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METABOTROPIC GLUTAMATE RECEPTORS MODULATE *N*-METHYL-D-ASPARTATE RECEPTOR FUNCTION IN NEOSTRIATAL NEURONS

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Abstract—The functional roles played by metabotropic glutamate receptors in the neostriatum is just beginning to be examined. One possibility, raised by previous studies, is that metabotropic glutamate receptors act to modulate responses mediated by ionotropic glutamate receptors. In the present study, we examined this possibility in a neostriatal brain slice preparation using intracellular recording and iontophoretic techniques.

We found that the iontophoretic application of the metabotropic glutamate receptor agonist 1-amino-cyclopentane-1,3-dicarboxylic acid markedly attenuated both the amplitude and duration of excitatory responses induced by the iontophoretic application of *N*-methyl-D-aspartate. These inhibitory effects were stereo-selective and relatively long-lasting. The metabotropic glutamate receptor antagonist 2-amino-3-phosphonopropionic acid applied either iontophoretically or in the bath prevented the inhibitory effects of 1-amino-cyclopentane-1,3-dicarboxylic acid. The inhibitory action of 1-amino-cyclopentane-1,3-dicarboxylic acid was specific to *N*-methyl-D-aspartate, as 1-amino-cyclopentane-1,3-dicarboxylic acid had no consistent action on the responses evoked by the iontophoretic application of glutamate, amino-3-hydroxy-5-methyl-4-isoazolepropionic acid or quisqualate. Bath application of 1-amino-cyclopentane-1,3-dicarboxylic acid inhibited the evoked depolarizing postsynaptic potentials recorded in neostriatal cells. Thus, activation of metabotropic glutamate receptors may play an important role in modulating *N*-methyl-D-aspartate receptor function in neostriatal neurons.

The mammalian neostriatum plays a critical role in motor function. The major source of excitatory innervation of the neostriatum are projections from the cerebral cortex which utilize the excitatory amino acid glutamate (or possibly aspartate) as a transmitter.[18,39] There are two general forms of glutamate receptors: (i) ionotropic receptors (iGluR), which are ligand-gated cation channels, and (ii) metabotropic receptors (mGluR), which are coupled to various signal transduction processes through GTP-binding proteins.[41,61] The iGluRs can be further classified according to their preferential agonists into *N*-methyl-D-aspartate (NMDA), quisqualate/amino-3-hydroxy-5-methyl-4-isoazole propionic acid (AMPA) and kainate receptors. Binding studies have demonstrated that each of these receptor subtypes are present within the neostriatum.[42,62] Electrophysiological studies suggest that the electrical stimulation of cortical inputs evokes excitatory postsynaptic potentials (PSPs) in neostriatal neurons which are largely mediated by the non-NMDA receptor subtypes, although NMDA receptors may also play a role.[7,11,20,21,53] The precise functional roles played by the NMDA and non-NMDA glutamate receptor subtypes at this synaptic connection are not yet clear.

There is also evidence to suggest that mGluRs may also have a role in neostriatal function. Both autoradiographic binding and *in situ* hybridization studies reveal that this receptor type is localized in the neostriatum.[9,22,37,57,58] In addition, biochemical studies indicate that activation of metabotropic receptors can regulate inositol phosphate metabolism in neostriatal tissue.[14,31,32,49,50,54] Finally, bath application of the mGluR agonist 1-amino-cyclopentane-1,3-dicarboxylic acid (t-ACPD) has been reported to inhibit synaptically-evoked responses in the neostriatum.[8,28,29] However, possible interactions between the metabotropic glutamate and NMDA receptors have not been investigated in neostriatal slices. Recent work raises the possibility that mGluR activation can modulate iGluR-mediated responses.[3,6,24] The present study was designed to test the possibility that interactions between the mGluRs and iGluRs also occur in the neostriatum. Such interactions may be particularly important in the neostriatum, where glutamate receptor activation may play a role in the

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Abbreviations: ACSF, artificial cerebrospinal fluid; AMPA, amino-3-hydroxy-5-methyl-4-isoazole propionic acid; AP-3, 2-amino-3-phosphonopropionic acid; AP-5, 2-amino-5-phosphonopentanoic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; EAA, excitatory amino acid; iGluR, ionotropic glutamate receptor; mGluR, metabotropic glutamate receptor; NMDA, *N*-methyl-D-aspartate; PSP, postsynaptic potential; t-ACPD, 1-amino-cyclopentane-1,3-dicarboxylic acid; TTX, tetrodotoxin.

pathology of neurodegenerative disorders such as Huntington's disease.[17]

The objectives of this study were to determine: (i) the effects of the mGluR agonist t-ACPD on striatal neurons *in vitro*; (ii) whether t-ACPD can modulate NMDA or non-NMDA glutamate receptor-induced responses in these neurons; (iii) the time course of such an effect; (iv) the effect of the mGluR antagonist 2-amino-3-phosphonopropionic acid (AP-3) on such a response; (v) the effects of t-ACPD and AP-3 on evoked PSPs recorded in neostriatal neurons.

EXPERIMENTAL PROCEDURES

Preparation of neostriatal slices

Adult, male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) were maintained in small groups with continuous access to food and water. After animals were killed by decapitation, brains were dissected and placed in cold oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 124, $NaHCO_3$ 25, KCl 5, $MgSO_4$ 2, NaH_2PO_4 1.25, $CaCl_2$ 2.4 and glucose 10 (pH 7.2–7.4). Transverse sections (400 μm) were cut and incubated in ACSF at 35–37°C for 1–2 h. Tissue was then transferred to the recording chamber at least 30 min prior to recording. The slice rested upon filter paper in the chamber and was superfused continuously by oxygenated ACSF (35–37°C). A warm, humidified gas (95% O_2, 5% CO_2) flowed over the top surface of the slice.

