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Activation of Metabotropic Glutamate Receptors Inhibits Calcium Currents and GABA-mediated Synaptic Potentials in Striatal Neurons

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The transmitter release from GABAergic synapses is thought to be calcium (Ca^{2+}) dependent. The pharmacological modulation of Ca^{2+} currents in central GABAergic neurons may strongly affect GABA release from synaptic sites. The source of striatal GABA-containing synapses is intrinsic to the striatum and mainly originates from axon collaterals of projecting medium-spiny neurons. In order to characterize the role of metabotropic glutamate receptors (mGluRs) in the modulation of central GABA release, we have combined the study of high-voltage-activated (HVA) Ca^{2+} currents in isolated striatal neurons with the analysis of GABA-mediated synaptic potentials evoked by local stimulation in striatal slices. The mGluR agonists *t*-ACPD and 1*S*,3*R*-ACPD produced a reversible and dose-dependent decrease of both HVA Ca^{2+} currents and GABA-mediated synaptic potentials. The mGluR-mediated inhibition of GABA-mediated synaptic potentials was not coupled with changes of the membrane responses to exogenously applied GABA, suggesting an effect on the transmitter release rather than on the GABA receptor sensitivity. The reduction of Ca^{2+} currents persisted in nifedipine, but not in ω-conotoxin, supporting the involvement of an N-type Ca^{2+} channel in this pharmacological effect. The GABA-mediated synaptic potentials were greatly reduced by ω-conotoxin. The inhibitory action of 1*S*,3*R*-ACPD on residual GABA-mediated potentials was fully occluded in the presence of ω-conotoxin. In neurons dialyzed with GTP-γ-S, the reduction of HVA currents was irreversible, suggesting an involvement of a G-protein–mediated mechanism. Preincubation in staurosporine blocked neither the reduction of Ca^{2+} currents nor the inhibition of synaptic potentials induced by mGluR activation, suggesting that staurosporine-sensitive kinases are not involved in these actions. L-AP3, a noncompetitive antagonist of mGluR-mediated alteration of phosphoinositide (PI) hydrolysis, failed to block both the mGluR-mediated reduction of Ca^{2+} current and the inhibition of GABA-mediated synaptic potentials. We conclude that activation of mGluRs depresses intrastriatal GABAergic transmission and Ca^{2+} currents recorded from putative GABAergic striatal cells. We suggest that a reduction of Ca^{2+} influx in the striatal GABAergic terminal may account for the mGluR-mediated inhibition of synaptic GABA release in this structure. The modulation of GABA release by mGluRs may have a profound implication in the physiopathology of basal ganglia activity.

[Key words: calcium channels, GABA-mediated potentials, glutamate metabotropic receptors, synaptic transmission, intracellular recordings, whole-cell recordings]

It is known that a large population (>90%) of striatal neurons are GABAergic neurons projecting to the pallidum and the substantia nigra (DiFiglia et al., 1976; Kitai et al., 1979; Wilson and Groves, 1980; Somogyi et al., 1981; Chang et al., 1982). The dendrites of these neurons are covered by a large number of spines, and are therefore referred to as medium-spiny neurons. The spiny projection neurons have local axon collaterals terminating on neighboring spiny cells (Kitai et al., 1979; Wilson and Groves, 1980; Somogyi et al., 1981). The GABAergic local axon collaterals form an inhibitory feedback circuit within the striatum. As a consequence of this synaptic organization, the intrastriatal GABA release can be affected strongly by changes of the electrical activity of the GABAergic spiny neurons. At present, however, it is not well understood how the activation of different transmitter receptors on the somatic region and/or the axon terminals of medium-spiny cells may ultimately influence intrastriatal GABA release.

In several brain areas inhibition of Ca^{2+} currents has been an attractive hypothesis for the mechanism of inhibition of release by neurotransmitters. Since changes of Ca^{2+} influx at the axon terminals are difficult to study by electrophysiological techniques, the modulation of somatic Ca^{2+} currents has been assumed as a possible correlate of the transmitter release. Yet, this extrapolation, in the absence of a concomitant analysis of the synaptic activity, may generate misleading information. For this reason, we have approached the analysis of the pharmacological modulation of intrastriatal GABA release by combining recordings of Ca^{2+} currents from dissociated striatal neurons with intracellular measurements of GABA-mediated synaptic potentials from striatal slices. In particular, the aim of the present study was to characterize the possible inhibitory effects of mGluRs agonists on GABA-mediated synaptic potentials and on voltage-dependent Ca^{2+} currents recorded from putative spiny neurons. In hippocampal and cortical neurons, activation of mGluRs causes both depression of excitatory synaptic transmission (Baskys and Malenka, 1991; Desai and Conn, 1991; Desai et al., 1992) and reduction of HVA Ca^{2+} currents (Lester and Jahr, 1990; Sayer et al., 1992; Swartz and Bean, 1992;

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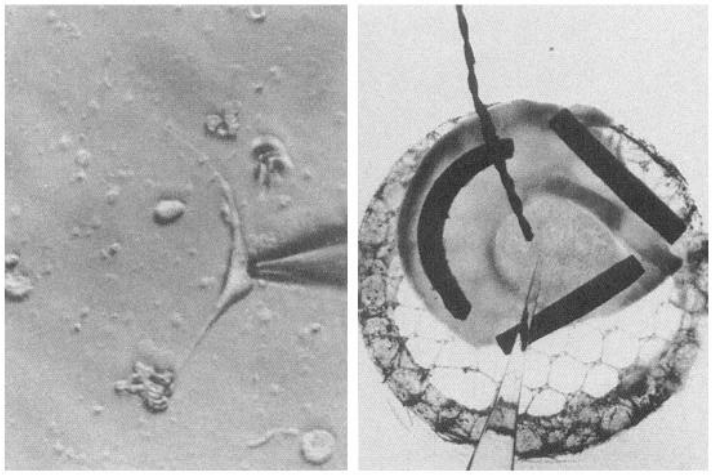

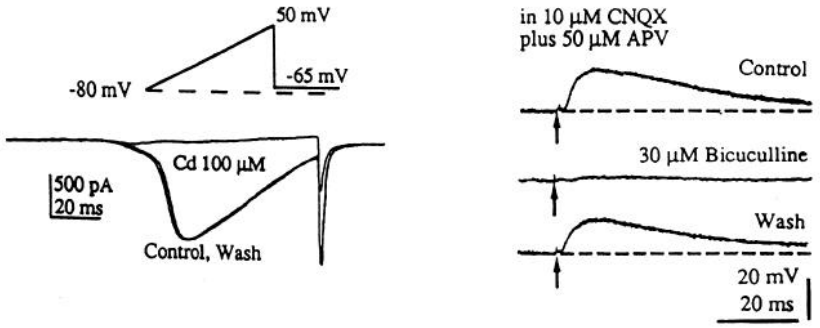

Figure 1. High-voltage-activated Ca^{2+} currents in isolated striatal neurons and GABA-mediated synaptic potentials evoked in striatal slices. *Top*, Photomicrographs of a freshly isolated striatal cell (*left*; magnification, 400×) and of a striatal slice (*right*). *Bottom: Left*, Ramp-activated (1 msec/1 mV) whole-cell Ba^{2+} currents (traces show current before, during, and after 100 μM $CdCl_2$). *Right*, Depolarizing postsynaptic potentials evoked by intrastriatal stimulation in the presence of 10 μM CNQX and 50 μM APV. Note that bicuculline (30 μM) fully abolished the synaptic potential showing that it is mediated by endogenous GABA.

