Role of Pertussis Toxin–Sensitive G-Proteins in Synaptic Transmission and Plasticity at Corticostriatal Synapses

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INTRODUCTION

The striatum is an important brain region for regulation of body movement as well as certain cognitive functions. Striatal neurons are strongly activated by afferents from the cortex that use glutamate as their neurotransmitter (Fonnum et al. 1981; Girault et al. 1986; Reubi and Cuenod 1979). High-frequency activation of these glutamergic afferents induces a long-term depression (LTD) of synaptic transmission at corticostriatal synapses, both in vivo (Garcia-Munoz et al. 1992, 1996; Wickers and Reynolds 1997) and in vitro (Calabresi et al. 1992, 1994; Choi and Lovinger 1997a,b; Lovinger et al. 1993; Walsh 1993). Striatal LTD has been proposed to be a cellular mechanism underlying motor learning and memory. In a recent study (Choi and Lovinger 1997b), evidence for a role of LTD in the development of corticostriatal synapses was presented.

The induction of LTD is blocked by L-type calcium channel blockers and intracellular dialysis of calcium chelators (Calabresi et al. 1992, 1994; Choi and Lovinger 1997b). These findings suggest that LTD induction after high-frequency synaptic activation involves postsynaptic membrane depolarization and increases in postsynaptic calcium concentration brought about by L-type calcium channel activation. The maintenance of LTD involves a decrease in the probability of neurotransmitter release at corticostriatal synapses (Choi and Lovinger 1997a,b).

At least two types of G-protein–coupled receptors have been implicated in striatal LTD, dopamine receptors and metabotropic glutamate receptors (Calabresi et al. 1992). In addition, a number of G-protein–coupled receptors have been shown to modulate transmission at corticostriatal synapses. Many of these receptors may be linked to pertussis toxin (PTX)–sensitive G-proteins. Thus, we sought to better understand the role of PTX-sensitive G-protein–coupled receptors in synaptic transmission and plasticity at corticostriatal synapses. To this end we examined the effects on corticostriatal synaptic transmission and LTD of PTX and N-ethylmaleimide (NEM), two agents that inhibit the function of these G-proteins. Some of these data have appeared in a recent abstract (Tang and Lovinger 1998).

METHODS

Acute brain slices were prepared from 14–28-day-old rats. Rats were killed by decapitation, and the brains were quickly removed and placed in ice-cold, modified artificial cerebrospinal fluid (aCSF) containing (in mM) 194 sucrose, 30 NaCl, 4.5 KCl, 1 MgCl2, 26 NaHCO3, 1.2 NaH2PO4, and 10 d-glucose. Modified aCSF was adjusted to pH 7.4 by bubbling with 95% O2-5% CO2. Coronal sections (400 μm in thickness) were cut in ice-cold modified aCSF using a manual vibroslice (World Precision Instruments, New Haven, CT). Slices were then transferred to a nylon net submerged in normal aCSF containing (in mM) 124 NaCl, 4.5 KCl, 2 CaCl2, 1 MgCl2, 26 NaHCO3, 1.2 NaH2PO4, and 10 d-glucose. Normal aCSF was oxygenated and maintained at pH 7.4 by bubbling with 95% O2-5% CO2 at room temperature (22–24°C). After incubation for 1 hour, a hemisphere containing the cortex and striatum at the level of the head of the caudate was completely submerged in a Plexiglas recording chamber and continuously superfused with normal aCSF at a flow rate of 2–3 ml/min. Normal aCSF or drugs in aCSF were delivered to the recording chamber by superfusion driven by gravity flow. The temperature of the bath solution was kept at 32–35°C and stable within ±1°C in a given experiment.

