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Cell-type specificity of mGluR activation in striatal neuronal subtypes

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Summary. The effects of metabotropic glutamate receptor (mGluR) activation were studied in medium spiny neurons and large aspiny (LA) interneurons by means of electrophysiological and optical recordings. DCG-IV and L-SOP, agonists for group II and III mGluRs, respectively, produced a presynaptic inhibitory effect on corticostriatal glutamatergic excitatory postsynaptic potentials (EPSPs) in both spiny and LA cells. Activation of group I mGluRs by the selective agonist 3,5-DHPG produced no effect on membrane properties and glutamatergic transmission in spiny neurons, whereas it did cause a membrane depolarization in LA interneurons coupled to increased input resistance. In combined optical and electrophysiological experiments, in spiny neurons 3,5-DHPG enhanced membrane depolarization and intracellular calcium (Ca^{2+}) levels induced by NMDA applications, but not in LA interneurons. These data suggest the existence of a positive interaction between NMDA and group I mGlu receptors only in medium spiny cells which might, at least partially, account for the differential vulnerability to excitotoxic damage observed in striatal neuronal subtypes.

Keywords: Amino acids – Striatum – Electrophysiology – Metabotropic glutamate receptors – Excitotoxicity

Introduction

Glutamate is the most diffuse excitatory transmitter in the mammalian brain. It acts through two main categories of receptors, ionotropic receptors (iGluRs), and metabotropic receptors (mGluRs). The first group of receptors is coupled to ligand-gated ion channels and include N-methyl-D-aspartate (NMDA), kainate and α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors (for review see Hollmann and Heinemann, 1994). At

present, at least eight subtypes of receptors form the family of mGluRs. According to their primary amino acid sequence, second messenger coupling, and pharmacological profile, mGluRs have been divided into three groups (for review, see Conn and Pin, 1997). Group I (mGluR1 and 5) promotes phosphoinositides hydrolysis, and it is preferentially activated by 3,5-dihydroxyphenylglycine (3,5-DHPG) (Schoepp et al., 1994). Group II (mGluR2, 3) and group III (mGluR4, 6, 7, 8) are negatively coupled to adenylate cyclase activity. (2S,1'R,2'R,3'R)-2-(2,3-dicarboxy-cyclopropyl)-glycine (DCG-IV) is a selective agonist for group II mGluRs (Ishida et al., 1993), whilst group III mGluRs are activated by L-2-amino-4-phosphonobutanoate (L-AP4) and L-serine-O-phosphate (L-SOP) (Nakanishi, 1992). Within the mammalian striatum, neuronal subtypes express a differential vulnerability to both energy deprivation and excitotoxic damage. Projecting cells, GABAergic medium spiny neurons, are very sensitive in both these pathological conditions (Pulsinelli, 1985; Beal et al., 1986). On the basis of electrophysiological, morphological and pharmacological criteria, three different classes of interneurons have been identified (Kawaguchi, 1993). Among these, a small percentage, 2–3%, is represented by large aspiny (LA) interneurons. These cells are known to be cholinergic and give rise to the intrinsic striatal cholinergic innervation (Bolam et al., 1984), exerting a strong influence on striatal circuitry, both in physiological and pathological conditions. Interestingly, cholinergic interneurons are selectively spared in pathological conditions involving exclusively, such as Huntington's chorea (Ferrante et al., 1985), or partially, such as brain ischemia, the striatum (Pulsinelli, 1985). Despite this experimental evidence, the reasons behind the differential vulnerability are not known yet. A possible involvement of glutamate excitotoxicity has been proposed in the pathogenesis of neurological diseases affecting the striatum such as Huntington's disease and global ischemia (Ferrante et al., 1986; Beal, 1992).

Recently, we have shown that spiny neurons are more sensitive than LA interneurons to application of iGluR agonists such as kainate, AMPA and NMDA. Moreover, we observed that high concentrations of these agonists, which cause irreversible membrane changes in spiny neurons do not produce similar effects in LA interneurons. Thus, the high sensitivity to iGluR activation expressed exclusively by spiny neurons might contribute to explain the differential vulnerability of this neuronal subtype to excitotoxic damage (Calabresi et al., 1998).

Here, we extend our observation, analysing the effects of mGluR activation in spiny neurons and in LA cells in order to define the role of metabotropic glutamate receptor activation in the pathophysiology of the striatum.