Intracellular recording

Intracellular recordings were obtained with glass microelectrodes filled with 3 M potassium acetate; d.c. resistances varied from 70 to 100 MΩ. The signal was amplified by an active bridge circuit (Axoclamp 2A amplifier in "bridge" mode) and stored on videotape for later analysis. After a neuron was impaled, a baseline recording was obtained to ensure that the cell's membrane properties were stable. This baseline membrane potential was maintained throughout the course of the experiment by manually adjusting the d.c. current. Hyperpolarizing current pulses (0.3–0.4 nA, 50 ms duration, 0.5 per second) were applied throughout all experiments to measure conductance changes. In some neurons, the current–voltage relationship was obtained by injection of depolarizing and hyperpolarizing pulses (100 ms duration); the cell's input resistance was determined from the linear portion of the current–voltage plots. Membrane excitability was measured as the response of the neuron to depolarizing current pulses (0.1 nA steps, 500 ms duration). Not all measurements were obtained for each cell. Most recordings were made from cells in the dorsomedial region of the neostriatum.

In order to investigate the effect of t-ACPD on other electrophysiological properties (e.g. action potential and afterhyperpolarization duration and amplitude), the first action potential evoked by the current pulses was analysed in more detail. The action potential amplitudes were measured from the start of the rising phase to the peak of depolarization, while durations were measured at half amplitude. The rise times were measured as the time from the start of the action potential to the peak, while the fall times were measured from the peak to the membrane's return to baseline potential. The amplitudes of afterhyperpolarizations were measured from start of the action potential to the maximal deflection of the hyperpolarization, while durations were determined at the time from the start of the hyperpolarization until the membrane potential returned to its value at the start of the action potential.

Stimulation of postsynaptic response

The stimulating electrode used to evoke PSPs consisted of a pair of 0.2 mm diameter Teflon-coated silver wires exposed at the tips. It was placed approximately 1–3 mm from the recording electrode. Typically, one tip was placed in the cortex while the other was placed in the dorsal neostriatum. Stimuli consisted of 100 μs square wave pulses of varying amplitudes. The PSP amplitudes were measured from the start of the rising phase to the peak depolarization, while durations were measured at half amplitude.

Iontophoresis

All drugs were applied iontophoretically. In addition, L-AP-3 and t-ACPD were also applied through the bath. For iontophoresis, a five-barreled pipette was placed close (100–200 μm) to the recording electrode. Each barrel contained one of the following solutions: AMPA (20 mM, pH 8; Research Biochemical), AP-3 (50 mM, pH 8; Research Biochemical) glutamate (100 mM, pH 8.5; Sigma), quisqualate (100 mM, pH 8; Tocris Neuramin), NMDA (100 mM, pH 8; Sigma), t-ACPD (1S*R*,3*RS*-ACPD, 50 mM, pH 8; Research Biochemical) 1*S*,3*R*-ACPD (50 mM, pH 8; Tocris Neuramin), 1*R*,3*S*-ACPD (50 mM, pH 8; Tocris Neuramin), and saline for current balancing and controls. Not all drugs were used in each experiment. All drugs were iontophoretically ejected as anions and were prevented from diffusing out of the barrel by a positive holding current of 15–20 nA. In some experiments, current balancing was carried out through a barrel containing 1 M NaCl. No differences were observed between experiments with or without current balancing. For bath application, the drugs were dissolved in ACSF. Bath application lasted for 5–10 min and was followed by a "washout" of up to 40 min.

In most cases, to study the effects of iontophoretically applied excitatory amino acid (EAA) agonists, ejection currents were adjusted to induce reproducible submaximal responses. Ejection times ranged from 2 to 10 s. The interval between ejection pulses varied from 2 to 3 min. Iontophoretic currents used to induce these responses ranged from 10 to 150 nA. Controls consisted of the application of negative currents of equal magnitude and duration through the saline-containing barrel of the pipette. In no case did the application of saline either cause membrane potential changes by itself or alter a cell's response to an EAA agonist.

The effects of the t-ACPD isomers on the iGluR agonist-evoked responses were determined by the concurrent ejection of t-ACPD and an ionotropic agonist. First, responses to the agonist were characterized as described above and then reassessed in the presence of t-ACPD. Typically, application of t-ACPD began 10 s before the iGluR agonist and continued during the agonist ejection. The response to the EAA alone was then retested. In order to determine the effects of the putative antagonist L-AP-3, the effects of t-ACPD were characterized as described above and then reassessed in the presence of AP-3 either applied iontophoretically or in the bath.

Statistical analysis

Differences between experimental and control values were evaluated using a paired *t*-test. Values were considered significantly different if $P < 0.05$. In the text, values are shown as means ± S.E.M.

RESULTS

Effect of 1-amino-cyclopentane-1,3-dicarboxylic acid on membrane properties of neostriatal neurons

Recordings were made from a total of 89 neurons in the neostriatum of 46 rats. The effect of t-ACPD alone (iontophoretic currents between 5 and 200 nA) was evaluated on 50 of these neurons. In most cases,

Table 1. The effect of the iontrophoretic application of 1-amino-cyclopentane-1,3-dicarboxylic acid on action potential parameters determined in nine neurons

	Control	t-ACPD	Wash
Input resistance (MΩ)	21.2 ± 1.6	20.7 ± 1.3	19.8 ± 1.7
Action potential amplitude (mV)	71.1 ± 2.0	70.2 ± 1.9	71.7 ± 1.9
Action potential duration (ms) (half-maximal amplitude)	0.70 ± 0.08	0.70 ± 0.03	0.70 ± 0.03
Action potential rise time (ms)	0.67 ± 0.06	0.62 ± 0.04	0.60 ± 0.04
Action potential fall time (ms)	1.45 ± 0.03	1.33 ± 0.04*	1.47 ± 0.08
Afterhyperpolarization amplitude (mV)	5.66 ± 0.43	7.60 ± 0.62†	6.11 ± 0.71
Afterhyperpolarization duration (ms)	30.4 ± 3.7	48.2 ± 8.5†	41.6 ± 5.8

In each of the cells, t-ACPD was found to inhibit NMDA-induced responses. Values are shown as means ± S.E.M. The magnitude of the depolarization used to evoke action potentials was similar under experimental and control conditions (39.2 ± 3.9 vs 38.6 ± 4.1 mV, respectively). The average resting membrane potential of these cells was −76.5 ± 4.8 mV ($n = 9$).

