Adenosine Acting on A1 Receptors Protects NO-Triggered Rebound Potentiation and LTP in Rat Hippocampal Slices

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INTRODUCTION

In the CNS, the production of the diffusible messenger, nitric oxide (NO), is typically brought about as a result of influx of Ca\(^{2+}\) through \(N\)-methyl-D-aspartate (NMDA) receptor channels, which stimulates the Ca\(^{2+}\)/calmodulin-dependent neuronal NO synthase (nNOS) enzyme tethered nearby (Brenman and Bretd 1997; Garthwaite and Boulton 1995). Although widespread, this is not the only mechanism, as activation of non-NMDA receptors and voltage-sensitive Ca\(^{2+}\) channels can also lead to NO formation (Marin et al. 1993; Okada 1992; Rodriguez-Alvarez et al. 1997; Southam et al. 1991). NO has now been implicated in numerous CNS functions, including long-term synaptic plasticity in several brain areas, such as the hippocampus, cerebellum, striatum, and cerebral cortex. Increasing evidence suggests that, as in other tissues, the principal pathway engaged by NO to elicit synaptic plasticity is activation of the NO receptor enzyme, soluble guanylyl cyclase (sGC), resulting in a rise in cGMP levels (Calabresi et al. 2000; Daniel et al. 1998; Hawkins et al. 1998).

Ordinarily, application of cGMP derivatives or low concentrations of NO does not, of itself, elicit the plastic changes, implying that the activity in the NO-cGMP pathway needs to be synchronized to other events, such as activity in presynaptic terminals or in the postsynaptic elements. In the CA1 subfield of hippocampal slices, however, perfusion of NO donor compounds during low-frequency synaptic stimulation was found to elicit a depression of excitatory synaptic transmission that was followed by a persistent potentiation on washout (Böhme et al. 1991; Bon and Garthwaite 2001a,b). The generation of the potentiation was taken, together with other evidence, to support of a role for NO in long-term potentiation (LTP). In apparent contradiction, a subsequent study reported that NO donors only produced a reversible depression (Boulton et al. 1994). This discrepancy has now been reconciled in that the NO-triggered potentiation is highly dependent on the baseline frequency of synaptic transmission, being present at 0.2 Hz but not at 0.033 Hz (Bon and Garthwaite 2001a). That such a low frequency of stimulation could generate long-term plastic changes in hippocampal synaptic transmission is in itself surprising. More unexpected still was the finding that the potentiation that followed exposure to NO was eliminated by NO synthase inhibition and by blockers of sGC and NMDA receptors. This suggests that the initial NO exposure elicited the rebound potentiation paradoxically by engaging the endogenous NMDA receptor-nNOS-sGC pathway (Bon and Garthwaite 2001b).

The identification of this mechanism provides an opportunity for understanding the important and unresolved issue of the conditions required for NO to potentiate hippocampal synaptic transmission. One question that arises is the relationship between the NO-induced depression and the rebound potentiation. Under 0.033-Hz stimulation, the depression was reported to be blocked by 8-cyclopentyl-1, 3-dipropylxanthine (DCPCX), suggesting that it is caused by adenosine release and the subsequent activation of presynaptic A1 receptors (Broome et al. 1994). The finding that an NO donor, at a concentration...
giving synaptic depression, enhances basal and stimulus-induced adenosine release in hippocampal slices supports this interpretation (Broad et al. 2000; Fallahi et al. 1996). At 0.2-Hz stimulation, the amplitude of the NO-triggered rebound potentiation correlates closely with the amplitude of the preceding depression (Bon and Garthwaite 2001a,b), raising the possibility that the two are causally related. The present experiments aimed to test this possibility.

METHODS

Experiments were performed on hippocampal slices from 6- to 8-wk-old male Sprague-Dawley rats. The slices were prepared as described in the previous paper (Bon and Garthwaite 2001a). Following a recovery period, they were placed in a submerged chamber at 30°C, perfused with an oxygenated (95% O₂-5% CO₂) artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 3 KCl, 1.25 NaH₂PO₄, 1 MgSO₄, 26 NaHCO₃, 2 CaCl₂, and 10 d-glucose.