Twenty-five-day-old rats were used in the PTX injection experiment. After rats were anesthetized with ketamine (80 mg/kg, ip), they were fixed in a stereotaxic instrument (Kopf Instruments, Tujunga, CA). An incision was made in the skin on the head using a scalpel to expose the skull. A small hole was drilled in the skull at coordinates AP1.5 mm anterior to bregma, L2.5 mm from the midline. The position of the nose clamp was –2.0 mm. PTX (1 μg) was dissolved in 2 ml of aCSF. Intrastriatal injections were performed stereotaxi-
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The PS and EPSC amplitudes were analyzed using peak-detection software provided in pClamp. These data were simply obtained by subtracting the peak values from the prerespone baseline values. For this measurement, a minimum was measured within a time window bounded by the increasing and decreasing phases of the synaptic responses. The other two cursors were placed at time points just before the occurrence of synaptic responses to obtain the baseline values. The amplitudes of synaptic responses were calculated by subtracting the peak values from the baseline values. Changes in PS and EPSC amplitudes during a time window 20–30 min after HFS or drug treatment are expressed as the percentage of the baseline response just before treatment. The representative PS and EPSC waveformss shown in the text are the average of 15–30 individual responses in a given recording. LTD was defined as a decrease in response amplitude (more than 20% below the baseline response amplitude) lasting at least 20 min after HFS. The magnitude of LTD was measured during a 10-min time window 20–30 min after the cessation of HFS. Paired-pulse facilitation (PPF) was elicited by paired stimuli with an interstimulus interval of 50 ms. Responses used for the PPF ratio were calculated as the ratio of the amplitude of the second EPSC to that of the first EPSC. All values are averaged from data obtained over 5–10 min and presented as mean ± SE. The statistical significance of changes in synaptic responses was measured using a two-tailed Student’s t-test. The statistical criterion for significance was P < 0.05.

PTX and NEM were purchased from Sigma. TTX was purchased from Alomone Labs and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) was purchased from RBI.

RESULTS

Effect of inhibition of PTX-sensitive G-proteins on LTD and synaptic transmission

Figure 1A illustrates that LTD could not be induced in eight of eight PTX-treated hemislices 24–48 h after PTX injection (PS = 123.9 ± 10.6% of baseline, P > 0.05, paired t-test, n = 8). In four of the eight hemislices, PS amplitudes were not significantly changed (<20% change relative to the baseline amplitude, P > 0.1, paired t-test, n = 4) after HFS, whereas in the remaining four hemislices, PS amplitudes were significantly potentiated (PS = 156.3 ± 11.6% of baseline, P < 0.01, paired t-test, n = 4). In these experiments, we used the opposite hemislices from the same animal as one control to minimize variability among animals or because of the anterior-posterior position of the slice. In opposite hemisphere control slices, LTD was induced in six of eight preparations (PS = 48.6 ± 9.8% of baseline, P < 0.01, paired t-test, n = 6). In one of the remaining two control hemislices, LTD was not induced after HFS. The PS amplitude was too small (maximal amplitude <0.2 mV) in the other control hemislice, and it was discarded.

Parallel experiments were performed in vehicle-injected animals of the same ages to ensure that the blockade of LTD induction was due to the action of PTX. Vehicle (2 μl) was unilaterally injected into striatum. In these vehicle-injected animals, LTD could be induced in either vehicle-injected (PS = 44.3 ± 8.9% of baseline, P < 0.01, paired t-test, n = 5) or noninjected (PS = 43.6 ± 8.4% of baseline, P < 0.01, paired t-test, n = 5) hemislices (Fig. 1B). The maximal PS amplitude recorded from PTX-treated hemislices was significantly smaller than that obtained in control hemislices (PS amplitude from PTX-treated hemislices: 0.55 ± 0.1 mV, n = 6; control hemislices: 0.89 ± 0.2 mV, P < 0.01, paired t-test,
examining LTD induction in vehicle-treated slices (n = 6). LTD could not be induced by high-frequency stimulation (HFS) in all tested PTX-treated hemislices, whereas LTD could be induced by HFS in six of seven hemislices. Field potentials a and b were evoked by single-pulse afferent stimulation before and 30 min after HFS in PTX-treated hemislices, respectively. Field potentials c and d were evoked by single-pulse afferent stimulation before and 30 min after HFS in control hemislices, respectively. Each field potential trace represents an average of 30 trials. Arrow: time of HFS application before and 30 min after HFS in control hemislices, respectively. Each field potential trace represents an average of 30 trials. Arrow: time of HFS application before and 30 min after HFS in vehicle-treated hemislices, respectively. Field potentials c and d were evoked by single-pulse afferent stimulation before and 30 min after HFS in control hemislices, respectively. Field potentials a and b were evoked by single-pulse afferent stimulation before and 30 min after HFS in PTX-treated hemislices, respectively.