Trombley and Westbrook, 1992; Sahara and Westbrook, 1993). However, the physiological and pharmacological characteristics of these actions are not homogeneous, and the functional relationship between the reduction of the somatic Ca^{2+} currents and the decrease of synaptic potentials is still unclear.

We have previously reported that activation of striatal mGluRs is involved in both short-term (Calabresi et al., 1992a) and long-term (Calabresi et al., 1992b) depression of excitatory synaptic transmission. These effects have different sensitivity to L-AP3 (Calabresi et al., 1993a) and to chronic lithium treatment (Calabresi et al., 1993b). These findings support the hypothesis of a functional heterogeneity of mGluRs as suggested by recent molecular studies (Nakanishi, 1992). Here we investigate the cellular mechanism underlying the depression of striatal GABAergic transmission caused by the activation of mGluRs; this modulation may influence the physiology of the basal ganglia and the motor behavior.

Materials and Methods

Slice preparation and intracellular recordings. Male Wistar rats (weighing 150–250 gm) were used. Rats were anesthetized with ether and killed by a heavy blow to the chest that severed major blood vessels. Coronal slices (200–300 μm) were prepared from tissue blocks of the brain with the use of a vibratome. These coronal slices included neostriatum, cortex, and corpus callosum (Fig. 1). A single slice was transferred to a recording chamber (0.5 ml vol) and submerged in a continuously flowing Krebs solution (36°C, 2–3 ml/min) gassed with 95% O_2, 5% CO_2. The composition of the solution was (in mM) 126 NaCl, 2–5 KCl, 1–2 $MgCl_2$, 1–2 NaH_2PO_4, 2.4 $CaCl_2$, 11 glucose, and 25 $NaHCO_3$.

Intracellular recording electrodes were filled with either 2 M KCl or 2 M K-acetate (30–60 MΩ). For synaptic stimulation, bipolar electrodes were used. The stimulating electrode was positioned inside striatum, close to the recording electrode (~0.5–3 mm apart). All the experiments concerning the modulation of GABA-mediated potentials by mGluRs were performed in the presence of antagonists of excitatory amino acids receptors (30–50 μM APV plus 10 μM CNQX). Intracellular potentials were recorded with an Axoclamp-2A amplifier, displayed on an oscilloscope, and stored on a digital system. The statistical significance of the experiments was evaluated with the use of Student's *t* test. 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.

Preparation of isolated cells and whole-cell recordings. Striatal neurons were dissociated from 50 male Wistar rats aged 1–4 months. Striatum was dissected under stereomicroscope from coronal slices 450 μm thick. Slices were then incubated in a HEPES-buffered Hank's balanced salt solution (HBSS), bubbled with 100% O_2 and warmed at 34°C. From 30 to 60 min later, one slice was transferred in HBSS medium supplemented with 1.5 mg/ml protease XIV (Sigma; see Mody et al., 1989). After 35–45 min of enzymatic treatment, the tissue was repeatedly rinsed in HBSS and mechanically triturated. The cell suspension was finally placed in a Petri dish mounted on the stage of an inverted microscope (Nikon). Cells were allowed to settle for 10–12 min. Neurons were chosen for recordings if presumed to be medium-spiny neurons by their morphology and size (usually bipolar, 15 μm major axis) (Fig. 1).

Whole-cell recordings were performed using pipettes (Corning 7052) pulled at a Flaming-Brown and fire polished just prior to use. Pipette resistance ranged from 3 to 8 MΩ when filled by the internal solution consisting of (in mM) *N*-methyl-D-glucamine, 160; HEPES, 40; EGTA, 10; Mg, 4; phosphocreatine, 20; ATP, 2–4; GTP, 0–0.2; leupeptin, 0.2; pH was adjusted to 7.3 with phosphoric acid, and the osmolarity was 265–275 mOsm/liter. After obtaining the whole-cell configuration, the cells were usually bathed in a medium composed of (in mM) NaCl, 135–

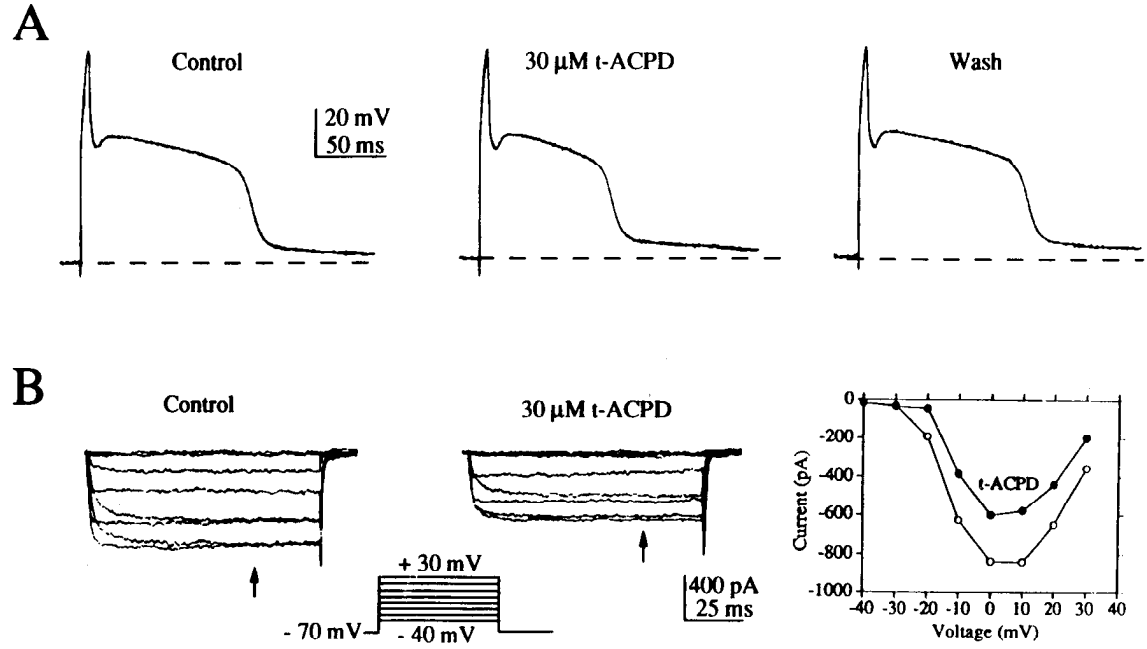

Figure 2. *t*-ACPD reduces Ca^{2+}-mediated plateau potentials as well as HVA currents. *A*, In a striatal neuron intracellularly recorded with a CsCl (2 M)-filled electrode (plus 10 mM external TEA), depolarizing steps (5 msec duration, 1 nA intensity) activated plateau potentials; 30 μM *t*-ACPD reduced the duration of the plateau potential (*middle trace*); the effect fully recovered after the interruption of the drug application (*right trace*). *B*, HVA Ca^{2+} currents were activated by voltage steps from −50 to +30 mV under control condition (*left traces*, holding potential −70 mV); 30 μM *t*-ACPD reduced HVA currents (*middle traces*; the inhibition at −10 mV was about 20%). *Right*, *I/V* plot of peak currents in control condition (*open circles*) and in the presence of the mGluR agonist (*solid circles*).