Stimulating and recording electrodes were positioned in the CA1-stratum radiatum and field excitatory postsynaptic potentials (fEPSPs) were evoked at 0.2 Hz, or occasionally 0.033 Hz, as described elsewhere (Bon and Garthwaite 2001a). Except where indicated, the connection between area CA3 and CA1 was severed to inhibit spontaneous synaptic activity exacerbated in presence of DPCPX (Alzheimer et al. 1989; Thummler and Dunwiddie 2000). LTP was induced by delivering a train of 100 shocks at 100 Hz at twice the baseline voltage. The slope of the EPSP was measured in the region between 20 and 40% of the peak amplitude, and the values were normalized relative to the mean values obtained during the first 15 min of recording in absence of any treatment. In experiments in which DPCPX was perfused, the stimulation voltage was reset (see RESULTS) and subsequent data renormalized the same manner. The averages of eight consecutive fEPSPs were used for analysis.

Drugs were applied to the slices in the perfusion fluid. Stock solutions of 1,1-diethyl-2-hydroxy-2-nitroso-hydrazine sodium (DEA/NO), d(-)-2-amino-5-phosphonopentanoic acid (d-AP5) and L-nitroarginine (L-NOArg) were prepared as described (Bon and Garthwaite 2001b). DPCPX was made up in ethanol and diluted ≥1,000-fold into the ACSF immediately before use. The final concentration of ethanol in the perfusion fluid had no effect on low-frequency synaptic transmission. DEA/NO was supplied by Alexis Corporation (Nottingham, UK); d-AP5, L-NOArg, and DPCPX were all obtained from Tocris Cookson (Bristol, UK). Degraded DEA/NO was obtained by leaving a solution (300 μM) to degrade in oxygenated ACSF at room temperature for ≥4 h.

Data are presented as means ± SE and were analyzed for statistical significance using the two-tailed, unpaired t-test or, when stated, the paired t-test; P values of <0.05 were considered significant. Values for the level of LTP given in the text refer to measurements made 60 min after the tetanus.

RESULTS

Role of area CA3 in NO-elicited depression and potentiation

Some forms of CA1 hippocampal synaptic plasticity are influenced by connections originating from the CA3 area, for example, the potentiation induced by perfusion of a metabotropic glutamate receptor agonist (Bortolotto and Collingridge 1995). The role of these connections in the NO-triggered effects had not been studied previously. Moreover, spontaneous activity in this pathway under conditions of enhanced excitability in the slice (see following text) could give rise to undesired complications. Consequently, a comparison was initially made between intact slices and slices from which connections from area CA3 had been severed.

A variety of classes of NO donor have been used in the past, some of which are now considered dubious because of uncertainties about their inherent chemical reactivity and/or the nature of the NO species derived from them. In the present experiments, we used the NONOate, DEA/NO, which breaks down to release authentic NO with a half-life of ~6 min at 30°C (calculated from Schmidt et al. 1997) and which has previously been shown to elicit the depression-rebound potentiation sequence in hippocampal slices (Bon and Garthwaite 2001a,b).

In agreement with these previous findings, in intact slices stimulated at 0.2 Hz, application of DEA/NO (300 μM) led to a profound synaptic depression (22 ± 9% of control at the maximum effect). On washout, a rebound potentiation emerged, reaching a stable value after ~30 min and lasting ≥1 h, at which time the fEPSP slope was 184 ± 24% of baseline (Fig. 1). In the CA3-lesioned slices, the DEA/NO-induced depression (21 ± 3% at the maximum effect) and rebound potentiation (188 ± 19% after 1 h) were indistinguishable in