Materials and methods

Adult male Wistar rats (150–250 g) were used for all the experiments. The preparation and maintenance of coronal slices have been described previously (Calabresi et al., 1990b, 1998; Pisani et al., 1997a, b). Animals were killed under ether anesthesia by cervical dislocation, the brain was removed and coronal slices (180–200μm thick), containing

cortex and striatum, were cut from tissue blocks with a vibratome. A single slice was transferred to a recording chamber and submerged in a continuously flowing Krebs solution (35°C, 2–3 ml/min) gassed with 95% O_2–5% CO_2. The composition of the control solution was (in mM): 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 11 Glucose, 25 $NaHCO_3$. In all the experiments the intracellular recording electrodes were filled with 2M KCl (30–60 MΩ). An Axoclamp 2A amplifier was used for recordings either in current-clamp or in voltage-clamp mode. In single-electrode voltage-clamp mode the switching frequency was 3 kHz. The headstage signal was continuously monitored on a separate oscilloscope. Traces were displayed on an oscilloscope and stored in a digital system. For synaptic stimulation, bipolar electrodes were used. These stimulating electrodes were located either in the cortical areas close to the recording electrode or in the white matter between the cortex and the striatum in order to activate corticostriatal fibres. For simultaneous optical and electrical recordings, the tip of the recording electrode was filled with a solution of 2 mM fura-2 (pentapotassium salt, Molecular Probes, The Netherlands) and 100 mM KCl, whilst the shank was filled with a 2 M KCl solution. After cell impalement, cells were loaded with fura-2 by injecting, through the recording electrode, 0.1–0.5 nA negative current for 10–15 min. The recording chamber was mounted on the stage of an upright microscope (Axioscop FS, Zeiss), equipped with a 40× water immersion objective (Zeiss). Fura-2 fluorescence was excited at 380 nm by using a 75 W xenon lamp. Emission light was filtered by a long-pass barrier filter (470 nm) and detected by a CCD camera (Photonic Science, UK). Images were stored and analysed with a software (IonVision, ImproVision, UK) running on PowerMac 8100. The background fluorescence was measured in a part of the slice with no fura-2-filled neurones and subtracted from the signal to obtain the basal fluorescence level (F). Changes in intracellular Ca^{2+} are expressed in terms of $\Delta F/F$, where ΔF is the normalized change in fluorescence, and F is the background-subtracted basal fluorescence. The $\Delta F/F$ value can be interpreted as changes in Ca^{2+} (Lev-Ram et al., 1992).

Values given in the text and in the figures are mean ± SEM of changes in the respective cell populations. Student's *t* test (for paired and unpaired observations) was used to compare the means. Drugs were applied by dissolving them to the desired final concentration in the saline and by switching the perfusion from control saline to drug-containing saline. All chemicals were from Tocris Cookson (UK), except phorbol-12,13-diacetate (PDAc) (Sigma, Italy) and bicuculline from RBI.

Results

Intrinsic and synaptic properties of the recorded cells

The large majority of the recorded neurons were electrophysiologically identified as spiny neurons. These cells had high resting membrane potential (−85 ± 5 mV), relatively low apparent input resistence (38 ± 6 MΩ) when measured at the resting potentials from the amplitude of small (<10 mV) hyperpolarizing electrotonic potentials, action potentials of short duration (1.1 ± 0.3 ms) and high amplitude (100 ± 4 mV). Most of these properties have been described previously both *in vivo* (Calabresi et al., 1990a) and *in vitro* (Kita et al., 1984; Calabresi et al., 1990b; Cepeda et al., 1994). Spiny neurons responded to a single cortical stimulation by producing an EPSP whose physiological and pharmacological characteristics have been previously described (Kita et al., 1984; Calabresi et al., 1990b, 1996). These EPSPs were almost completely abolished by 10 μM CNQX, an AMPA glutamate receptor antagonist (data not shown), the NMDA component being negligible. Another group of recorded cells had elecrophysiological characteristics that have previously been attributed to LA cholinergic