FIG. 1. Pertussis toxin (PTX) blocks induction of striatal long-term depression (LTD). A: population spike (PS) amplitude (normalized) plotted as a function of time for each evoked potential observed during the course of experiments examining LTD induction in PTX-treated slices (n = 8) and control hemislices (n = 6). LTD could not be induced by high-frequency stimulation (HFS) in all tested PTX-treated hemislices, whereas LTD could be induced by HFS in six of seven hemislices. Field potentials a and b were evoked by single-pulse afferent stimulation before and 30 min after HFS in PTX-treated hemislices, respectively. Field potentials c and d were evoked by single-pulse afferent stimulation before and 30 min after HFS in control hemislices, respectively. B: PS amplitude (normalized) plotted as a function of time for each evoked potential observed during the course of experiments examining LTD induction in vehicle-treated slices (n = 5) and control hemislices (n = 5). LTD could be induced by HFS in either vehicle-treated or control hemislices. Field potentials a and b were evoked by single-pulse afferent stimulation before and 30 min after HFS in vehicle-treated hemislices, respectively. Field potentials c and d were evoked by single-pulse afferent stimulation before and 30 min after HFS in control hemislices, respectively. Each field potential trace represents an average of 30 trials. Arrow: time of HFS application. Each control experiment was performed in the contralateral hemislice of the same coronal section.

The magnitude of afferent stimulation needed to elicit a maximal amplitude PS did not differ between the PTX-exposed and control slices. No difference in PS amplitude between control and injected hemislices was observed in tissue from vehicle-injected animals. This suggests that prolonged PTX exposure decreases synaptic responses. In a subset of hemislices from PTX-treated animals we observed PS amplitudes that were within the range of those observed in control slices. These PTX-treated slices did not exhibit LTD after HFS. Thus, we do not believe that the absence of LTD induction in PTX-treated slices was related to the smaller response amplitude after PTX exposure.

We attempted to examine PTX effects using whole cell recording. Three animals were unilaterally injected with 1 μg of PTX in striatum, and whole cell recordings were performed 24 h later. However, the amplitude of evoked EPSCs was generally quite small (maximal amplitude <50 pA) in these PTX-injected animals, and thus we were unable to obtain accurate measures of synaptic plasticity in these experiments. The current levels at the holding potential did not differ in these neurons relative to neurons that were not exposed to PTX, indicating that the PTX-treated neurons were healthy and had input resistances similar to the control cells.

Effect of NEM on LTD and synaptic transmission

We also used NEM to block PTX-sensitive G-protein action in acute slice experiments. NEM is a sulphydryl alkylating agent that can selectively inhibit PTX-sensitive G-protein-mediated effects in central (Morishita et al. 1997) and peripheral (Shapiro et al. 1994) mammalian neurons, invertebrate neurons (Fryer 1992), and HEK 293 cells (McCool et al. 1996). The advantage of using NEM was that it allowed us to examine synaptic transmission before and after inhibition of PTX-sensitive G-proteins within a given recording. The main concern with the use of NEM was that drug specificity, because NEM has been reported to inhibit a number of processes, such as membrane fusion reactions, in the mM concentration range (Lledo et al. 1998; Macaulay and Forbes 1996; Meffert et al. 1996). In the present study, a 200 μM concentration of NEM was used, which is in the range of concentrations previously shown to block PTX-sensitive G-protein effects in slice preparations (Morishita et al. 1997). We examined the ability of NEM to block inhibition of synaptic transmission produced by bath application of 100 micromolar adenosine as a positive control for NEM blockade of a response mediated by a PTX-sensitive G-protein (Munshi et al. 1991; Scholz and Miller 1992). In two slices we observed that adenosine inhibited synaptic transmission in the absence of NEM (PSs decreased to 30.4% of control in the presence of adenosine), but not in the presence of NEM (PS = 98.2% of control; data not shown).

Induction of LTD was prevented in slices treated with NEM (200 μM) before and during HFS, and in fact the PS amplitude was larger after HFS in these animals (PS = 144.8 ± 12.2% of baseline, P < 0.05, paired t-test, n = 5; Fig. 2B). In contrast, LTD (PS = 55.0 ± 3.3% of baseline, n = 5; Fig. 2A) was induced in paired control slices that did not receive NEM treatment. The data shown in Fig. 2 were obtained using extracellular field potential recording. Similar results were obtained in experiments using whole cell voltage-clamp recording (Fig. 3). The induction of LTD was blocked in slices treated with NEM before and during HFS, and a significant potentiation in EPSC amplitude was observed after HFS in these slices (EPSC = 150.3 ± 13.2% of baseline, P < 0.01, paired t-test, n = 5; Fig. 3B). On the other hand, LTD (EPSC = 68.0 ± 3.2% of baseline, n = 5) was inducible in control slices (Fig. 3A).

