-induced effects. Since inhibitors of the MAPK pathway also reduced mGlu2/3-induced initial depression, it was conceivable that the reduction of LTD was simply a consequence of insufficient receptor activation. From the dose response curves of mGlu2/3-induced initial depression and LTD (Fig. 1C), it is clear that a 30–35% initial depression is sufficient to induce maximal LTD. Thus, inhibition of the MAPK pathway indeed reduced mGlu2/3-LTD. The inhibition of mGlu2/3-LTD was only partial, suggesting the participation of other signaling cascades or the incomplete blockade of the MAPKK.

This report of a mGlu2/3-LTD at corticostriatal synapses adds to the growing body of evidence showing the importance of mGlu2/3 in LTD. The mGlu2/3-LTD use various transduction pathways: synapses of the hippocampus and the amygdala show a cAMP/PKA-dependent LTD whereas prefrontal cortex and corticostriatal LTD involve MAPK.

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