interneurons (Kawaguchi, 1993; Calabresi et al., 1998). LA cells had relatively low membrane potential (-60 ± 3 mV), high input resistance (155 ± 45 MΩ). Spontaneous firing occurred in some of these cells. In these neurons depolarizing current pulses of small amplitude (200–400 pA) and short duration (5–30 ms) elicited few action potentials followed by a long-lasting afterhyperpolarization (amplitude 8.9 ± 9 mV, duration 350 ± 130 ms). A single activation of corticostriatal fibres produced EPSPs in most of the recorded LA interneurons. Since the resting membrane potential of LA interneurons was less negative than that of spiny neurons, these EPSPs were rather small in amplitude and often triggered an action potential. Thus, in most of the experiments on LA interneurons, the cells were hyperpolarized by injecting negative current to hold the membrane potential at about -75 mV. Under this experimental condition the EPSP amplitude increased and it was possible to characterise the physiological and pharmacological properties of these potentials. Compared to medium spiny neurons, the pharmacology of the EPSP was different. In fact, in LA interneurons unlike spiny cells, bath application of 50 μM APV significantly reduced both the amplitude and the duration of the EPSP ($n = 4$; $p < 0.001$). When 10 μM CNQX was added to the medium containing APV, a further reduction of the EPSP amplitude was observed ($n = 4$, $p < 0.001$). The complete suppression of the depolarizing potential was obtained by adding 30 μM bicuculline (data not shown).

Effects of group I mGluRs activation on spiny neurons and LA interneurons

3,5-DHPG, a selective agonist for group I mGluRs, when bath-applied (up to 300 μM, 5 min), caused no detectable changes in membrane properties (resting membrane potential, input resistance, firing activity; $n = 18$, $p > 0.05$) and cortically-evoked EPSPs of spiny neurons (data not shown). Conversely, 3,5-DHPG (10–300 μM, 1 min) produced a dose-dependent and reversible membrane depolarization in LA interneurons (Fig. 1) which was coupled to increased input resistance. Activation of group I mGluRs is known to promote polyphosphoinositides (PI) hydrolysis and intracellular Ca^{2+} mobilization (Conn and Pin, 1997). Thus, we performed some combined electrophysiological and optical recordings to monitor the effects of 3,5-DHPG on intracellular Ca^{2+} concentration. As shown in figure 1, 100 μM 3,5-DHPG produced an increase in intracellular Ca^{2+} concentration in LA interneurons.

Functional interplay between iGluRs and mGluRs in spiny neurons and LA interneurons

As recently reported, activation of group I mGluRs by 3,5 DHPG enhances NMDA-responses in striatal spiny neurons via protein-kinase C (PKC) activation (Fig. 2) (Pisani et al., 1997a). Conversely, after incubation with 100 μM 3,5-DHPG (3–5 min), the NMDA-induced membrane depolarization was not potentiated in LA interneurons (Fig. 2; $n = 10$, $p > 0.05$). The depolarizing effects of 3,5-DHPG on LA cells was balanced by applying

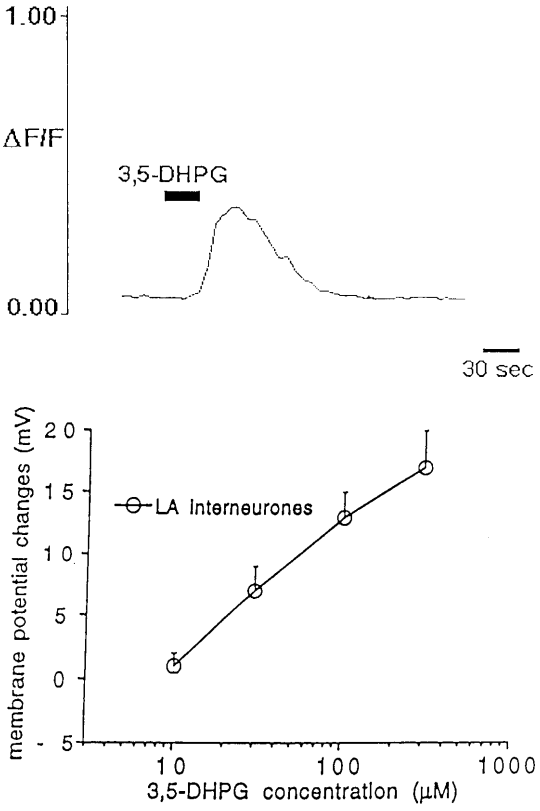

Fig. 1. 3,5-DHPG induces an increase in intracellular Ca^{2+} levels and membrane depolarization in LA interneurons. The upper graph shows that bath-application of 100 μM 3,5-DHPG (30 sec) produced a transient rise in Ca^{2+} in a large aspiny interneuron. Ca^{2+} changes are expressed in terms of changes in normalized fura-2 fluorescence intensity ($\Delta F/F$). The lower graph shows that 3,5-DHPG caused a dose-dependent membrane depolarization in LA cells (open circles). Before the application of 3,5-DHPG, LA interneurons were hyperpolarized to -75 mV in order to avoid action potential discharge during drug application