![FIG. 1. Effect of cutting connections from CA3 on nitric oxide (NO)-induced depression and potentiation. Intact slices (controls; n = 4) or slices where the area CA3 had been removed (n = 4) were perfused with 1,1-diethyl-2-hydroxy-2-nitroso-hydrazine sodium (DEA/NO) for 10 min. One hour after the washout of the drug, slices were then subjected to high-frequency stimulation (HFS, at ). The 40- to 50-s delay in the perfusion system (see METHODS) has not been corrected for in this and subsequent figures. The first data after HFS are off-scale. The insets show typical fEPSPs (average of 4 consecutive traces) taken at the time points indicated by the letters in intact slices (left) or CA3-lesioned slices (right).](image-url)
time course and amplitude from those found in intact slices (Fig. 1). Furthermore, in both cases, the potentiations occluded subsequent HFS-induced LTP, such that 1 h after HFS, the fEPSP slopes were not significantly different from their respective pretetanus values or from each other (207 ± 26% for the lesioned slices; 203 ± 18% for the intact slices). All subsequent experiments were carried out using CA3-lesioned slices.

**Effect of DPCPX on NO-induced depression at 0.033 Hz**

The action of the adenosine A1 antagonist DPCPX on the depression induced by DEA/NO at 0.033 Hz, was investigated to determine if the findings of Broome et al. (1994), who used a nitrosothiol donor, are applicable to authentic NO.

At this low frequency of stimulation, exposure of slices to DEA/NO resulted in a depression of the fEPSP slope that returned to baseline after drug washout (Fig. 2), whereas subsequent delivery of a brief high-frequency stimulation (HFS) led to a sustained increase in the fEPSP as before (Bon and Garthwaite 2001a; Boulton et al. 1994). Perfusion of DPCPX (1 μM) by itself produced an increase of fEPSP slope that stabilized within 30 min as reported previously (Fig. 2; Broome et al. 1994). The stimulation voltage was then reduced to restore the fEPSP slope to the baseline value (as in Broome et al. 1994), and after a further 15 min, DEA/NO was applied. Compared with control slices, the subsequent peak depression was reduced by ~69%. On washout, the fEPSP slope returned to baseline within ~10 min. DPCPX was washed out at the same time as the DEA/NO, but it is known that the action of this drug is effectively irreversible over the duration of these experiments (Alzheimer et al. 1989; Thummler and Dunwiddie 2000). Tetanic stimulation 1 h after washout of DEA/NO gave rise to an enduring increase in the fEPSP slope (159 ± 5% after 1 h).

**Effect of DPCPX on NO-induced depression and rebound potentiation at 0.2 Hz**

The foregoing results indicate that the major part of the DEA/NO-induced depression at 0.033 Hz is due to adenosine acting on the A1 adenosine receptors. When examined using 0.2-Hz stimulation, DPCPX caused an increase in fEPSP slope similar to that found at 0.033 Hz (Fig. 3). The depression produced by DEA/NO was also inhibited, albeit to a somewhat lesser degree (55%) than observed at 0.033 Hz. Unexpectedly, however, the rebound potentiation normally seen at 0.2-Hz stimulation was abolished, the mean fEPSP slope recorded 1 h after DEA/NO application (87 ± 8%) being not significantly different from that obtained 15 min after resetting the stimulation voltage (98 ± 1%; P > 0.22 by paired t-test). Furthermore, subsequent HFS-induced LTP was abolished (107 ± 18% after 1 h).

**Origin of the blockade of NO-triggered potentiation and LTP by DPCPX**

A simple explanation for the loss of the rebound potentiation and LTP after administration of DPCPX during 0.2-Hz stimulation could be that following the lowering of the stimulation voltage, the numbers of fibers being stimulated was insufficient to generate the necessary cooperativity in the synaptic input required to generate the plastic change (Lee 1983; McNaughton et al. 1978). Comparison of the input-output relationships in slices stimulated at 0.033 and 0.2 Hz, however, indicated that this was unlikely. Just before application of DPCPX, the fEPSP slopes at the two stimulation frequencies were not significantly different (26 ± 2 and 28 ± 4% of the maximum slopes, respectively; n = 3). Likewise, after the voltage reset when the effect of DPCPX was maximal, the values were also not significantly different (18 ± 1 and 19 ± 3% of maximum, respectively; n = 3).