*Significantly different ($P < 0.05$) compared to control. †Significantly different ($P < 0.01$) compared to control.

t-ACPD application had no effect on membrane potential ($n = 36$). In nine neurons, t-ACPD produced a small (<2.5 mV) membrane depolarization, while in five neurons it produced a larger depolarization (between 2.5 and 10 mV). These depolarizations were only seen when larger (>100 nA) iontophoretic currents were used. In addition, t-ACPD applied iontophoretically ($n = 14$) or in the bath (50 μM; $n = 5$) did not affect the current–voltage relationship of the neurons examined (Table 1). Finally, the effects of t-ACPD were determined on several parameters that would be expected to reflect the overall level of cell excitability. Iontophoretic application of t-ACPD had no measurable effect on input resistance, action potential amplitude, half-amplitude duration or rise time (Table 1). In contrast, t-ACPD did cause modest but significant increases in the amplitude (137 ± 10% of control values) and duration (165 ± 27% of controls) of the afterhyperpolarization of the action potential, as well as in the action potential's fall time (Table 1). This effect was only partially reversible.

N-*Methyl*-D-*aspartate-induced responses*

The iontophoretic application of NMDA consistently depolarized cell membranes and, in many cases, induced repetitive action potentials (e.g. top trace in Fig. 1). The magnitude of the response varied with the intensity of current applied to the iontophoretic electrode and, in most cases, ejection currents were adjusted to induce reproducible submaximal responses (iontophoretic current varied between 15 and 150 nA with an average of 51.8 ± 4 nA). NMDA application produced an average depolarization of 24 ± 2 mV, with half-maximal depolarization occurring 5.3 ± 0.3 s after the beginning of the NMDA treatment ($n = 58$). Thus, the peak of the membrane depolarization normally occurred after NMDA ejection had ended. The membrane potential returned to baseline levels within 20–40 s after peak depolarization (average time for membrane repolarization was 30.3 ± 2.3 s). Finally, although the data are not shown, the NMDA-induced excitations were prevented by the application of the NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid (AP-5) and were not blocked by the AMPA/kainate

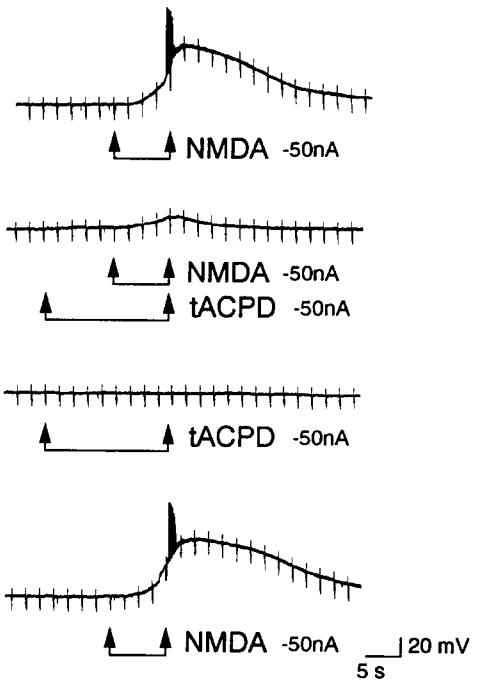

Fig. 1. The iontophoretic application of t-ACPD inhibits NMDA-evoked responses in neostriatal neurons. NMDA and t-ACPD were delivered iontophoretically from a multibarreled microelectrode. Top trace: NMDA produced a depolarization of the membrane potential accompanied by action potentials. Second trace: t-ACPD was applied 10 s before the start of the ejection of NMDA and was continued throughout the application of NMDA. NMDA-induced excitation was inhibited. Third trace: t-ACPD by itself produced no noticeable response. Bottom trace: The NMDA-induced response returned to control levels within 2 min after the t-ACPD application stopped. In this and other figures, the arrows under the traces indicate the onset and offset of treatments and calibration refers to all traces. Hyperpolarizing current pulses (0.4 nA, 50 ms duration, 0.5 per second) were applied as a measure of membrane conductance. Resting membrane potential was −75 mV.

glutamate receptor antagonist 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX).

Effect of 1-*amino-cyclopentane*-1,3-*dicarboxylic acid on* N-*methyl*-D-*aspartate-induced responses*

The effects of the iontophoretic application of the mGluR agonist t-ACPD on NMDA-evoked responses was evaluated in 37 neostriatal neurons. In all but one case, the application of t-ACPD reversibly reduced responses to NMDA (Fig. 1). t-ACPD treatment (iontophoretic current between 5 and 200 nA) started 10 s prior to NMDA ejection and was continued throughout the application of NMDA. Typically, t-ACPD inhibited but did not eliminate the cell's response to NMDA. At maximal ejection current, t-ACPD significantly reduced both the magnitude ($36 \pm 3.4\%$ of control; $P < 0.01$) and duration ($55 \pm 5\%$ of control; $P < 0.01$) of the NMDA-evoked responses ($n = 37$). t-ACPD also inhibited NMDA-evoked responses in the presence of tetrodotoxin (TTX; bath applied, 1.0 μM, $n = 5$). The magnitude of the inhibitory action of t-ACPD was a function of the intensity of the current applied to the iontophoretic electrode (Fig. 2). The bath application of t-ACPD (10–50 μM) also reversibly inhibited the response to NMDA ($n = 8$; Fig. 3).

The time course of t-ACPD's action on NMDA-evoked responses was investigated in 10 neurons by applying a fixed treatment (10 s in duration) of t-ACPD at various intervals prior to the application of NMDA (Fig. 4). For most neurons (seven of 10), the application of t-ACPD followed by a delay of 20–40 s produced the most effective inhibition of the NMDA-induced response. For all neurons examined, the application of t-ACPD followed by at least 10 s of no treatment produced a stronger inhibition than the simultaneous application of t-ACPD and NMDA.