140; $BaCl_2$, 5; $CsCl_2$, 5; HEPES, 10; TTX, 0.001; pH was adjusted to 7.4 with NaOH and the osmolarity to 300–305 mOsm/liter with glucose. In a subset of recordings (n = 20), 150 mM TEA was substituted for Na. Control as well as drug solutions were applied with a linear array of six gravity-fed capillaries positioned within 500 μm of the patched neuron. Recordings were made with an Axopatch 1D at room temperature (21–22°C). Series resistance compensation (70–80%) was employed. Data were low-pass filtered (corner frequency = 5 kHz). For data acquisition and analysis, pCLAMP 5.51 running on a PC486 computer was used. Ba^{2+} currents were studied with voltage steps and ramps. Ramp speed (0.8–1 mV/msec) was chosen to maximize the agreement with the current–voltage relationship obtained with this method and that derived from short (30 msec) step depolarizations.

Drugs. Aminophosphonovalerate (APV), bicuculline, GABA, guanosine-5′-triphosphate (GTP), guanosine-5′-γ-triphosphate (GTP-γ-S), nifedipine, pronase E, staurosporine, tetrodotoxin (TTX), and tetraethylammonium (TEA) were obtained from Sigma (St. Louis, MO). ω-Conotoxin was obtained from Sigma and from Bachem (Bubendorf, CH). 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX), *trans*-(±)-1-amino-1,3-cyclopentanedicarboxylic acid (*t*-ACPD), 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD), and L-2-amino-3-phosphonopropionic acid (L-AP3) were obtained from Tocris Neuramin (Bristol, UK). Bay K 8644 was a gift of Bayer.

Results

Characterization of Ca^{2+} currents and of GABA-mediated synaptic potentials

Whole-cell patch-clamp recordings were obtained from 102 acutely dissociated striatal neurons. Recordings were taken from cells with medium-sized cell bodies that previous retrograde labeling and single-cell expression profiling have revealed to be medium-spiny projecting neurons (Surmeier et al., 1992).

As shown in Figure 1, in these cells voltage ramps from −80 mV to +50 mV elicited inward barium (Ba^{2+}) currents that were blocked by 100 μM Cadmium (Cd^{2+}), indicating that they could be attributed to permeation through Ca^{2+} channels.

Ba^{2+} was used as a charge carrier to minimize current rundown during pharmacological analysis. Previous work in these cells (Bargas et al., 1994) has shown that, in adult medium-sized neurons, currents are predominantly of the HVA type. Low-voltage-activated (LVA) currents have consistently been observed in cultured striatal cells (Bargas et al., 1991) and in 40% of isolated neurons from young animals (3–4 weeks; Hoehn et al., 1993). In our cells, obtained from adult rats (1–4 months), LVA currents were only rarely seen (7 of 90 neurons), and their small amplitude did not allow further pharmacological characterization. Even by utilizing voltage steps instead of voltage ramps and Ca^{2+} as a charge carrier instead of Ba^{2+}, LVA were only rarely observed (unpublished observations).

Intracellular recordings, obtained from corticostriatal slice preparations, revealed that intrastriatal stimulation evokes depolarizing synaptic potentials (DSPs) that are only partially blocked by glutamate ionotropic receptors antagonists (Calabresi et al., 1991, 1992a; Jiang and North, 1991). In the presence of 10 μM CNQX, an AMPA-like glutamate receptor antagonist, plus 30–50 μM APV, an NMDA receptor antagonist, intrastriatal synaptic stimulation evoked a DSP that was reversibly blocked by 30 μM bicuculline (Fig. 1). This finding indicates that endogenous GABA, acting on bicuculline-sensitive $GABA_A$ receptors, mediates part of the intrastriatal synaptic transmission. Even with electrodes that contained potassium acetate, the GABA-mediated synaptic potentials were depolarizing at the resting membrane potential (−85 ± 3 mV, n = 25). The GABA-mediated DSPs were reduced in amplitude by depolarization and reversed in polarity at −55 ± 4 mV (n = 5) when recorded with potassium acetate electrodes. In cells recorded with potassium chloride–filled electrodes, the extrapolated reversal potential was −31 ± 2 mV (n = 5).

Effects of mGluRs agonists on Ca^{2+}-mediated plateau potentials and HVA Ca^{2+} currents

Intracellular recordings obtained from striatal neurons in brain slice preparations by utilizing cesium chloride (2 M)–filled electrodes showed that, in the presence of 10 mM external TEA, brief (5–20 msec) depolarizing pulses produced Ca^{2+}-mediated plateau potentials (Misgeld et al., 1986; Calabresi et al., 1987). Bath application of *t*-ACPD (3–100 μM) produced a reversible decrease of the duration of these plateau potentials. In most of the cases (seven of nine), this effect was not coupled with significant changes of their membrane potential (Fig. 2); in two cells, the depression of the plateau potential was coupled with a slight membrane depolarization (5 and 4 mV, respectively; data not shown).

In acutely dissociated striatal cells, Ca^{2+} currents were isolated by blocking voltage-dependent sodium channels with TTX and by virtually eliminating potassium currents (see Materials and Methods). As shown in Figure 2, from an holding potential of −70 mV, 100 msec depolarizing steps of progressively increasing amplitude (from −50 up to +30 mV) evoked sustained inward Ba^{2+} currents through HVA Ca^{2+} channels. The current showed little inactivation even with 400 msec voltage jumps (data not shown). The inward current activated above −40 mV, peaked at 0 mV, and then declined (see *I/V* plot in Fig. 2). Rapid perfusion with *t*-ACPD (3–300 μM) reversibly decreased this HVA Ca^{2+} current in the large majority of cells (43 of 45). In the presence of *t*-ACPD there was a minimal change in the current time course, and the current–voltage plot showed a near-uniform current suppression at all voltages, suggesting that the activation parameters are not shifted along the voltage axis (plot in Fig. 2). The inhibitory action of *t*-ACPD on Ca^{2+} currents was also evident when studied utilizing voltage ramps (Fig. 3). The *t*-ACPD–mediated inhibition of the HVA Ca^{2+} current was dose dependent; the minimal effective concentration was 3 μM, whereas the effect was maximal at 100 μM (Fig. 3). The inhibitory action of *t*-ACPD on HVA currents was mimicked by the active isomer 1*S*,3*R*-ACPD, which showed a dose-dependent curve similar to that observed for *t*-ACPD (data not shown). In 15 neurons, we used 2.4 mM external Ca^{2+} instead of Ba^{2+} as the charge carrier for HVA currents; dose–response curves for both agonists were similar to those observed when 5 mM Ba^{2+} were utilized (data not shown). In particular, also in the presence of Ca^{2+} a maximal inhibition was obtained with 100 μM *t*-ACPD (−36.2 ± 3%, $n = 4$).

Effect of mGluRs agonists on GABA-mediated synaptic potentials

As shown in Figure 3, *t*-ACPD induced a dose-dependent decrease of the GABA-mediated synaptic potential evoked in striatal slices by intrastriatal stimulation. In most of the cases this effect was not coupled with significant changes of membrane potential and input resistance of the recorded neurons (18 of 22). In the remaining cells, a slight (3–5 mV) membrane depolarization was observed during the application of *t*-ACPD (data not shown). The minimal concentration required to obtain significant inhibition of GABA-mediated DSPs was 3 μM, whereas 100 μM was the dose producing maximal inhibition of the synaptic potentials (Fig. 3). As well as for the inhibition of HVA Ca^{2+} currents, also the *t*-ACPD–mediated depression of DSPs was mimicked by 1*S*,3*R*-ACPD ($n = 9$). Also in this case, the dose–response curve was similar to that obtained for *t*-ACPD.