Another concern was whether the increase in fEPSP slope occurring in the presence of DPCPX prior to the voltage reset could have influenced subsequent events at 0.2 Hz but not at 0.033 Hz. To test this, the slope of the fEPSP (elicited at 0.2 Hz) was continuously adjusted back to the baseline during perfusion of DPCPX by lowering the stimulation voltage (Fig. 4). With this protocol, the DEA/NO-induced depression (65 ± 10%) remained the same as observed normally (64 ± 5%). Similarly, there was no rebound potentiation (88 ± 11% 1 h after washout of DEA/NO) nor a subsequent LTP (94 ± 15% 1 h after HFS). Moreover, there were no significant differences in the positioning of the fEPSPs on the input-output curve regardless of whether the voltage was continuously adjusted or
stepped down once the effect of DPCPX had reached steady state. The values (as percentage maximum fEPSP slopes) were 29\% and 28\% before DPCPX and 19\% and 19\% afterward, respectively (n = 4).

A third possibility was that DPCPX alone could have effects at 0.2 Hz but not at 0.033 Hz that result in an inhibition of LTP. To test this, slices were perfused with DPCPX alone for 55 min (the stimulation voltage being reset after 30 min) followed, as usual, by a 60-min perfusion period without DPCPX prior to HFS (Fig. 5). Clear LTP ensued, the fEPSP slope 1 h after HFS (159\% ± 18\%) being significantly different from the pretetanus value (94\% ± 9\%).

Finally, a check was made on the degradation products of DEA/NO by allowing a solution of 300 \( \mu \)M DEA/NO to decay in oxygenated ACSF for \( \geq 4 \) h (Schmidt et al. 1997). At 0.2-Hz stimulation and in the presence of DPCPX, no depression was observed (94\% ± 5\%) when degraded NO was applied 25 min after the voltage reset, whereas fresh NO perfused 15 min later was effective (68\% ± 6\% at the maximal effect, n = 4). At 0.033 Hz, again following DPCPX treatment and the voltage reset, degraded NO also produced no depression (100\% ± 4\%, n = 4) and subsequent LTP was unaffected (155\% ± 17\%, n = 4; data not shown).

Restoration of NO-induced potentiation by an NMDA antagonist

Prior activation of NMDA receptors either synaptically or pharmacologically is able to inhibit subsequent LTP (Coan et al. 1989; Hsu et al. 2000; Huang et al. 1992; Izumi et al. 1992). Conceivably, such an effect could be occurring, particularly under conditions of enhanced excitation imposed by DPCPX. When DEA/NO was applied to DPCPX-treated slices in the presence of the NMDA antagonist, \( \alpha \)-AP5 (50 \( \mu \)M), there was no rebound potentiation (fEPSP slopes 1 h after washout were 87 ± 8 and 76 ± 7\% in the absence and presence of \( \alpha \)-AP5, respectively; \( P > 0.39 \)) and subsequent HFS failed to produce LTP (Fig. 6A). A more prolonged application of \( \alpha \)-AP5 was therefore examined. When \( \alpha \)-AP5 was applied simultaneously with DPCPX (Fig. 6B), the rate of increase in fEPSP slope was slowed, but the value after 30 min was not significantly different (146 ± 10\%) from that observed in absence of \( \alpha \)-AP5 (162 ± 28\%; \( P > 0.57 \)). In the continued presence of \( \alpha \)-AP5, however, a clear rebound potentiation took place following subsequent application of DEA/NO (142 ± 19\% after 1 h).

To calibrate this “restored” rebound potentiation, a comparison was made with control slices (no DPCPX or \( \alpha \)-AP5 treatment) stimulated at the intensity typically used following DPCPX treatment (20\% of maximum fEPSP slope; Fig. 7). The resulting rebound potentiation measured 1 h after washout of DEA/NO was not significantly different (152 ± 9\%, \( P > 0.68 \)). When HFS was given, no significant further potentiation of the fEPSP was observed (174 ± 10\%, \( P > 0.15 \), by paired t-test). This level of potentiation was similar to that found...
when LTP was induced in slices not treated beforehand with DEA/NO (186 ± 28%; data not shown).