The t-ACPD used in this study was an equimolar mixture of 1*S*,3*R*-ACPD and 1*R*,3*S*-ACPD. In order to determine which of these isomers was biologically active, the effects of 1*S*,3R-ACPD were first assessed. In all neurons tested ($n = 18$), the iontophoretic application of this isomer reversibly inhibited the NMDA-evoked response (Fig. 5); the NMDA-evoked response of those neurons treated with 1*S*,3*R*-ACPD (maximal ejection current) was $35 \pm 5\%$ of controls. These results were very similar to those obtained with the application of t-ACPD. In 15 of these same neurons, 1*R*,3*S*-ACPD was also evaluated. This isomer decreased the magnitude of the NMDA-evoked response in 11 of 15 neurons (Fig. 5). Overall, the NMDA-induced response in the presence of 1*R*,3*S*-ACPD (maximal ejection current) was reduced to $73.3 \pm 9\%$ of controls; in these same 15 neurons, 1*S*,3*R*-ACPD inhibited the NMDA-induced response to $36 \pm 6\%$ of control values. Thus, although 1*R*,3*S*-ACPD had a clear inhibitory effect on NMDA-induced responses, these actions were weaker and less consistent than those produced by 1*S*,3*R*-ACPD.

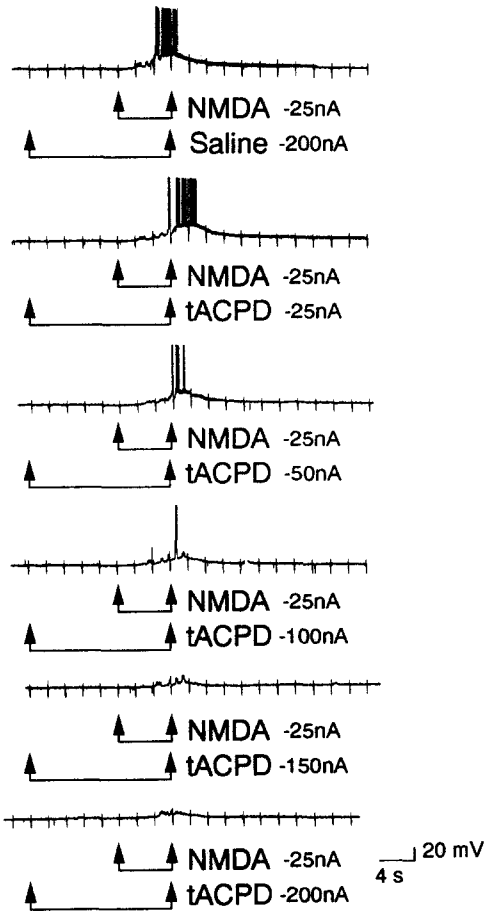

Fig. 2. The inhibitory action of t-ACPD varied with the magnitude of the current applied to the iontophoretic electrode. Top trace: an NMDA-induced depolarization. Saline controls (−200 nA) had no effect on the NMDA-induced response. The six subsequent traces show the NMDA-induced response in the presence of increasing ejection currents of t-ACPD. The NMDA-induced response returned to control levels within 2 min after the t-ACPD application stopped (data not shown). Resting membrane potential was −80 mV.

Effect of 1-*amino-cyclopentane*-1,3-*dicarboxylic acid on other glutamate receptor agonists*

The effects of t-ACPD on membrane potential depolarization evoked by glutamate, AMPA and quisqualate were also evaluated. Under the conditions in which these experiments are performed, the iontophoretic application of glutamate acts primarily through AMPA/kainate receptors, i.e. glutamate-induced responses are blocked by CNQX but not by AP-5. Responses to these other EAA agonists were largely unaffected by the application of t-ACPD (iontophoretic current between 100 and 200 nA; Fig. 6). Similar to experiments with NMDA, t-ACPD

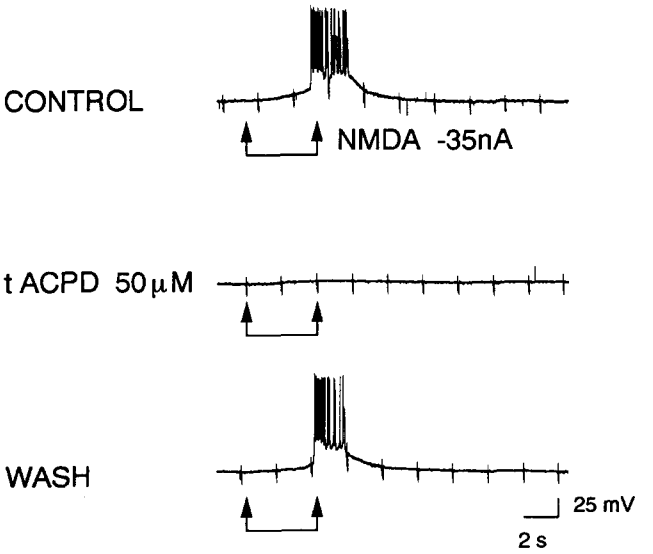

Fig. 3. Bath application of t-ACPD (50 μM) also inhibited NMDA-induced responses. Top trace: an NMDA-induced depolarization. Middle trace: NMDA-induced excitation was inhibited after a 5 min exposure to t-ACPD (50 μM). Bottom trace: 10 min after the t-ACPD application ended, the NMDA-induced response returned to control levels. Resting membrane potential was −80 mV.