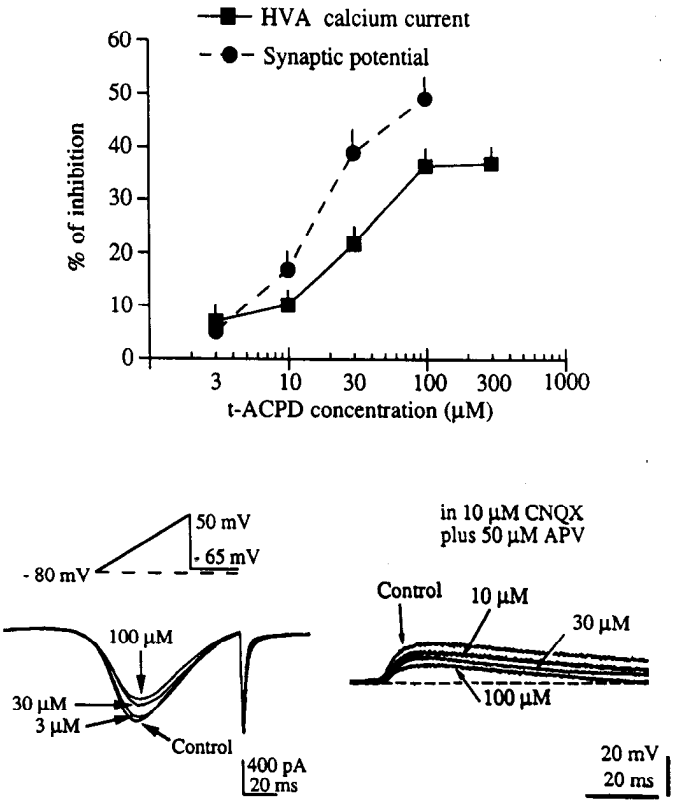

Figure 3. Dose–response curves of mGluR-mediated effects on Ca^{2+} currents and GABA-mediated synaptic potentials. *Top*, Percentage of inhibition of HVA Ca^{2+} currents and of GABA-mediated synaptic potentials by different concentrations of *t*-ACPD. Each point represents the mean of at least four experiments. *Bottom*, Representative dose-dependent modulation by the mGluR agonist on ramp-activated whole-cell Ba^{2+} currents (*left*) and GABA-mediated postsynaptic potentials (*right*).

In order to study whether the ACPD-mediated depression of the DSPs was caused by changes of $GABA_A$ receptor sensitivity, we characterized the membrane responses of striatal neurons to the application of exogenous GABA before and during the application of *t*-ACPD. As shown in Figure 4, activation of mGluRs by *t*-ACPD or by 1*S*,3*R*-ACPD (10–100 μM) affected neither membrane depolarization ($n = 6$) nor inward current ($n = 6$) caused by brief applications of exogenous GABA (300 μM). In fact, in control condition the GABA-induced membrane depolarization and inward current were, respectively, 16 ± 5 mV ($n = 6$) and +320 ± 80 pA ($n = 6$). In ACPD, the GABA-induced depolarization and inward current were, respectively, 17 ± 5 mV ($n = 6$) and 305 ± 95 pA ($n = 6$). These differences were not statistically significant, suggesting that the postsynaptic sensitivity of $GABA_A$ receptor is not significantly altered by mGluR activation.

Type of HVA Ca^{2+} channels inhibited by the activation of mGluRs

Striatal neurons have multiple types of HVA Ca^{2+} channels (Bargas et al., 1991, 1994; Hoehn et al., 1993). At present, the best distinction of these subtypes of these Ca^{2+} channels is obtained by utilizing a pharmacological approach. For this reason, we have utilized the dihydropyridine (DHP) agonist Bay K 8644, which is known to promote long-lasting Ca^{2+} tail current in central neurons (Nowicky et al., 1985). Also in striatal cells, Bay

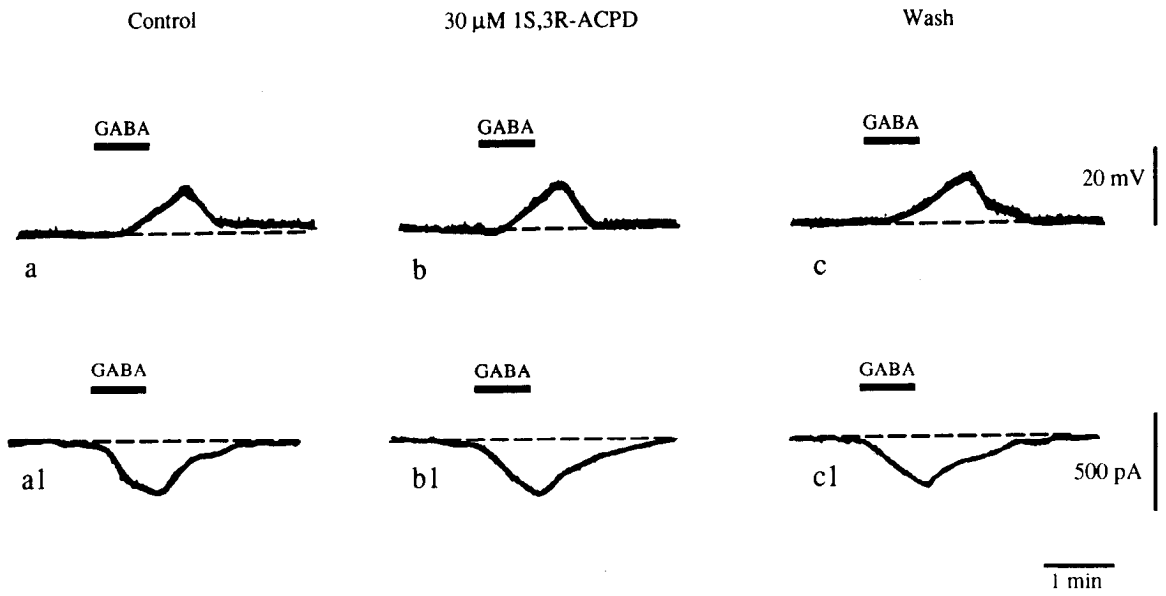

Figure 4. The mGluRs agonists do not modify the postsynaptic responses to exogenously applied GABA in striatal slice: current-clamp (*top*) and voltage-clamp (*bottom*) recordings of neuronal responses to GABA application. *a/a1,* Responses in control condition. *b/b1,* Responses in the presence of 30 μM 1*S*,3*R*-ACPD. *c/c1,* Wash. Neither GABA-mediated depolarization nor GABA-mediated inward current was significantly affected by the mGluR agonist.

K 8644 (2 μM) enhanced both HVA Ca^{2+} current and Ca^{2+} tails (Fig. 5*A*). In the presence of Bay K 8644, *t*-ACPD was still effective in producing inhibition of HVA currents ($n = 5$). However, the activation of mGluRs did not alter the Ca^{2+} tails recorded in the presence of the DHP agonist (Fig. 5*B*; $n = 5$), suggesting that L-type Ca channels are not strongly involved in the mGluR-induced modulation of HVA Ca^{2+} currents. A further evidence against a role of L-type Ca^{2+} channels in the mGluR-mediated modulation of HVA Ca^{2+} currents was obtained from experiments utilizing the DHP antagonist nifedipine (5 μM). As shown in Figure 5*C*, nifedipine produced a significant reduction of HVA currents, yet the mGluR-mediated response was not significantly altered by nifedipine. On the other hand, ω-conotoxin, an N-type Ca-channel antagonist, reduced by itself HVA currents and fully occluded the mGluR-mediated action on these currents (Fig. 5*D*). These findings suggested that an N-type, rather than an L-type Ca^{2+} channel, is involved in the modulatory action of mGluRs.