continuous hyperpolarizing current through the recording electrode. Bath-application of S-MCPG (300–500 μM), a broad spectrum antagonist of group I mGluRs prevented the 3,5-DHPG-induced enhancement of membrane depolarization caused by NMDA in spiny neurons ($n = 4$, $p < 0.01$; data not shown). PDAc, an irreversible protein kinase C activator, mimicked the potentiating action of 3,5-DHPG on NMDA responses recorded from spiny neurons ($n = 4$, $p < 0.01$), but not in LA interneurons ($n = 3$, $p > 0.05$) (Fig. 2). These data suggest the presence of a functional coupling between group I mGluRs and NMDA receptors, *via* PKC activation, expressed in striatal spiny neurons but not in LA interneurons. We also studied the effects of group I mGluR activation on combined NMDA-induced membrane potential and intracellular Ca^{2+} concentration changes following bath application of NMDA on spiny neurons. In control conditions, bath-application of 30 μM NMDA (1 min) produced reversible membrane depolarization and intracellular Ca^{2+} increase in striatal spiny neurons. After incubating the slices with 100 μM 3,5-

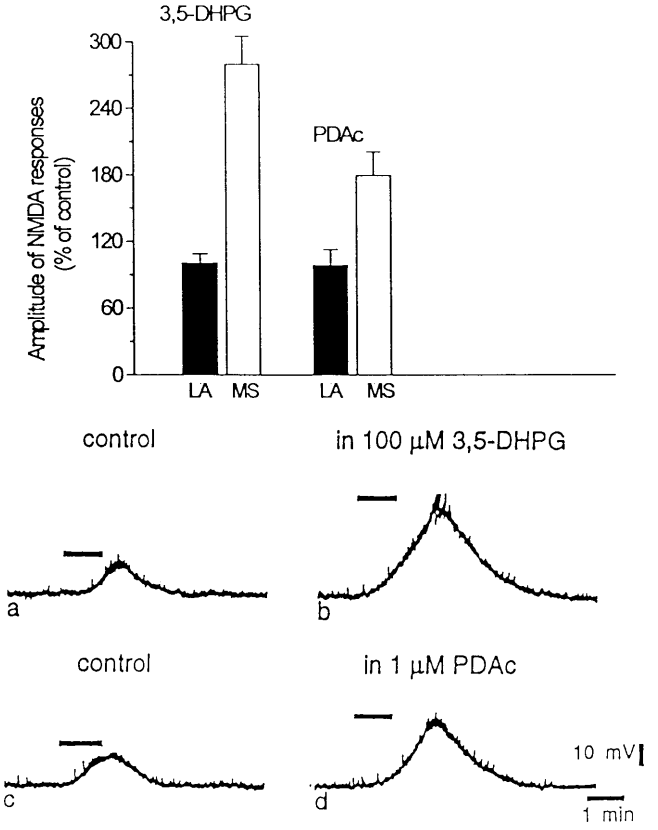

Fig. 2. Activation of mGluRs of group I and stimulation of protein kinase C enhance the NMDA-mediated responses in spiny neurons but not in LA interneurons. The experiments obtained from spiny neurons were performed in the presence of 3μM scopolamine. The graph in the upper part of the figure were obtained from LA interneurons (black bar, *LA*) and spiny neurons (white bar, *MS*). Values are expressed in terms of percent of control NMDA response (10μM, 1 min), compared to those obtained after application of 100μM 3,5-DHPG and during the application of 1μM PDAc. In the lower part of the figure, in the upper row the traces represent a single experiment showing that the membrane depolarization induced by 10μM NMDA in a spiny neuron (*a*) was increased during the application of 100μM 3,5-DHPG (*b*). The RMP (−85 mV) was constant during the experiment. Lower row: the traces represent a single experiment showing that the membrane depolarization induced by 10μM NMDA in a spiny neuron (*c*) was increased during the application of 1μM PDAc (*d*). The RMP (−86 mV) was constant during the experiment

DHPG, the same application of NMDA induced prominent and irreversible membrane potential changes and intracellular Ca^{2+} accumulation ($n = 10$, $p < 0.01$; not shown).

Group II and III activation in spiny neurons and LA interneurons

We tested the effects of agonists for group II and III mGluRs on membrane properties and corticostriatal EPSPs in spiny neurons and LA interneurons.