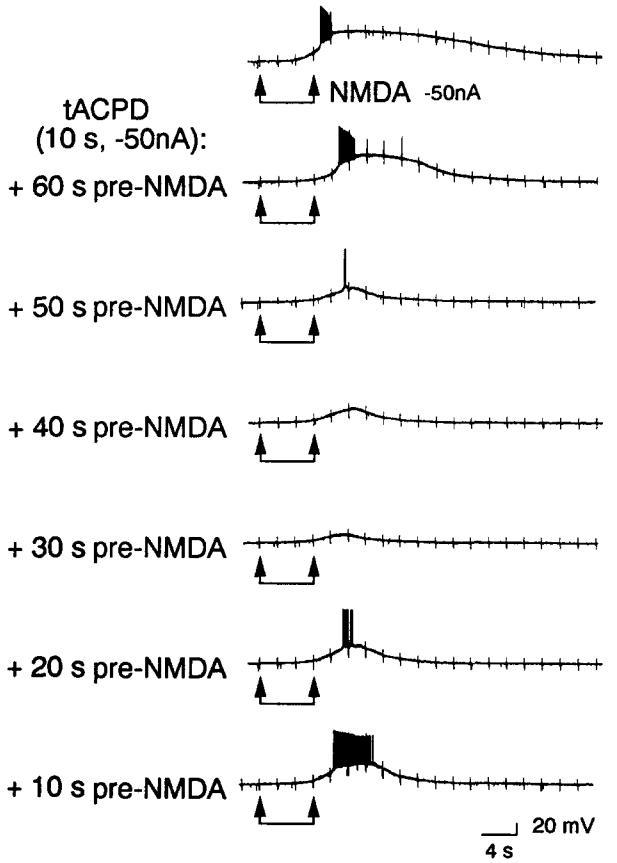

Fig. 4. The time course of t-ACPD's inhibition of NMDA-evoked responses. A fixed treatment of t-ACPD (10 s, −50 nA) was applied at various intervals prior to application of NMDA. The delay was measured from the end of the t-ACPD application. The NMDA-induced response returned to control levels within 2 min after the t-ACPD application stopped (data not shown). Resting membrane potential was −75 mV.

treatment was started 10 s prior to the start of the ejection of the EAA agonist and was continued throughout the application of the agonist. The magnitudes of the depolarizations induced by glutamate, AMPA or quisqualate in the presence of t-ACPD were found to be $96 \pm 5\%$ ($n = 10$), $92 \pm 6\%$ ($n = 8$) and $105 \pm 8\%$ ($n = 8$) of the respective control values. In all but one of these same neurons, t-ACPD inhibited NMDA-evoked responses; thus, this lack of an effect was not due to a failure to deliver the t-ACPD.

Effect of 1-*amino-cyclopentane*-1,3-*dicarboxylic acid in the presence of* 2-*amino*-3-*phosphonopropionic acid*

L-AP-3 is an effective antagonist of EAA-stimulated inositol phosphate metabolism in neostriatal tissue.[31] The possibility that this compound is also an effective antagonist electrophysiologically was evaluated in 16 cells. For these experiments, the effect of t-ACPD on NMDA-evoked responses was first determined. Then AP-3 was applied iontophoretically for a long duration (1–5 min; 50–200 nA), during which time the effects of NMDA alone and NMDA plus t-ACPD were assessed. After the end of AP-3 treatment, the effects of NMDA and of NMDA plus t-ACPD were again examined. The iontophoretic application of AP-3 attenuated t-ACPD's inhibition of the NMDA-evoked response (in 10 of 11 cells; Fig. 7). During AP-3 treatment, the magnitude of the depolarization evoked by NMDA in the presence of t-ACPD (maximal ejection current) was $89 \pm 9\%$ of controls. In contrast, before AP-3 treatment of these same neurons, the response evoked by NMDA plus t-ACPD was $41 \pm 5\%$ of controls. Similar results were obtained after AP-3 treatment. In several cases, the depolarizations evoked by t-ACPD plus NMDA in the presence of AP-3 were larger than those evoked by NMDA alone; however, this effect was not explored in detail. Shorter (< 30 s) treatments of AP-3 were ineffective at preventing t-ACPD inhibition of NMDA-evoked responses. Bath application of AP-3 (50 μM) also reversibly prevented the inhibitory actions of t-ACPD ($n = 5$).

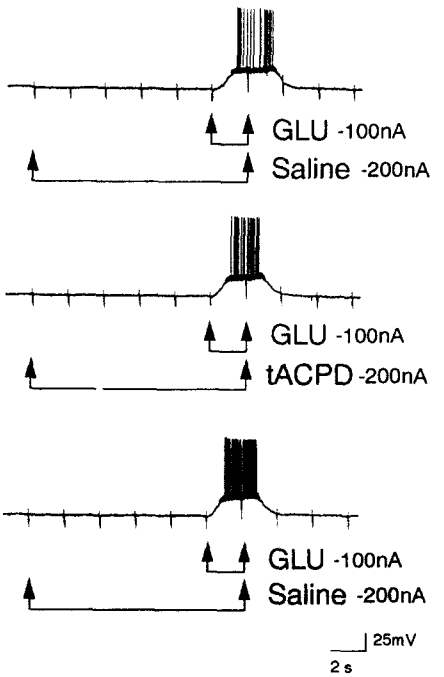

Fig. 6. The iontophoretic application of t-ACPD did not alter glutamate-evoked responses in neostriatal neurons. Top trace: glutamate (GLU) produced a depolarization of the membrane potential accompanied by action potentials. Middle trace: t-ACPD was applied 10 s before the start of the ejection of glutamate and was continued throughout the application of glutamate. Glutamate-induced excitation was not affected. Bottom trace: the glutamate-induced response returned to control levels within 2 min after the t-ACPD application stopped. In this cell, t-ACPD application did inhibit NMDA-induced responses (data shown in Fig. 2). Hyperpolarizing current pulses (0.4 nA, 50 ms duration, 0.5 per second) were applied as a measure of membrane conductance. Resting membrane potential was -80 mV.