In normal slices, the NO-triggered rebound potentiation is blocked by the NO-synthase inhibitor, L-NOArg, indicating that endogenous NO is involved (Bon and Garthwaite 2001b). To investigate if the restored potentiation had this same property, slices were exposed to L-NOArg starting at the same time as the DEA/NO was given and for 1 h afterward. The potentiation was completely blocked (Fig. 8), the mean slope of the fEPSP obtained 30 min after washout of L-NOArg and d-AP5 (98 ± 5%) being not significantly different from the control value obtained 15 min after the voltage reset in presence of DPCPX (100 ± 1%). Moreover, after subsequent HFS, only a small persistent potentiation of the fEPSP slope was observed (112 ± 5%; P < 0.05 by paired t-test). This presumably reflects the slow reversibility of the effect of L-NOArg on NO-synthase (Dwyer et al. 1991).

**DISCUSSION**

**Adenosine and the NO-induced depression**

The finding that the depression elicited by DEA/NO was partly blocked by DPCPX either at 0.2- or 0.033-Hz stimulation frequency is broadly in agreement with the result of Broome et al. (1994) using a nitrosothiol NO donor, except that this previous study found a complete block whereas, at the same stimulation frequency, a 70% reduction was observed in the present experiments. The reason for this relatively minor discrepancy is unclear, but it could be related to the different methods used for recording and quantifying the fEPSPs or to differences in the rates of NO release from the two donors.

A previous study concluded that the depression was mediated by cGMP because it could be mimicked by the cGMP phosphodiesterase inhibitor, zaprinast (Boulton et al. 1994). We found, however, that the concentration-response relationship for the depression produced by DEA/NO was unrelated to that for raising cGMP levels and that the depression was unaffected by inhibition of sGC, pointing to a cGMP-independent mechanism (Bon and Garthwaite 2001b). Similarly, direct measurements of adenosine release from hippocampal slices exposed to an NO donor indicated that cGMP was not involved (Broad et al. 2000). Instead, the depression observed in our experiments appeared to be related to mild metabolic inhibition because DEA/NO, at a concentration that generated a near-complete depression (300 μM), partially inhibited hippocampal oxygen consumption (50%) without affecting ATP levels (Bon and Garthwaite 2001b). Furthermore, the depression-potentiation sequence could be replicated by brief perfusion of the metabolic inhibitor, 2,4-dinitrophenol (Bon and Garthwaite 2001b). The likely site of action of NO is the mitochondrial cytochrome c oxidase enzyme, which is responsible for most cellular oxygen consumption and which is inhibited in a competitive manner by NO (Brown 1999; Brown and Cooper...
A release of adenosine occurs in the hippocampus (and elsewhere) early on under conditions of metabolic inhibition, and this event contributes to the synaptic depression seen in this condition. Indeed, the partial effect of DPCPX observed in our experiments agrees with observations made following hypoxia or aglycemia (Calabresi et al. 1997; Gribkoff et al. 1990; Lucchi et al. 1996). The origin of residual depression is unclear, but may include a contribution from GABAB receptor activation (Dutar and Nicoll 1988; Gahwiler and Brown 1985; Newberry and Nicoll 1984).

Adenosine A1 receptors, NO, and the block of synaptic plasticity

A primary aim of the present experiments was to determine the relationship between the depression and rebound potentiation induced by NO during low-frequency (0.2 Hz) synaptic activity. First of all, this sequence as a whole cannot be attributed just to adenosine release because application of adenosine to hippocampal slices causes only a reversible depression, whether the stimulation frequency is 0.033 or 0.2 Hz (Dunwiddie and Hoffer 1980; Masino and Dunwiddie 2000). Given the unexpected finding that the NO-triggered rebound potentiation is NMDA-receptor dependent and requires the NO synthase-sGC pathway (Bon and Garthwaite 2001b), it was anticipated that the results would help identify the conditions under which the endogenous pathway becomes active in such a way that it contributes to synaptic plasticity. It was hypothesized that the 0.2-Hz stimulation superimposed on a mild metabolic inhibition might result in intracellular Ca2+ accumulation to levels ordinarily observed after tetanic stimulation. The raised Ca2+ could then engage NO synthase and other physiological downstream pathways, resulting in LTP-like synaptic plasticity (Fig. 9A). In this case, the depression would be an epiphenomenon of the transient metabolic inhibition not a necessary precursor of the potentiation. This leads to the prediction that blockade of the depression should not interfere with the potentiation. At first glance, the finding that both the rebound potentiation and tetanus-induced LTP were abolished under conditions where the depression was reduced (by DPCPX) appears to negate this hypothesis. However, such an interpretation ignores other consequences of blocking adenosine A1 receptors.