Application of NEM 30 min after LTD induction increased synaptic response amplitude to the point that responses were potentiated relative to pre-HFS baseline values. This effect of NEM was observed in both field potential (PS = 153.7 ± 14.8% of baseline, P < 0.01, paired t-test, n = 5; Fig. 2C) and whole cell (EPSC = 118.4 ± 9.9% of baseline, P > 0.05, paired t-test, n = 4; Fig. 3C) recordings. Interestingly, we also found that treatment with NEM alone potentiated the ampli-
tudes of the PS (PS = 146.8 ± 13.4% of baseline, P < 0.01, paired t-test, n = 5; Fig. 2 D) in field potential recordings and the EPSC (EPSC = 131.2 ± 17.0% of baseline, P < 0.05, paired t-test, n = 5; Fig. 3D) in whole cell recordings. Application of NEM did not alter the baseline current amplitude at the holding potential in the whole cell voltage-clamp experiments. Thus, it is unlikely that NEM effects on synaptic transmission are due to alteration of the passive membrane properties of the postsynaptic neuron.

In past studies we observed that blockade of adenosine A1 receptors potentiates synaptic transmission in striatal slices (Lovinger and Choi 1995). NEM may potentiate transmission solely by preventing synaptic depression produced by endogenous adenosine acting on A1 receptors. We examined this possibility by first applying the A1 receptor antagonist DPCPX (1 μM) to slices during field potential recording and then determining whether NEM produces potentiation of the PS response after stable potentiation by DPCPX. In two slices treated in this manner, we still observed robust potentiation by 200 μM NEM after DPCPX treatment (PS amplitude increased by 63% after NEM exposure), even though DPCPX produced an increase in PS amplitude of 15%. This finding indicates that the potentiation by NEM is not due solely to prevention of the actions of endogenous adenosine acting on A1 receptors.

We compared the magnitude of the increases in PS and EPSC amplitude after NEM alone and under conditions in which NEM was applied before and during HFS. There was no difference in potentiation by NEM under these two conditions (unpaired t-test, P > 0.1 for both PS and EPSC measures).

Because NEM alone potentiated synaptic transmission, it was difficult to determine whether the blockade of LTD induction resulted from the slow development of the NEM facilitatory effect overriding LTD expression or from NEM inhibition.
of specific mechanisms of LTD induction. To address this issue, we examined whether LTD could be induced by HFS applied in a state of stable potentiation after NEM exposure. We consistently observed that NEM alone potentiated PS amplitudes. A stable potentiated PS was observed; 45 min after NEM application (average potentiated PS = 172.8 ± 8.8% of baseline, P < 0.001, paired t-test, n = 4) as shown in Fig. 4, A and B. In two of these slices, responses were recorded for 1 h after NEM exposure before delivery of HFS. Application of HFS during this stable potentiation by NEM could not induce LTD (Fig. 4, A and B). We also performed an experiment in which the stimulus amplitude was reduced after potentiation by NEM to elicit a PS with an amplitude similar to that recorded before NEM exposure. HFS was then delivered at the original stimulus intensity and the responses after HFS were recorded at the lower stimulus intensity. No consistent evidence of LTD was observed after NEM exposure using this paradigm (percent of pre-HFS PS amplitude = 98%, n = 2 slices).

**Mechanism(s) of NEM effects on transmission: presynaptic versus postsynaptic actions**

Next, we assessed the mechanism by which NEM potentiates synaptic transmission using analysis of spontaneous EPSCs, miniature mEPSCs (mEPSCs) measured in the presence of the sodium channel blocker TTX, and measurement of paired-pulse facilitation (PPF). Figure 5A shows that NEM alone (200 μM) increased the frequency of spontaneous EPSCs (before NEM: 1.8 ± 0.6 Hz; after NEM: 4.2 ± 0.7 Hz, P < 0.01, paired t-test, n = 5). This effect could be due to NEM actions on either axons or axonal terminals of the presynaptic neuron. To investigate, we examined NEM’s effects on mEPSCs by applying TTX (200 nM), which blocked evoked action potentials before NEM treatment and determined whether the change in frequency persisted when mEPSCs were isolated in this manner. Figure 5B shows that the NEM-induced increase in spontaneous EPSC frequency persisted even in the presence of specific mechanisms of LTD induction. To address this issue, we examined whether LTD could be induced by HFS applied in a state of stable potentiation after NEM exposure. We consistently observed that NEM alone potentiated PS amplitudes. A stable potentiated PS was observed; 45 min after NEM application (average potentiated PS = 172.8 ± 8.8% of baseline, P < 0.001, paired t-test, n = 4) as shown in Fig. 4, A and B. In two of these slices, responses were recorded for 1 h after NEM exposure before delivery of HFS. Application of HFS during this stable potentiation by NEM could not induce LTD (Fig. 4, A and B). We also performed an experiment in which the stimulus amplitude was reduced after potentiation by NEM to elicit a PS with an amplitude similar to that recorded before NEM exposure. HFS was then delivered at the original stimulus intensity and the responses after HFS were recorded at the lower stimulus intensity. No consistent evidence of LTD was observed after NEM exposure using this paradigm (percent of pre-HFS PS amplitude = 98%, n = 2 slices).