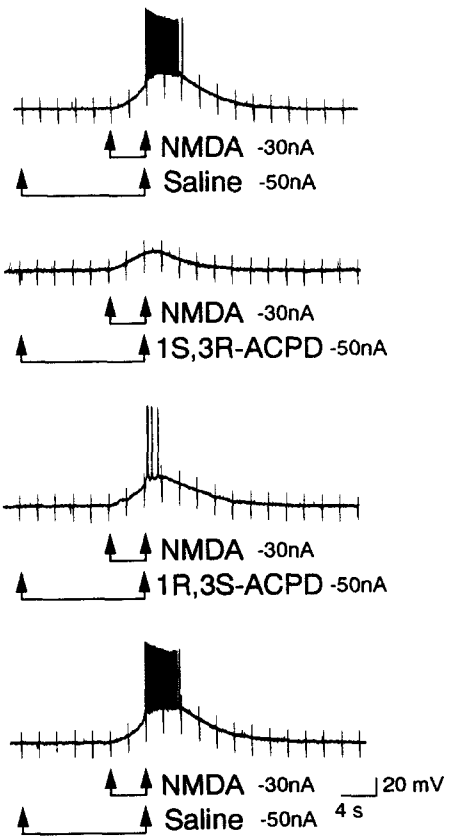

Fig. 5. The inhibition of NMDA-evoked responses by t-ACPD is stereo-selective. Top trace: an NMDA-induced depolarization. Saline controls had no effect on the NMDA-induced response. Second trace: 1*S*,3*R*-ACPD inhibited the response. Third trace: 1*R*,3*S*-ACPD also inhibited the NMDA-evoked response. This inhibition was consistently weaker than that produced by 1*S*,3*R*-ACPD. Bottom trace: the response to NMDA was restored to control levels within 2 min after the application of t-ACPD. Resting membrane potential was -79 mV.

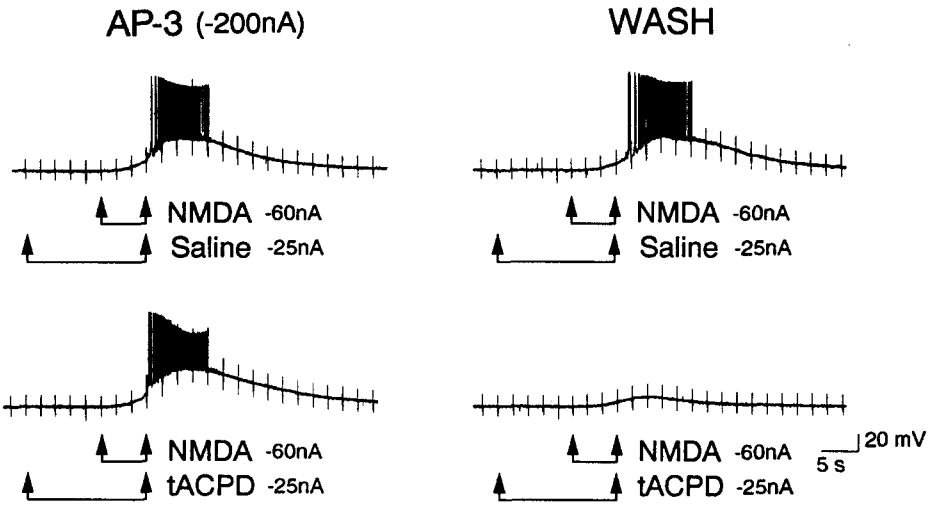

Fig. 7. The putative mGluR antagonist AP-3 attenuates the inhibitory effects of t-ACPD. In the two left traces, AP-3 was continuously applied by iontophoresis, while the two right traces were obtained 2 min after AP-3 application had stopped. Top traces: NMDA-induced depolarizations. Control injections of saline (−25 nA) did not affect the NMDA-induced responses. Bottom traces: t-ACPD is applied 10 s before NMDA ejection. AP-3 blocked the inhibitory action of t-ACPD on the NMDA-induced response. In this cell, t-ACPD inhibited NMDA-induced responses both before (data not shown) and after the application of AP-3. Resting membrane potential was −85 mV.

Effect of 1-*amino-cyclopentane*-1,3-*dicarboxylic acid on synaptic responses*

The effects of bath-applied t-ACPD and AP-3 on depolarizing PSPs recorded in response to local stimulation were determined (Fig. 8). Application of t-ACPD (50 μM) reversibly inhibited the amplitude of the depolarizing PSP to 54.3 ± 6.4% of control values ($P < 0.01$, $n = 14$). The t-ACPD treatment also reduced the half-amplitude duration of the PSP to 88.4 ± 4.0% of control values; however, this effect was not significant. Bath application of AP-3 (50 μM) alone did not affect the magnitude or duration of the evoked PSP in the five neurons examined.

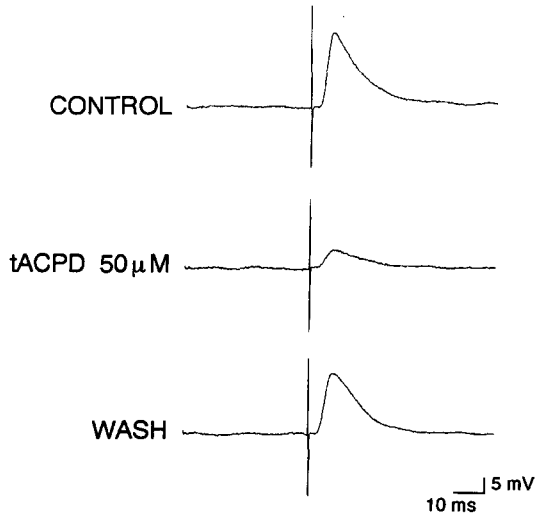

Fig. 8. Bath application of t-ACPD (50 μM) decreases the amplitude of the depolarizing PSP elicited by local extracellular stimulation. Top trace: PSP under control conditions. Middle trace: PSP after a 5 min exposure to t-ACPD (50 μM). Bottom trace: 20 min after the t-ACPD application ended, the PSP amplitude returned to control levels. Traces shown are the average of five synaptic potentials evoked by 900 μA stimulus (0.1 ms pulse duration). Stimulation occurrence is indicated by the stimulus artifact. Resting membrane potential was −85 mV.