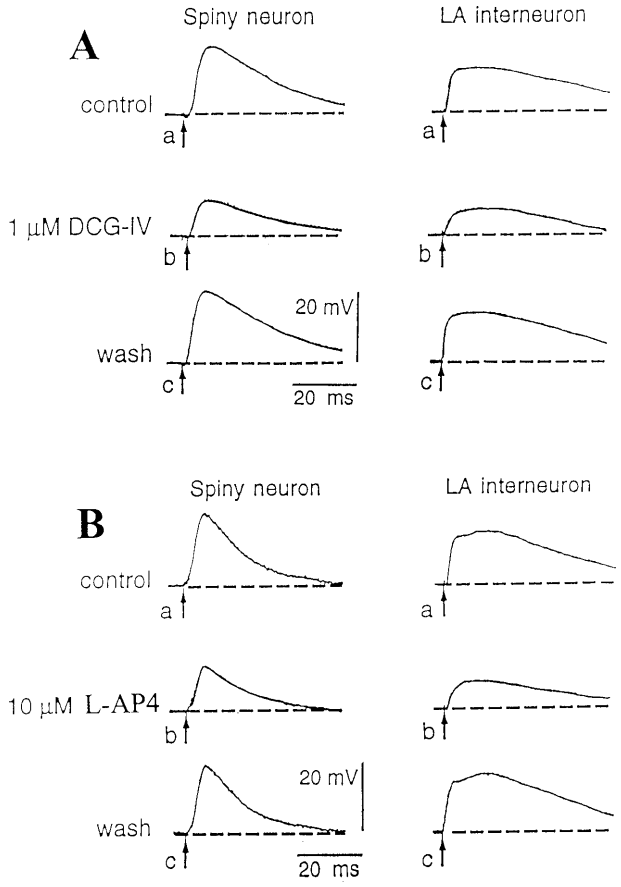

Fig. 3. DCG-IV and L-AP4 mediate a presynaptic inhibitory effect on EPSPs recorded from both spiny neurons and LA interneurons. **A** Left, the EPSP amplitude recorded in control condition from a spiny neuron (*a*) was reduced by 1 μM DCG-IV (*b*); this inhibitory effect was reversed after 15 minutes of wash out (*c*). The resting membrane potential (RMP, −84 mV) was constant during the experiment. Right, the EPSP amplitude recorded in control condition from a LA cell (*a*) was reduced by 1 μM DCG-IV (b); this inhibitory effect was reversed after 15 minutes of wash out (*c*). The resting membrane potential (RMP, −60 mV) was constant during the experiment. **B** L-AP4 mediates a presynaptic inhibitory effect on EPSPs recorded from both spiny neurons and LA interneurons. Left, the control EPSP from a spiny neuron (*a*) was reduced by 10 μM L-AP4 (*b*); this inhibitory effect was reversed after 15 minutes of wash out (*c*). The resting membrane potential (RMP, −85 mV) was constant during the experiment. Right, the EPSP amplitude recorded in control condition from a LA cell (*a*) was reduced by 10 μM L-AP4 (*b*); this inhibitory effect was reversed after 10–15 minutes of wash out (*c*). The resting membrane potential (RMP, −61 mV) was constant during the experiment

Both DCG-IV and L-AP4, agonists for group II and III mGluRs, respectively, failed to affect membrane properties of spiny (n = 21) and LA (n = 18) neurons (data not shown). Conversely, either DCG-IV or L-AP4 significantly reduced the amplitude of the EPSPs recorded in both neuronal subtypes (n = 18 for each cell type, $p < 0.01$; Fig. 3). These effects were dose-dependent and reversible after wash-out (Fig. 3). Furthermore, the inhibitory effects of

DCG-IV and L-AP4 on EPSPs recorded from both spiny and LA cells were additive, suggesting that both group II and III mGluRs are functionally important in both neuronal subtypes. Previous evidence suggests that activation of group II and III mGluRs modulates excitatory glutamatergic transmission in the striatum by inhibiting glutamate release from synaptic terminals (East et al., 1995; Pisani et al., 1997b). To verify this hypothesis in spiny neurons and LA interneurons, we considered synaptic responses to a pair of stimuli before, during and after the application of DCG-IV and L-AP4 in these two different neuronal subtypes. In these experiments interstimulus interval was of 60 msec for spiny neurons and 80 ms for LA interneurons. Paired-pulse modification of neurotransmission has been studied extensively and is attributed to a presynaptic change in release probability (Schulz et al., 1994). An increase in the ratio of the second pulse response to the first pulse response (EPSP2/EPSP1) indicates a decrease in the release probability. The decrease in transmitter release probability is consistent with the observations that manipulations depressing transmitter release usually increase the magnitude of this ratio also at corticostriatal synapses. Both DCG-IV (1 μM) and L-AP4 (10 μM) depressed corticostriatal EPSPs increasing paired-pulse facilitation (PPF) in spiny neurons and in LA interneurons, suggesting that in these inhibitory effects a presynaptic site of action was implicated ($n = 12$, $p < 0.01$; not shown).