Effects of ω-conotoxin on the GABA-mediated synaptic potentials and on the inhibitory action of 1S,3R-ACPD

Since ω-conotoxin was able to occlude the action of mGluR agonists on Ca^{2+} channels, we also tested the role of ω-conotoxin–sensitive channels in the generation of GABA-mediated synaptic potentials. Bath application of 5 μM ω-conotoxin produced a large reduction of GABA-mediated synaptic potentials ($-81 \pm 13\%$, $n = 5$; see Fig. 6*Aab,B*). We also tested the inhibitory action of 1*S*,3*R*-ACPD in the presence of ω-conotoxin. For this reason, considering that ω-conotoxin per se, in some cases, almost completely abolished the synaptic potentials, before the application of 1*S*,3*R*-ACPD we usually had to increase the intensity of the synaptic stimulation. Under this condition, 1*S*,3*R*-ACPD (30–100 μM) did not cause significant reduction of synaptic potentials ($n = 4$, $p > 0.05$; Fig. 6*Acd,C*). However, even in this condition, bath application of 30 μM bicuculline fully abolished the GABA-mediated synaptic potentials ($n = 3$; Fig. 6*Ae*).

Coupling between receptor and channel involves G-proteins

We tested the involvement of a G-protein in the coupling between mGluRs and HVA Ca^{2+} channels in striatal neurons by comparing cell dialyzed with GTP with cells dialyzed by its nonhydrolyzable analog GTP-γ-S. This approach has previously been utilized in the study of G-protein–mediated pharmacological actions in other central neurons. As earlier shown for hippocampal and cortical cells (Lester and Jahr, 1990; Sayer et al., 1992; Swartz and Bean, 1992), in striatal neurons dialyzed for 6–10 min with 300 μM GTP-γ-S, the inhibition of HVA Ca^{2+} currents by mGluR activation was irreversible. In fact, only ACPD applications occurring within 2–5 min from the onset of the intracellular dialysis were fully reversible, while application of mGluR agonists after this period produced irreversible effects on HVA currents ($n = 6$, Fig. 7*A*). In contrast, in neurons dialyzed with 300 μM GTP, the suppression of HVA currents by mGluR agonists was readily reversible during all the recording time ($n = 5$, Fig. 7*B*). These findings suggest that in striatal neurons, as in other neuronal types, the mGluR-mediated modulation of HVA currents involves a G-protein–linked mechanism.

Lack of effect of staurosporine on mGluR actions on HVA currents and GABA-mediated synaptic potentials

G-protein–coupled receptors can act via diffusible second messengers (Trautwein et al., 1986; Dunlap et al., 1987) or via the direct interaction with the α subunit of the ion channel (Brown and Birnbaumer, 1988; Lipscombe et al., 1989; Toselli et al., 1989). We examined whether protein kinase activation was required for coupling of mGluRs to Ca^{2+} channels and for the inhibition of GABA-mediated synaptic transmission by mGluR

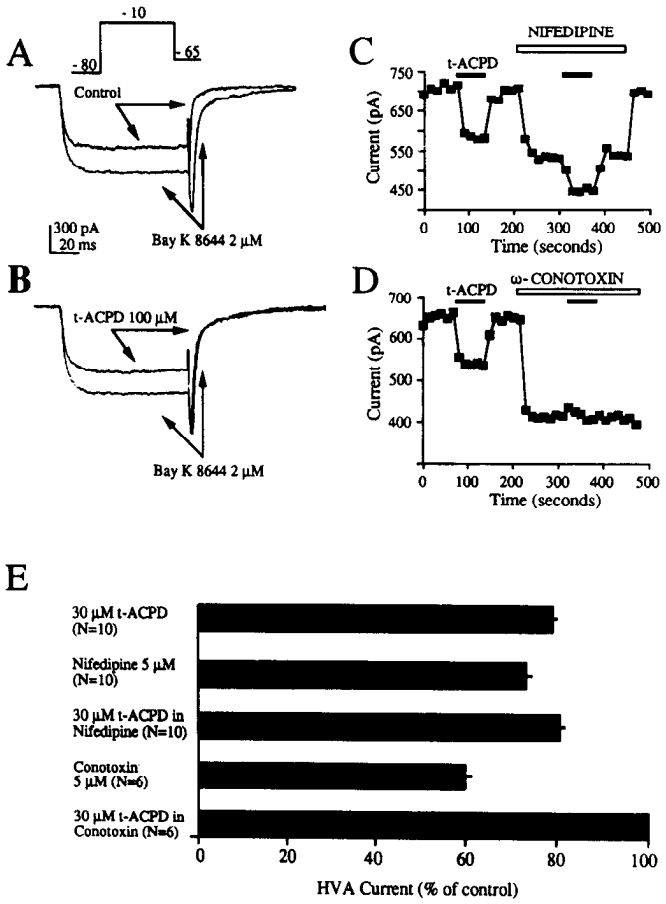

Figure 5. Type of HVA Ca^{2+} channel involved in mGluR-mediated modulation. *A* and *B*, The slow tail currents enhanced by the DHP agonist Bay K 8644 are not suppressed by *t*-ACPD. *A*, Ba^{2+} currents evoked by a step pulse from −80 to −10 mV were increased in the presence of 2 μM Bay K 8644; note the long-lasting tail at −65 mV (*arrows*). *B*, *t*-ACPD at 100 μM decreased Ba^{2+} currents without significantly affecting the Bay K 8644–induced tail (*arrows*; same finding in other four neurons). *C* and *D*, Representative responses to mGluR agonist in the presence of 5 μM nifedipine and 5 μM ω-conotoxin. *C*, Persistence of the *t*-ACPD–mediated modulation in nifedipine (analogous findings in other nine neurons). *D*, ω-Conotoxin suppressed the *t*-ACPD–mediated modulation (same finding in other five cells). *E*, The histograms summarize the percentage of modulation of striatal HVA Ca^{2+} currents by different pharmacological agents.