Interestingly, AP-3 did not attenuate the effect of t-ACPD on PSP amplitude. In five cells, it was possible to compare directly the inhibition caused by t-ACPD (50 μM) alone with the inhibition caused by t-ACPD in combination with AP-3 (both drugs at 50 μM). The two treatments caused an indistinguishable inhibition of the PSP amplitude (64.6 ± 5.9% of control values vs 62.2 ± 6.0% for t-ACPD and t-ACPD plus AP-3, respectively). Thus, t-ACPD inhibited evoked PSPs in neostriatal cells in an AP-3-insensitive manner.

DISCUSSION

The results of this study demonstrate that the mGluR agonist t-ACPD inhibits responses of neostriatal neurons evoked by the application of NMDA. Application of t-ACPD markedly attenuated both the amplitude and duration of excitatory responses induced by NMDA. These inhibitory effects were stereo-selective, relatively long-lasting and attenuated by the application of the mGluR antagonist AP-3. In contrast, excitatory responses induced by activation of non-NMDA receptors were unaffected by the application of t-ACPD.

Previous electrophysiological studies have shown that bath application of t-ACPD inhibits PSPs evoked by local electrical stimulation in neostriatal slices.[8,28,29] In addition, these studies found that

t-ACPD did not inhibit responses induced by non-NMDA receptor agonists, nor did it produce dramatic changes in the membrane properties of these neurons. Because the depolarizing PSP is primarily mediated by activation of non-NMDA glutamate receptors in neostriatal neurons, both studies concluded that t-ACPD acts presynaptically to inhibit transmitter release at the corticostriatal synapse. The present findings are in agreement with these studies. Bath applied t-ACPD (50 μM) caused an approximate 50% reduction in the magnitude of the evoked PSPs. Similar inhibitory actions of t-ACPD have been observed at other excitatory synapses.[5,13,15,19,40,45,48,63]

Characterization of 1-*amino-cyclopentane*-1,3-*dicarboxylic acid's inhibition of* N-*methyl*-D-*aspartate-evoked responses*

The t-ACPD used throughout most of this study was an equimolar mixture of 1*S*,3*R*-ACPD and 1*R*,3*S*-ACPD. Previous studies have generally reported that 1*S*,3*R*-ACPD is the active form; however, exceptions have been found.[33,49] For example, in rat neostriatal slices, both enantiomers of t-ACPD stimulate phosphoinositide hydrolysis, but 1*S*,3*R*-ACPD is more effective.[50] In contrast, in cultured mouse neostriatal neurons, 1*R*,3*S*-ACPD was more potent than 1*S*,3*R*-ACPD in stimulating intracellular calcium and phosphoinositide hydrolysis.[33] In the present study, although both 1*S*,3*R*-ACPD and 1*R*,3*S*-ACPD inhibited NMDA-induced responses, 1*S*,3*R*-ACPD was more effective. The reasons for these differences are presently unknown; however, it appears that in neostriatal tissue both 1*S*,3*R*-ACPD and 1*R*,3*S*-ACPD show biological activity.

If t-ACPD's action on NMDA-evoked excitation is mediated through a second messenger system, then t-ACPD's inhibitory action should exhibit a time course consistent with this mechanism of action. The application of t-ACPD for 10 s followed by a delay of 20–40 s before the application of NMDA produced a more effective inhibition than simultaneous application of t-ACPD and NMDA. In primary cultures of neostriatal neurons, quisqualate (a potent mGluR agonist) caused an increase in inositol phosphate accumulation which was observed within 15 s of treatment.[2] Thus, the time course of t-ACPD's actions are at least consistent with the hypothesis that t-ACPD is acting through a second messenger system to inhibit NMDA-evoked responses.

AP-3 has been reported to be a partial agonist or antagonist of phosphoinositide-linked mGluR in brain slices and cultured neurons.[49] Furthermore, in *Xenopus* oocytes injected with rat brain mRNA, t-ACPD's activation of calcium-sensitive chloride currents were blocked by AP-3.[56] In cultured neostriatal neurons, AP-3 has been shown to antagonist t-ACPD-induced phosphoinositide hydrolysis.[31] In this preparation, AP-3 appears to act as a partial mGluR agonist, as it also showed some ability to stimulate phosphoinositide hydrolysis.[32] In the present study, AP-3 was an effective antagonist of t-ACPD's inhibition of NMDA-mediated excitation, but was ineffective in preventing t-ACPD's inhibition of the PSP amplitude. Thus, in neostriatal tissue, t-ACPD must act through both AP-3-sensitive and AP-3-insensitive mechanisms. Similar findings have been reported in other regions of the CNS. For example, in the hippocampus, AP-3 inhibited t-ACPD-stimulated phosphoinositide hydrolysis but did not antagonize other actions of t-ACPD on membrane properties of these neurons.[16] Never the less, AP-3 does appear to be an effective antagonist of at least some of t-ACPD's actions in the neostriatum.

1-*Amino-cyclopentane*-1,3-*dicarboxylic acid's actions are likely to be mediated by metabotropic glutamate receptors*

t-ACPD is well established as a selective agonist of mGluRs, and this is likely to be the mechanism by which this agonist inhibits NMDA-evoked responses. Although with iontophoresis the actual concentration of t-ACPD reaching the neuron is unknown, bath application of t-ACPD at 10 and 50 μM also inhibited NMDA-evoked responses. At these concentrations, t-ACPD is selective and does not interfere with [^{3}H]CGS-19755 or NMDA-sensitive [^{3}H]glutamate binding.[49,50] Furthermore, the findings that t-ACPD's actions are stereo-selective and attenuated by AP-3 argue against any non-specific action of t-ACPD. Finally, t-ACPD has not been reported to non-specifically block NMDA receptors in other preparations.[49] These results strongly support our suggestion that t-ACPD is acting through mGluR activation to inhibit NMDA-induced responses in the neostriatum.