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of TTX (200 nM; before TTX: 1.3 ± 0.6 Hz; after TTX alone: 1.0 ± 0.4 Hz, after TTX + NEM: 34.2 ± 9.4 Hz, n = 4). The spontaneous EPSC frequency was significantly increased in TTX + NEM relative to the either the control (before TTX) or TTX alone (P < 0.01, paired t-test, n = 4). We also examined the effect of TTX exposure after NEM application and observed that TTX did not significantly reduce the frequency of spontaneous EPSCs after NEM exposure (data not shown). These findings suggest that all the spontaneous EPSCs we recorded in this preparation either in the absence or presence of NEM are action potential–independent mEPSCs and that NEM increases mEPSC frequency.

Another piece of evidence suggesting that NEM could act on a presynaptic site was provided by the observation that NEM decreased PPF ratio (ratio before NEM: 1.33 ± 0.04; ratio after NEM: 0.9 ± 0.07, P < 0.01, paired t-test, n = 5). The current traces in Fig. 5C show that the amplitude of the EPSC was significantly increased and PPF ratio was decreased after NEM application.

The amplitude of spontaneous EPSCs was also increased by 200 μM NEM (before NEM: 11.4 ± 2.1 pA; after NEM: 17.5 ± 1.6 pA, P < 0.01, paired t-test, n = 5) in conjunction with the increase in frequency as shown in Fig. 5A. This observation suggests that NEM could enhance synaptic transmission partly via postsynaptic actions. Figures 5B and 6A show that application of NEM also increased the amplitude of TTX-insensitive mEPSCs (control before TTX: 9.2 ± 1.3 pA; TTX alone: 8.8 ± 0.9 pA; TTX + NEM: 15.8 ± 1.9 pA). The increase in mEPSC amplitude was significant in the TTX + NEM condition relative to the control (before TTX) or TTX alone (paired t-test, P < 0.05, n = 4) conditions. When TTX was applied after NEM exposure, the amplitude of spontaneous EPSCs was not significantly decreased (paired t-test, P > 0.05, n = 5; inset in Fig. 6A). NEM increased the amplitude of mEPSCs as shown in a cumulative plot (Fig. 6A) in which the amplitude distribution of mEPSCs shifted to the right in the presence of TTX + NEM. Examining the data with a histogram distribution (Fig. 6B) also revealed that the mEPSC amplitude was increased in the presence of NEM. Our findings indicate that the spontaneous EPSCs we observed under all conditions were mEPSCs and that the amplitudes of the mEPSCs were enhanced by NEM.

We attempted to examine this apparent postsynaptic effect of NEM further by perfusing the postsynaptic neuron with NEM (200 μM) in the patch pipette. In these recordings, we observed an increase in spontaneous EPSC frequency compared with cells without NEM perfusion (basal EPSC frequency: 1.5 ± 1.0 Hz, n = 11) in 4 of 11 cells perfused with NEM (EPSC frequency: 6.3 ± 0.4 Hz, P < 0.0001, unpaired t-test). In these four cells we also observed a slight increase in the spontaneous EPSC amplitude to 11.2 ± 2.4 pA, but this increase was not significant (compared with the predrug control (10.3 ± 1.7 pA, P > 0.1, unpaired t-test, n = 9)). A loss of PPF (PPF ratio = 0.89 ± 0.1, P < 0.01, unpaired t-test) in the presence of NEM was also observed in these four cells. In addition, we tried to induce LTD in two of the NEM-perfused cells showing a loss of PPF. LTD could not be induced in these two cells. In the remaining seven cells, no increase in spontaneous EPSC frequency (2.0 ± 0.5 Hz, P > 0.4, unpaired t-test), or amplitude (9.4 ± 0.8 pA, P > 0.2, unpaired t-test), or loss of PPF (PPF ratio = 1.25 ± 0.04, P > 0.2, unpaired t-test) was observed. Moreover, we also tried to induce LTD in two NEM-perfused cells that showed no change in PPF ratio. LTD could be induced in these two cells. Bath application of NEM (200 mM) after a 30-min intracellular perfusion of NEM (200 mM) still increased the spontaneous EPSC frequency (before NEM bath application: 3.5 Hz; after NEM bath application: 48.3 Hz) and amplitude (before NEM bath application: 10.4 pA; after NEM bath application: 16.5 pA) in two NEM-perfused cells. We also perfused four cells with 5 mM NEM to examine whether a stronger effect of NEM could be observed. NEM at the 5 mM concentration produced a larger increase in the spontaneous EPSC frequency (34.2 ± 8.1 Hz, P < 0.001, unpaired t-test) and amplitude (14.5 ± 1.5 pA, P < 0.01, unpaired t-test). After synaptic perfusion 5 mM NEM also changed PPF to paired-pulse depression (0.45 ± 0.04, P < 0.001, unpaired t-test).