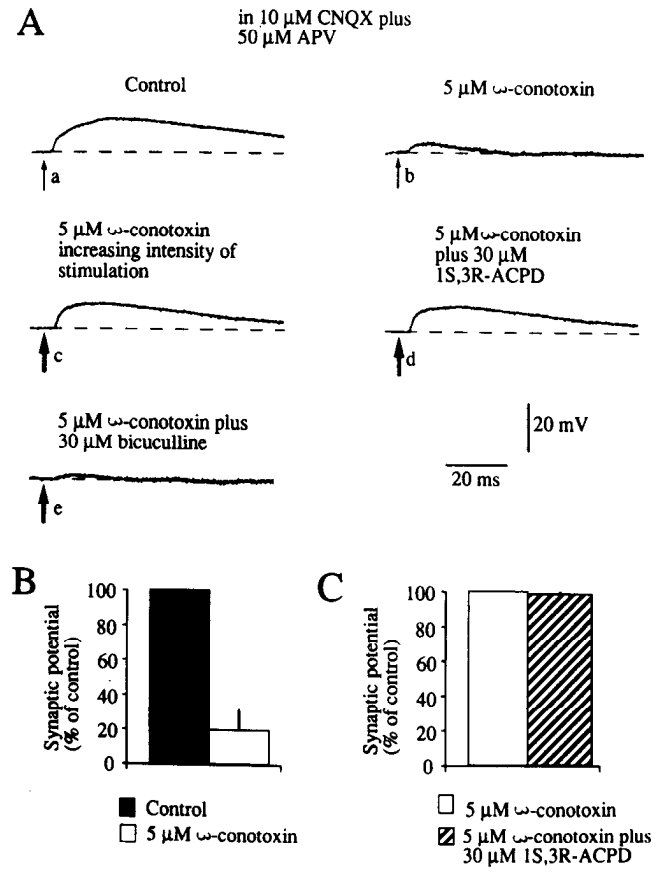

Figure 6. ω-Conotoxin decreases GABA-mediated potentials and occludes the action of 1*S*,3*R*-ACPD. *A*, Depolarizing postsynaptic potential evoked by intrastriatal stimulation in the presence of 10 μM CNQX and 50 μM APV (*a*). ω-Conotoxin (5 μM) greatly reduced the GABA-mediated synaptic potentials (*b*). In the presence of ω-conotoxin, the stimulus intensity was enhanced to restore a consistent synaptic potential (*c*). Under this condition, 30 μM 1*S*,3*R*-ACPD was ineffective on the GABA-mediated potentials (*d*). Bicuculline (30 μM) almost abolished the GABA-mediated synaptic potentials in the ω-conotoxin–added bath (*e*). *B*, The histogram shows the effect of ω-conotoxin in reducing GABA-mediated synaptic potentials (n = 5). *C*, The histogram shows that, in the presence of ω-conotoxin, the inhibitory action of 1*S*,3*R*-ACPD is abolished (for details, see text).

agonists. Therefore, we incubated (for at least 10–15 min) both dissociated neurons and striatal slices in a medium containing 50 nM staurosporine, a kinase inhibitor (Hidaka and Kobayashi, 1992), which at this concentration is able to block striatal LTD (Calabresi, unpublished data). As shown in Figure 8, incubation in staurosporine significantly affected neither mGluR-mediated inhibition of HVA currents (n = 7) nor ACPD-mediated depression of GABAergic transmission (n = 10), suggesting that staurosporine-sensitive protein kinases are not involved in these effects produced by mGluR activation.

Lack of effect of L-AP3 on mGluR-mediated actions on HVA currents and GABA-mediated synaptic potentials

L-AP3 has been reported to block noncompetitively the phosphoinositide hydrolysis mediated by the activation of mGluRs (Schoepp et al., 1990) and to alter synaptic plasticity in different brain areas (Stanton et al., 1991; Zheng and Gallagher, 1992; Calabresi et al., 1993a). Yet, this antagonist has been reported not to be effective in counteracting some electrophysiological effects induced by mGluR agonists in different brain areas (Calabresi et al., 1993a; Schoepp and Conn, 1993). We tested the possibility that L-AP3 might antagonize mGluR-induced inhibition of HVA Ca^{2+} currents and of GABA-mediated synaptic potentials in the striatum. As shown in Figure 9, 100 μM L-AP3 did not affect the mGluR-induced depression of HVA currents (n = 7). However, higher concentrations of L-AP3 (300–500 μM) in some cells (two of five) caused a reduction (−12% and −18%) of HVA currents.

Similarly, the inhibition of GABA-mediated potentials by *t*-ACPD was not blocked by the incubation of the slice in 30 μM L-AP3 (Fig. 9; n = 5). L-AP3 (50–100 μM) produced by itself a depression of GABA-mediated synaptic transmission (−15 ± 5 mV, n = 5). Even in this condition, the mGluR agonist–mediated inhibition of GABA-mediated potentials was not significantly affected (n = 4; data not shown).

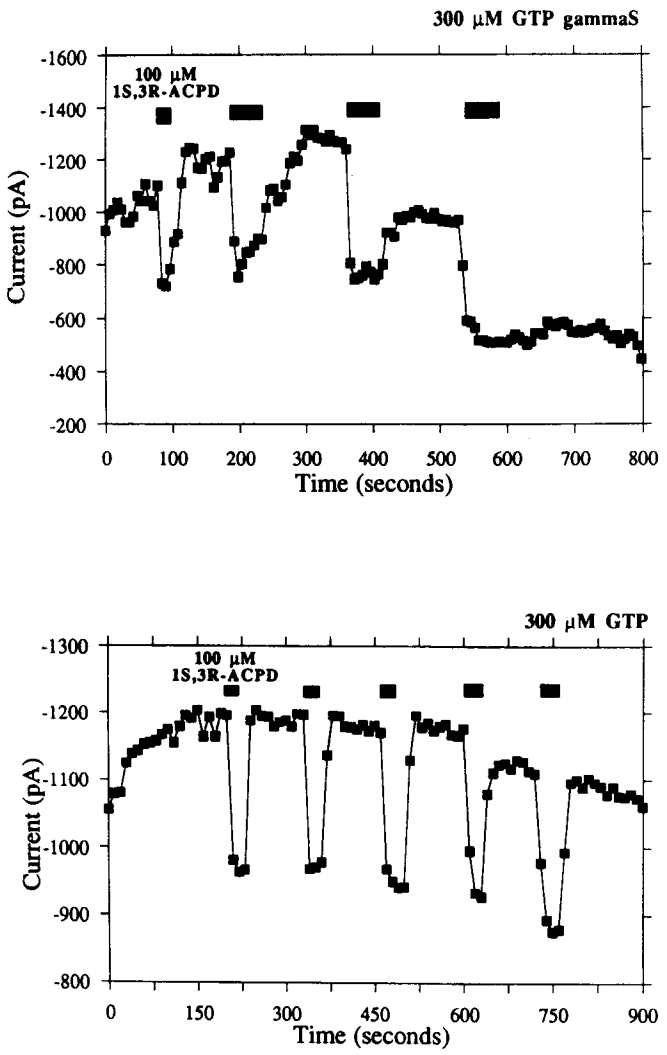

Figure 7. A G-protein couples mGluRs to Ca^{2+} channels. *A*, In a cell dialyzed with 300 μM GTP-γ-S, only the first two applications of 100 μM 1*S*,3*R*-ACPD reversed (note the slight run-up of the current); however, after 7–8 min of dialysis, the current modulation was irreversible (analogous finding in other four neurons). *B*, In a cell dialyzed with 300 μM GTP, the modulation of Ba^{2+} currents by 100 μM 1*S*,3*R*-ACPD was still reversible after five applications.

Discussion

Heterogeneity of mGluRs

Although the first studies concerning the function of mGluRs in central neurons have shown that activation of these receptors causes excitatory effects (for reviews, see Schoepp et al., 1990; Miller, 1991; Schoepp and Conn, 1993), recent studies have provided evidence in favor of more complex roles of these receptors in the brain. Furthermore, a variety (at least six subtypes) of mGluRs have been cloned recently (for a review, see Nakanishi, 1992). Some of the responses mediated by mGluRs involve intracellular Ca^{2+} mobilization as a consequence of an altered PI metabolism (Schoepp and Conn, 1993). L-AP3 is an effective antagonist of these responses (Irving et al., 1990). L-AP3–sensitive mGluRs have been implicated in the formation of synaptic plasticity in hippocampus (Otani and Ben-Ari, 1991), septum (Zheng and Gallagher, 1992), cerebellum (Linden et al., 1991), and striatum (Calabresi et al., 1992b). A major role of L-AP3–sensitive mGluRs in the generation of long-term changes of synaptic function has been recently provided in the striatum (Calabresi et al., 1993a); lithium treatment, a procedure that is known to alter PI metabolism and intracellular Ca^{2+} mobilization (Nahorski et al., 1991), blocks the formation of striatal LTD (Calabresi et al., 1993b).