Discussion

The present study accomplished two main goals: 1) activation of group II and III mGluRs produced similar presynaptic inhibitory effects on both striatal spiny neurons and LA interneurons and 2) activation of group I mGluRs enhances NMDA-mediated responses in spiny neurons but not in LA interneurons. The characterization of the functional effects coupled to mGluR activation in the striatum appears of primary importance for several reasons. First, ligand binding and *in situ* hybridization studies demonstrated the presence of mGluRs in the striatum (Tallaksen-Greene et al., 1998; Testa et al., 1994). In particular, mGluR5, a group I PI-linked mGluR, is highly expressed in medium spiny neurons (Testa et al., 1995). *In situ* hybridization and RNA amplification studies have shown that receptors from group II and group III mGluRs are present in rat striatum (Testa et al., 1994, 1998). Second, mGluRs have been involved in synaptic plasticity processes in the striatum, like in other brain structures (Calabresi et al., 1996; see also Conn and Pin, 1997). Third, recently, evidence began to accumulate in favour of an involvement of mGluRs in excitotoxicity. In particular, group I mGluRs have been shown to play a cooperative role in NMDA-mediated excitotoxic injury (Bruno et al., 1998). Likewise, antagonists of group I mGluRs protect against cell damage caused by NMDA receptor activation in the striatum (Bruno et al., 1999). Conversely, experimental evidence demonstrates that activation of group II and III mGluRs exerts a protective action in excitotoxic processes (Buisson et al., 1996; Conn and Pin, 1997).

Role of group II and III mGluRs

Glutamatergic EPSPs evoked by cortical stimulation were strongly reduced either in medium spiny or in LA cells by activation of both group II and III mGluRs. This effect was not coupled to changes in intrinsic membrane properties; moreover, the increase in PPF suggests an involvement of a presynaptic site of action in this effect. These data are in line with previous electrophysiological and morphological studies. It has been reported that activation of group II and III mGluRs reduces EPSPs recorded from spiny neurons (Pisani et al., 1997b). Here we extended this observation to the EPSPs recorded from LA interneurons. These electrophysiological findings are also in agreement with recent immunohistochemical data showing the presence of mGluR2/3 on the terminals of corticostriatal afferents (Testa et al., 1998), where they are thought to regulate glutamate release. It is conceivable that in the striatum group II and III mGluRs exert a powerful inhibitory action on glutamate release from cortical and thalamic inputs to the striatum, thereby limiting neuronal excitability.

Functional interaction NMDA/group I mGluR in striatal neurons

The interplay between NMDA receptors and group I mGluRs in spiny neurons and in LA interneurons might, to some extent, clarify the differential vulnerability of striatal neuronal subtypes. The positive modulation of NMDA responses by group I mGluR agonists occurs in spiny, but not in LA neurons. The postsynaptic mechanism seems to involve a rise in intracellular Ca^{2+} concentration, and activation of PKC as a second messenger. NMDA-induced membrane depolarizations, in fact, are increased by 3,5-DHPG or PDAc in spiny neurons but not in LA interneurons. Furthermore, 3,5-DHPG enhanced the intracellular Ca^{2+} increase induced by NMDA in spiny neurons. Further work is required in order to determine whether this cytosolic Ca^{2+} elevation is due to the opening of voltage-dependent Ca^{2+} channels or on the release of Ca^{2+} from internal stores. Support to the present electrophysiological findings is provided by recent morphological work. As determined by *in situ* hybridization and immunocytochemical techniques (Testa et al., 1995; Tallaksen-Greene et al., 1998), spiny cells expressing enkephalin or substance P are particularly enriched in group I mGluRs mRNA (mGluR5 > mGluR1). Interestingly, LA interneurons have been shown to express high levels of mGluR1 but not mGluR5 mRNA.

The ability to mitigate intracellular Ca^{2+} overload by endogenous buffers may also play an important role in the differential neuronal vulnerability in the striatum. In view of these experimental findings, we pursue the idea that the pharmacological modulation of this pathway might be a target for new possible therapeutic approaches to movement disorders, such as Huntington's disease and Parkinson's disease.

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