**DISCUSSION**

We observed evidence that PTX-sensitive G-proteins have multiple roles in corticostriatal synaptic transmission and plasticity. G-protein inhibition on an acute time scale (i.e., during recording from a single-slice preparation) produces potentiation of synaptic transmission that appears to involve both presynaptic and postsynaptic mechanisms. Prolonged inhibition produced by PTX injection appears to decrease synaptic transmission, at least on average. Striatal LTD could not be induced after either PTX injection or acute NEM application. This finding indicates that PTX-sensitive G-proteins play a role in LTD.

**FIG. 4.** A: PS amplitude (normalized) plotted as a function of time for each evoked potential observed during the course of an experiment in which HFS was applied after a stable potentiation induced by NEM (200 μM). Note that NEM potentiated the PS amplitudes as shown in Fig. 2D. However, LTD could not be elicited by NEM during a state of stable NEM potentiation. In addition, no further potentiation of PS amplitude was observed. Line: time from beginning to end of NEM perfusion. B: summary plot (n = 4) showing that HFS was unable to induce LTD or potentiation in NEM-pretreated slices. HFS was applied after a stable potentiation by NEM was obtained. Arrow: time of HFS application.
Although LTD was not observed in PTX-treated slices, it was observed under several control conditions, suggesting that a PTX-sensitive G-protein signaling pathway is necessary for LTD induction or expression. Because PTX was injected locally into the striatum, it may inactivate G-proteins on both presynaptic terminals and postsynaptic cell bodies. Thus, it is difficult to determine the synaptic location of the receptors and G-proteins involved in LTD at this time. LTD induction is known to involve both dopamine receptors and metabotropic glutamate receptors (Calabresi et al. 1992). There is ample evidence for both pre- and postsynaptic metabotropic glutamate receptors (Calabresi et al. 1993; Lovinger and McCool 1995; Testa et al. 1998) and dopamine D2 receptors in the striatum (Freund et al. 1984; Smith and Bolam 1990). The location of the G-proteins and G-protein–coupled receptors involved in LTD will have to be determined in future experiments.

In agreement with the findings obtained in PTX-treated slices, LTD could not be induced by HFS in the presence of NEM. This observation supports our conclusion that activation of PTX-sensitive G-proteins is necessary for LTD induction. However, the mechanism of blockade of LTD induction by NEM is not entirely certain from this study. The blockade of PTX-sensitive G-protein activity by PTX or NEM may interfere with signaling cascades activated by receptors such as the dopamine D2 receptors that are required for LTD induction (Calabresi et al. 1992). Our positive control experiment with adenosine indicates that NEM can block adenosine A1 receptor-mediated inhibition of transmission, a process that likely involves a PTX-sensitive G-protein (Munshi et al. 1991; Scholz and Miller 1992). Other mechanisms, such as indirect interactions of NEM on voltage-dependent calcium channels (Shapiro et al. 1994), should be considered as having possible roles in the synaptic actions of NEM in striatum.

Application of NEM enhances transmission before LTD induction, and it could well be the case that this enhancement of transmission simply occludes LTD induction and expression. In addition to the experiments explicitly performed to examine this possibility, which are described in the RESULTS section, three pieces of evidence argue against this interpretation. First, a prolonged exposure to PTX leads to a reduction in synaptic response amplitude and also blocks LTD (Fig. 1A).