Several other actions mediated by mGluRs activation are not sensitive to L-AP3. Among these, the inhibition of excitatory synaptic transmission (Desai et al., 1992; Glaumm and Miller, 1992; Calabresi et al., 1993a; Lovinger et al., 1993) and the depression of Ca^{2+} currents (Sayer et al., 1992). The L-AP3–insensitive responses have been linked either to a direct interaction between the G-protein and the ion channel or to an inhibitory effect on cAMP cascade (Nakanishi, 1992). Taken together, these findings suggest that the mGluRs that mediate the short- and long-term modulation of synaptic transmission have rather different physiological and pharmacological properties.

Modulation of GABA-mediated synaptic potentials by mGluRs

Together with the reduction of synaptic transmission mediated by excitatory amino acids, mGluR-mediated reduction of GABAergic synaptic potentials has been described in the striatum (Calabresi et al., 1992a), nucleus tractus solitarius (Glaumm and Miller, 1992), and hippocampus (Desai and Conn, 1991). In the hippocampus, the mGluR-mediated reduction of inhibitory postsynaptic potentials was partially ascribed to concomitant decrease of synaptic excitation of GABAergic interneurons, thus leading to disinhibition. In contrast, we have shown that in the striatum the reduction of GABA-mediated synaptic potentials was observed even in the presence of ionotropic glutamate receptor antagonists, a condition that allows the pharmacological isolation of GABA-mediated synaptic potentials. Furthermore, the inhibition of striatal GABA-mediated synaptic potentials was not coupled with significant changes of the membrane responses to the applications of exogenous GABA; therefore, the sensitivity of GABA receptors located on medium-spiny cells does not seem to be altered by the activation of mGluRs. These findings, taken together, strongly support the hypothesis that mGluR agonists reduce the release of GABA in the striatum.

Reduction of HVA Ca^{2+} currents by mGluRs activation and its implication for GABA release

Anatomical and functional data indicate that a significant part of the recurrent GABAergic innervation in the striatum is provided by axon collaterals originating from medium-spiny projecting neurons (DiFiglia et al., 1976; Kitai et al., 1979; Somogyi et al., 1981). In fact, GABAergic aspiny interneurons represent only a minority of the neuronal population of the mammalian striatum (Wilson and Groves, 1980). Thus, the modulation of HVA Ca^{2+} currents recorded from medium-spiny cells may have a profound impact in the control of GABA release within the striatum. In the present study we have shown that agonists of mGluRs, at approximately the same doses that inhibited GABA-mediated potentials, reduced HVA Ca^{2+} currents from these neurons.

A reduction of HVA Ca^{2+} currents by mGluRs agonists have been previously reported in hippocampal (Lester and Jahr, 1991; Swartz and Bean, 1992) and cortical (Sayer et al., 1992) neurons. However, a main difference concerning the identification of the subtype of HVA Ca^{2+} channels modulated by mGluRs arose from these studies. Whereas in hippocampal cells N-type channels were implicated in this modulatory action (Swartz and

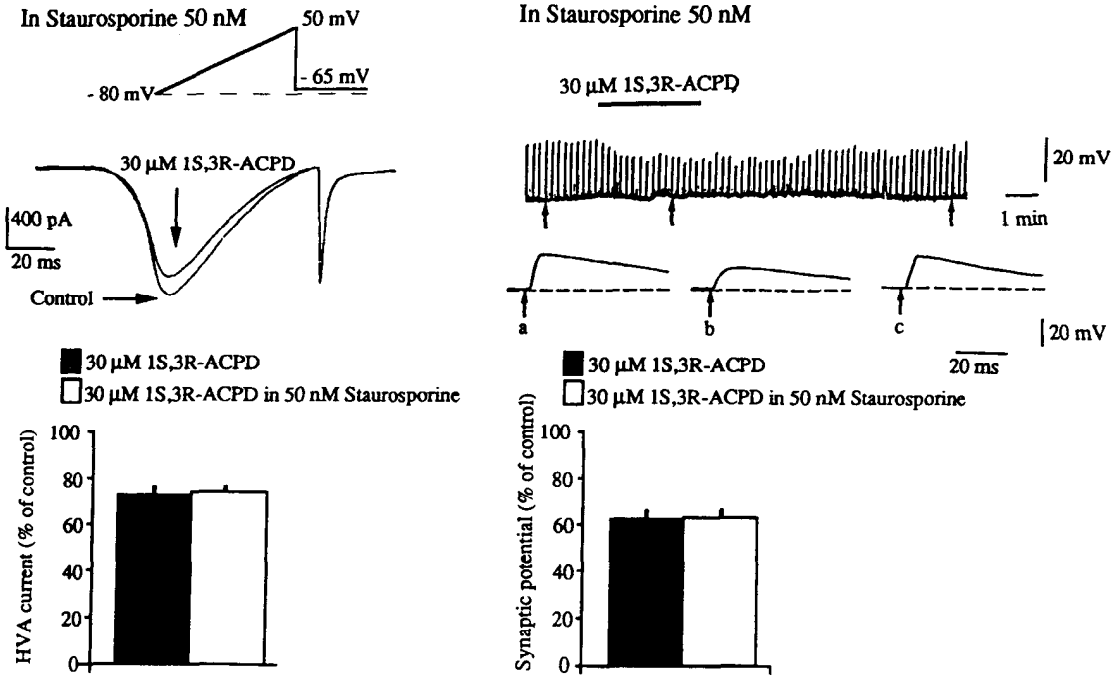

Figure 8. The mGluR-mediated modulation of HVA Ca^{2+} channels and GABA potentials is not prevented by preincubation in staurosporine. *Left*, 1*S*,3*R*-ACPD at 30 μM inhibited by about 19% the ramp-activated Ba^{2+} currents despite prolonged application (10 min) of 50 nM staurosporine. The histogram *below* shows that no significant difference in the ACPD-mediated inhibition of HVA currents is detected between control (n = 10) and pretreated (n = 7) cells. *Right*, The chart record at low speed (*upper trace*) shows the membrane potential (−85 mV) and the GABA-mediated potentials (*upward deflections*) of striatal cell recorded from a slice incubated in 50 nM staurosporine. In the *lower traces* synaptic potentials selected before (*a*), during (*b*), and after (*c*) ACPD application are shown at higher sweep speed. The histogram *below* shows the efficacy of mGluRs agonist in reducing GABA potentials even in the presence of staurosporine (n = 10).