It is likely that more than one subtype of mGluR is mediating the effects of t-ACPD in the neostriatum. Recently, the cDNA clones for several different subtypes of the mGluR (mGluR1–6) have been isolated.[1,22,37,44,46,57] These different mGluRs are coupled to a variety of signal transduction mechanisms. For example, mGluR1 and mGluR5 activate a phosphoinositide/Ca^{2+} cascade,[1,4,22,37] while others (mGluR2–4) appear to inhibit cAMP formation.[57,58] The receptor subtypes also show distinct distributions, and mGluR1, mGluR3 and mGluR5 (but not mGluR2 or mGluR4) are expressed in the neostriatum.[1,36,46,51,57,58] When mGluR1 and mGluR5 are expressed in *Xenopus* oocytes and Chinese hamster ovary cells, t-ACPD stimulates phosphoinositide hydrolysis and inositol-1,4,5-triphosphate-mediated intracellular calcium release through a mechanism insensitive to AP-3.[1,4] This suggests that another mGluR receptor subtype (one sensitive to AP-3) may be expressed in the neostriatum and may be responsible for mediating t-ACPD's inhibition of NMDA-induced responses.

1-Amino-cyclopentane-1,3-dicarboxylic acid's site of action: pre- or postsynaptic?

Both pre- and postsynaptic mGluRs have been described and could mediate the effects of t-ACPD described in this study. The present data support a presynaptic mechanism of action for t-ACPD's inhibition of PSP amplitude (see previous discussion). On the other hand, the site of action of t-ACPD's inhibition of NMDA-induced responses is likely to be postsynaptic. The observation that t-ACPD inhibits NMDA-induced responses under conditions in which synaptic transmission is blocked (bath-applied TTX) supports a postsynaptic mechanism of action. An alternative possibility is raised by the recent description of a "presynaptic" glutamate receptor sensitive to NMDA,[55] i.e. NMDA-induced responses could be due to a presynaptic stimulation of glutamate release and t-ACPD's actions on these responses could also be presynaptic. However, there are reasons indicating that this latter alternative is unlikely. First, NMDA-induced responses in the neostriatum are completely blocked by the NMDA antagonist AP-5, while NMDA responses mediated by the presynaptic receptor are only partially inhibited by this antagonist.[55] Second, under the conditions under which the present experiments were performed, glutamate-induced responses are mediated by AMPA/kainate glutamate receptors and are blocked by the antagonist CNQX.[7,11,20,21] Thus, if NMDA were acting through a presynaptic mechanism to cause a release of glutamate, these responses would also be blocked by CNQX. In contrast, preliminary data indicate that CNQX has a limited impact on NMDA-induced responses in the neostriatum and t-ACPD still inhibits NMDA-induced responses in the presence of CNQX. These observations suggest that, in the neostriatal brain slice, the iontophoretic application of NMDA acts primarily via postsynaptic receptors and that t-ACPD's inhibition of these responses is also due to a postsynaptic mechanism.

Possible cellular mechanisms

t-ACPD could act to inhibit NMDA-induced responses through a number of mechanisms. In neostriatal tissue, EAA agonists including t-ACPD have been shown to stimulate inositol phosphate metabolism,[14,31,34,49,50,54] as well as to decrease forskolin-stimulated cAMP formation.[34,47] Alterations in either of these two second messenger systems could affect the NMDA receptor/ion channel's phosphorylation state and thus regulate NMDA-induced responses. Recently, a variety of studies have provided evidence that the phosphorylation state of NMDA receptors can determine their activity.[10,23,26,35,43,59,60] Alternatively, previous studies have described a calcium-dependent inactivation or desensitization of NMDA-induced responses,[12,27,38,64] and a similar process could be at work in the neostriatum. t-ACPD's activation of inositol phosphate metabolism does result in the mobilization of calcium from intracellular stores in neostriatal cells.[32,33] However, at present, the mechanism by which t-ACPD acts to inhibit NMDA-evoked response in neostriatal neurons is unknown.

Comparison with previous studies

The finding that t-ACPD can modulate NMDA-induced responses has been reported in other areas in the brain.[3,6,24] In the CA1 area of the hippocampus, t-ACPD potentiated NMDA- but not AMPA-induced currents.[3] Similarly, 1*S*,3*R*-ACPD enhanced NMDA-induced currents in *Xenopus* oocytes injected with rat brain mRNA.[23] In both cases, the potentiation of the NMDA-induced currents appeared to be mediated through a protein kinase C-dependent mechanism. Furthermore, in rat spinal cord cells, 1*S*,3*R*-ACPD increased both NMDA- and AMPA-induced currents.[6] In these three cases, t-ACPD potentiated rather than inhibited NMDA-induced currents. However, in cultured neocortical neurons,[25] as well as the retina,[52] injection of 1*S*,3*R*-ACPD protects against NMDA-induced cell death. Perhaps these regions are similar to the neostriatum in that t-ACPD inhibits NMDA-induced responses. Interestingly, the NMDA receptor activates an ion channel permeable to calcium[30] and the resulting increase in intracellular calcium concentration is considered a critical step in the mechanism of neurotoxicity. In the neostriatum and other areas, activation of mGluRs by t-ACPD may result, after a delay, in an attenuation of NMDA receptor-mediated calcium influx. This negative regulation could serve to protect these neurons from over-exposure to calcium during periods of high levels of synaptic input.

CONCLUSIONS

The activation of mGluRs by t-ACPD appears to have two distinct actions on neostriatal neurons *in vitro*. First, t-ACPD inhibits responses induced by NMDA. These inhibitory effects were stereoselective, relatively long-lasting and attenuated by the application of the mGluR antagonist AP-3. In addition, t-ACPD apparently acts through a presynaptic mechanism at the corticostriatal connection to inhibit the release of an EAA transmitter.[8,28,29] Although the functional significance of this regulation is currently unknown, both of these actions will have the result of decreasing the excitatory response of neostriatal neurons and may play a role in maintaining the low level of neural activity characteristic of these cells.

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