![FIG. 5. Evidence for presynaptic effects of NEM at corticostriatal synapses. A, left: representative spontaneous EPSCs obtained in the control condition (before NEM application); right: representative spontaneous EPSCs obtained 20 min after beginning the application of NEM (200 μM). NEM strongly increased the frequency and amplitude of spontaneous EPSCs (n = 5). B: representative records showing spontaneous EPSCs in the control condition (before 200 nM TTX), mEPSCs in the presence of TTX, and mEPSCs in the presence of TTX (200 nM) plus NEM (200 μM). Frequency of EPSCs was not altered in the presence of TTX when compared with that in the predrug control period (P > 0.1, paired t-test, n = 4). However, the frequency of mEPSCs was considerably augmented in the presence of TTX plus NEM when compared with that in the predrug control period (P < 0.01, paired t-test, n = 4). C: (top) representative current traces showing loss of paired-pulse facilitation (PPF) after NEM (200 μM) perfusion. Current traces a and b were obtained before and 30 min after the application of NEM, respectively. The first EPSC amplitude was greatly increased after the perfusion of NEM, and the PPF ratio (calculated as the second EPSC peak current divided by the first EPSC peak current) was decreased. In this case, although the second EPSC amplitude was also increased, the extent of increase was less than the increase in the first EPSC amplitude. Bottom: PPF ratio plotted as a function of time during the course of experiments examining the effect of NEM (200 μM) alone on PPF ratio (n = 5). PPF ratio was decreased after NEM application. Each current trace represents an average of 30 trials. Line: time from beginning to end of NEM perfusion.](http://jn.physiology.org/ by 10.220.32.247 on May 18, 2017)
amplitude as those observed in untreated slices, suggests that the inability to induce LTD was not simply related to the small baseline response amplitude in the PTX-treated slices. Second, NEM blockade of LTD induction is observed regardless of the time between NEM exposure and high-frequency stimulation (compare Fig. 2B and 4). This indicates that LTD is not masked by the slowly developing NEM potentiation. These findings also indicate that LTD induction is blocked even before NEM potentiation is complete, indicating that blockade of LTD is not due to the large response amplitude after the full NEM effect. Third, other treatments that potentiate synaptic transmission, including blockade of adenosine A1 receptors and treatment with aniracetam (Lovinger and Choi 1995; Lovinger et al. 1993), do not prevent synaptic depression. That LTD can be observed even in the face of increased synaptic transmission supports the idea that the effects of NEM interact directly with mechanisms involved in LTD induction and/or maintenance.

We cannot, however, determine whether blockade of the function of PTX-sensitive G-proteins prevents LTD by blocking a specific set of molecular steps linking receptor activation to LTD induction or by antagonizing the synaptic mechanisms involved in LTD expression. Indeed, the expression of LTD has been postulated to involve a decrease in the probability of neurotransmitter release (Choi and Lovinger 1997b), and NEM appears to have an opposite action. We observed that NEM increased the probability of neurotransmitter release, as indicated by the increase in spontaneous EPSC frequency and the loss of PPF observed after NEM treatment. This effect would probably counteract the decrease in neurotransmitter release probability during LTD expression and contribute to the reversal of LTD by NEM. It should be noted, however, that evidence of decreased probability of neurotransmitter release associated with LTD has not been consistently observed in experiments on brain slices from adult rat (Calabresi et al. 1999). However, a recent report (Dos Santos Villar and Walsh 1999) that an increase in PPF ratio is observed during LTD expression is consistent with our earlier findings. Thus, there may be two types of striatal LTD. One type does not appear to involve a change in release probability, whereas the other type involves decreased release probability and may be more highly expressed in developing striatum.

Potentiation by NEM in the absence of HFS suggests that tonic synaptic inhibition involving PTX-sensitive G-proteins occurs at corticostriatal synapses. This could be due to either agonist-independent receptor-mediated G-protein activation (Schütz and Freissmuth 1992) or tonic activity of a G-protein–coupled receptor by an endogenous agonist present within the slice. With respect to the latter mechanism, it is well known that adenosine is present in brain slices at concentrations that can activate inhibitory adenosine A1 receptors. Indeed, inhibition of corticostriatal synaptic transmission by endogenous adenosine has been observed in striatal slices (Lovinger and Choi 1995). Thus, it is tempting to speculate that inhibition of endogenous adenosine receptor activity contributes to the potentiation of transmission by NEM. However, our observations in the experiments with combined DPCPX and NEM exposure suggest that NEM effects on synaptic transmission are not due solely to elimination of A1 receptor-mediated synaptic modulation. In addition, NEM appears to have postsynaptic actions that contribute to potentiation, whereas adenosine A1 receptor effects are believed to be predominantly presynaptic (Malenka and Kocsis 1988). Furthermore, NEM reversal of adenosine A1-mediated inhibition cannot account for the blockade of LTD induction because adenosine A1 receptors are not involved in this process (Lovinger and Choi 1995). Thus, we believe that relief of tonic G-protein activity is the most likely explanation for the effects of NEM on striatal synaptic transmission and plasticity.