Bean, 1992), in cortical neurons L-type channels were involved in the effects of ACPD (Sayer et al., 1992). Our data suggest that the inhibitory effects of mGluRs on striatal HVA currents are sustained by the modulation of N-type channels. In fact, ω-conotoxin, but not nifedipine, occluded the mGluR-mediated inhibition of HVA Ca^{2+} currents. In agreement with these evidences, in the present report we have also shown that ω-conotoxin dramatically decreases GABA-mediated synaptic poten-

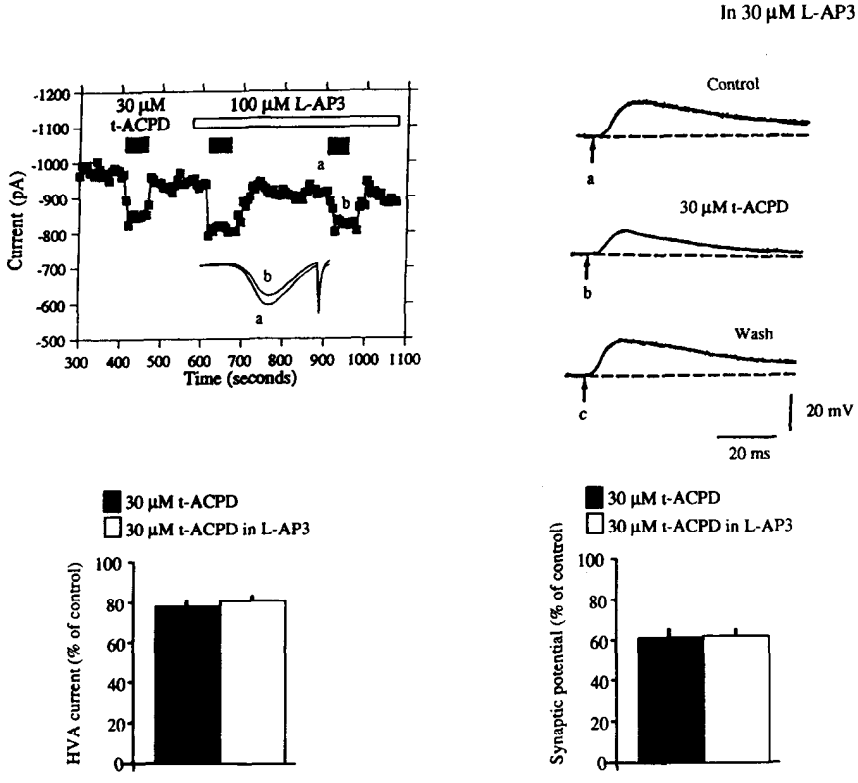

Figure 9. ACPD effects are not antagonized by L-AP3. *Left*, Time course of the effect of *t*-ACPD on HVA currents before and after L-AP3 (100 μM) application is shown. The *inset* shows representative ramp-activated currents (0.2 Hz, 1 mV/1 msec). The histogram *below* compares the efficacy of the mGluR agonist in control and L-AP3–treated preparations (n = 7); no significant difference was observed. *Right*, *t*-ACPD responses on GABA-mediated potentials evoked in a striatal slice were not antagonized by 30 μM L-AP3 are shown. The histogram *below* shows the results obtained from similar experiments (n = 5).

tials and fully occludes the inhibitory effects of mGluR activation on these potentials. Yet, the percentage block of GABA-mediated DSPs by ω-conotoxin is much greater than the percentage inhibition of HVA. This difference might be explained by two considerations: (1) the nonlinear relationship existing between presynaptic Ca^{2+} entry and transmitter release (Takahashi and Momiyama, 1993); (2) the possibility that ω-conotoxin–sensitive channels are differentially distributed between somatic region and axon terminals. However, further biochemical and physiological studies should be performed in order to investigate the role of ω-conotoxin–insensitive Ca^{2+} in the control of GABA release within the striatum (Pin and Bockaert, 1990). According with this consideration, in our recordings, a significant proportion of HVA currents are blocked neither by DHP antagonists nor by ω-conotoxin. Interestingly, this finding has been previously reported for younger striatal cells (Hoehn et al., 1993). These observations suggest that either P-type channel (Hillman et al., 1991) or other yet unidentified types of Ca^{2+} channels contribute to HVA Ca^{2+} currents in the striatum. Recently, it has been shown that P-type Ca^{2+} channels may play a role in the control of GABA synaptic potentials in different CNS structures (Takahashi and Momiyama, 1993; Toth et al., 1993). Nevertheless, our finding that the large part of the striatal GABA-mediated synaptic potential is ω-conotoxin sensitive seems to suggest that P-type channels do not play a major role in striatal GABA-mediated transmission.

Possible postreceptor mechanisms underlying mGluR-mediated modulation of HVA channels

In the presence of internal GTP-γ-S, the HVA current suppression by mGluR agonists was irreversible, suggesting a G-protein involvement in the observed modulation. Furthermore, the speed and reversibility of ACPD action on HVA currents were similar to those of other transmitters that have been found to inhibit Ca^{2+} channels via G-protein–mediated processes (Brown and Birnbaumer, 1988; Lipscombe et al., 1989; Toselli et al., 1989). In CA3 pyramidal neurons, Swartz and Bean (1992) have previously described similar physiological and pharmacological characteristics of the mGluR-mediated action on HVA currents.

In most of our whole-cell recordings, high concentrations of the Ca^{2+} chelator, EGTA, were present in the internal dialyzing solution and Ba^{2+} replaced Ca^{2+} as charge carrier of HVA channels. Therefore, we can hypothesize that mobilization of internal Ca^{2+} is not a limiting step in the modulation of HVA currents by ACPD. Further evidence in favor of this hypothesis was the finding that the efficacy of the inhibitory effect of ACPD in the presence of external Ca^{2+} was similar to that observed in the presence of Ba^{2+}. Differently from our observation, the mGluR-mediated modulation in neocortical neurons (Sayer et al., 1992) was relatively slow and required the presence of Ca^{2+} in the external medium, suggesting differential transduction mechanisms between cortical and striatal neurons. It is interesting to stress that, in hippocampal neurons, the kinetics of ACPD-mediated modulation of HVA currents were dependent upon Ca^{2+} buffering; only the increase in the steady state intracellular Ca^{2+} could in fact reveal a slow component of the inhibition, which presumably involved L-type channels (Sahara and Westbrook, 1993).

In this study, we have also shown that staurosporine, which is known to block protein kinase C (Hidaka and Kobayashi, 1992), does not alter either GABA-mediated synaptic potentials or HVA currents. Our findings, however, do not rule out other possible postreceptor mechanisms such as increase in cAMP accumulation (Winder and Conn, 1993) and inhibition of cAMP formation (Cartmell et al., 1992; Schoepp et al., 1992). Nevertheless, it should be stressed that both these effects have been shown to be blocked by L-AP3 (Schoepp and Johnson, 1992; Winder and Conn, 1992), which, at least at the concentration used in our experiments, did not significantly affect the mGluR-mediated modulation of GABA-mediated potentials and HVA currents.

Functional implications

Our results show that L-AP3–insensitive mGluRs modulate ω-conotoxin–sensitive HVA Ca^{2+} channels as well as GABA-mediated potentials in the striatum. We propose that these two actions can be functionally linked. In fact, the inhibition of Ca^{2+} conductances, if also occurring at the axon terminals, may produce a decrease of the GABA release within the striatum. As previously described (Calabresi et al., 1992a; Lovinger et al., 1993), mGluRs mediate also a reduction of glutamate release from corticostriatal terminals.

In addition to these effects, we have also recently shown that activation of mGluRs may play a role in the generation of striatal LTD. However, the mGluRs involved in this form of synaptic plasticity seem to be functionally and pharmacologically different from those involved in short-term modulation of transmitter release. In fact, L-AP3 blocks the long-term changes of synaptic transmission induced by tetanic stimulation of corticostriatal pathway but it does not alter the mGluR-mediated inhibition of glutamate and GABA release. All these findings taken together suggest a complex modulatory role of mGluRs in the physiology of the striatum.

It has been recently shown that the mGluR2 subtype is involved in the mGluR-mediated regulation of GABA-mediated synaptic potentials in olfactory bulb (Hayashi et al., 1993). It is possible that an mGluR2/mGluR3 subgroup may also contribute to the effects we have observed in the present study. Further studies in order to address this issue are in progress in our laboratory.

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