We have examined in some detail the mechanism(s) by which NEM facilitates synaptic transmission. NEM alone increased the frequency and amplitude of spontaneous EPSCs, and this effect was also seen for mEPSCs recorded in the presence of TTX. According to classic interpretation of miniature synaptic response analysis (del Castillo and Katz 1954), this finding would suggest that NEM acts on presynaptic terminals at corticostriatal synapses to increase the probability of
glutamate release. This interpretation is also consistent with the observation that NEM abolishes PPF. A change in the number of postsynaptically “silent” synapses may also account for the NEM-induced increase in spontaneous EPSC frequency (Isaac et al. 1995; Liao et al. 1995). However, it is not clear how this would lead to a loss of PPF.

We also obtained evidence that NEM increases the amplitude of spontaneous and miniature EPSCs, a finding that would be interpreted classically as indicating a postsynaptic NEM effect. However, another possible interpretation of these data is that NEM increases the amount of neurotransmitter released per quantum (Liu et al. 1999), an effect that would also increase mEPSC amplitude. It is also possible that the increase in mEPSC amplitude could result from simultaneous multiquantal release in the presence of NEM.

In the hippocampus, Morishita et al. (1997) observed that NEM could only increase GABA receptor–mediated miniature inhibitory postsynaptic current (mIPSC) frequency but not mIPSC amplitude. They observed an increase in mIPSC amplitude by NEM in medium containing 0 mM calcium and 8 mM magnesium. The difference between these findings and our results could be due to the different brain regions and neurotransmitters investigated. It has also been demonstrated that PTX treatment can block the induction of long-term potentiation (LTP) at stratum radiatum CA1 synapses (Goh and Pennefather 1989). In addition, cerebellar LTD involves activation of metabotropic glutamate receptors that most likely act through PTX-sensitive G-proteins (Linden and Connor 1993). Thus involvement of PTX-sensitive G-proteins is a common feature of many forms of synaptic plasticity including striatal LTD.

Potentiation of transmission was also observed after NEM treatment combined with HFS in whole cell and field potential recording experiments. It is possible that this increase reflects the slow potentiation observed with NEM treatment alone and not any LTP produced by specific synergism between the effects of HFS and NEM. Indeed, we did not observe any difference in the magnitude of response potentiation by NEM alone compared with potentiation after NEM and HFS in data from either field potential or whole cell recording experiments. This finding is consistent with the idea that the increase in synaptic response amplitude after combined NEM and HFS is most likely due to the effects of NEM alone.

It is not clear why inhibition of synaptic responses was observed after prolonged PTX exposure, whereas acute NEM exposure enhanced transmission. It is possible that constant, prolonged inhibition of G_{i/o} type G-proteins leads initially to a tonic increase in neurotransmitter release that eventually begins to deplete neurotransmitter pools, because PTX-catalyzed ADP ribosylation and inactivation of G-proteins are irreversible biochemical reactions (Katada et al. 1984; Ui 1984). However, we cannot rule out the possibility that the potentiation of transmission produced by acute NEM exposure involves different mechanisms than those produced by PTX exposure.

The effects of postsynaptic perfusion with NEM indicate that NEM must have a local action at synapses, because the compound could not diffuse too far and maintain an effective concentration, even if it escapes the postsynaptic neuron. Several mechanisms could contribute to these effects. First, NEM may act only at a postsynaptic site. Second, NEM may initiate as yet unknown intracellular pathways in the postsynaptic neuron to stimulate formation of a retrograde messenger. Third, NEM may diffuse through the postsynaptic plasma membrane and across the synaptic cleft to act on a presynaptic site. That NEM increased mEPSC amplitude is consistent with a postsynaptic action. However, the possibility of NEM’s producing only postsynaptic actions appears unlikely, given the increase in frequency of spontaneous EPSCs observed after NEM perfusion. So far, there is no known pathway by which inhibition of PTX-sensitive G-proteins by NEM can stimulate formation of a retrograde messenger. However, activation of G-proteins has been previously reported to be involved in the generation of a known retrograde messenger at developing neuromuscular synapses (Harish and Poo 1992). NEM is reasonably hydrophobic and can pass through membranes when applied extracellularly. It is quite possible that NEM could diffuse back to the presynaptic terminal after postsynaptic perfusion. At present we cannot distinguish between these different mechanisms, but the possibility that NEM possesses or activates retrograde signals is intriguing and may indicate potential use as an experimental tool for future studies.

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REFERENCES


TESTA, C. M., FRIBERG, I. K., WEISS, S. W., AND STANDAERT, D. G. Immunohistochemical localization of metabotropic glutamate receptors mGluR1a and mGluR2/3 in the rat basal ganglia. J. Comp. Neurol. 390: 5–19, 1998.