Several trivial explanations for the loss of plasticity were examined, including an effect of DPCPX per se, the increase in the iEPSP occurring as a result of DPCPX treatment prior to adjustment of the stimulation voltage, and a resulting insufficient stimulus strength; but none of these explanations stood up to experimental testing. In summary, the results suggest that the loss of synaptic plasticity caused by transient exposure to NO requires two conditions: 0.2-Hz (as opposed to 0.033 Hz) stimulation, and block of A1 receptors (Fig. 9B).

A clue as to the mechanism underlying the loss of plasticity was the finding that the rebound potentiation could be

![FIG 8. The DEA/NO-induced potentiation restored by D-AP5 requires endogenous NO. Slices were treated with DPCPX and DEA/NO in the presence of D-AP5 (n = 5) with that obtained when slices are exposed to DEA/NO at a corresponding stimulation voltage giving 20% of maximum of EPSP slope (n = 4). Insets: representative iEPSPs (average of 4 consecutive traces) recorded at the times indicated by the letters from a slice exposed only to DEA/NO.](image)

![FIG 7. Calibration of the NO-induced potentiation restored by D-AP5. A: comparison between the amplitude of the potentiation produced by DEA/NO in the presence of DPCPX and d-AP5 (n = 5) with that obtained when slices are exposed to DEA/NO at a corresponding stimulation voltage giving 20% of maximum of EPSP slope (n = 4). Insets: representative iEPSPs (average of 4 consecutive traces) recorded at the times indicated by the letters from a slice exposed only to DEA/NO.](image)
fully restored by prolonged perfusion of the NMDA antagonist, d-AP5. Again, at first sight, this appears paradoxical as we had previously shown (Bon and Garthwaite 2001b) that the NO-triggered rebound potentiation is inhibited by d-AP5 at the concentration used here. Nevertheless, untimely NMDA receptor activity is well known to be inhibitory to LTP, as shown by the activation of synaptic NMDA receptors (Coan et al. 1989; Hsu et al. 2000; Huang et al. 1992) or by application of NMDA receptor agonists (Izumi et al. 1992), prior to HFS. The available evidence indicates that the inhibition of LTP results from Ca\(^{2+}\) influx and may involve protein kinase C (Hsu et al. 2000). It is already known, in the CA1 area, that inhibition of A1 receptors enhances synaptic NMDA receptor activity during low-frequency stimulation (De Mendoça et al. 1995; Li and Henry 2000). In addition, a stimulation frequency of 0.2 Hz, by partially reducing GABA\(_B\) receptor-mediated inhibition, may further increase NMDA receptor activity (Davies and Collingridge 1993). The combination of DPCPX and 0.2-Hz stimulation, however, did not in itself cause a block of LTP (Fig. 5), implying that the additional transient exposure to NO was critical. NMDA receptor activity just during the exposure to NO was not instrumental (Fig. 6A), unlike in the case of the normal NO-triggered rebound potentiation (Bon and Garthwaite 2001b). Instead, we suggest that it is the partial inhibition of mitochondrial function by NO that under these conditions disables the rebound potentiation and LTP (Fig. 9B). A plausible mechanism would be that reduced mitochondrial function would lead to impaired Ca\(^{2+}\) homeostasis and therefore further Ca\(^{2+}\) accumulation (Brown 1999; Nowicky and Duchen 1998).

In this respect, it is interesting that the impairment of hippocampal LTP produced by prior NMDA treatment has been reported to require NO synthase activity (Zorumski and Izumi 1998), and hypoglycemia has a similar effect through a mechanism involving both NMDA receptors and NO synthase (Izumi et al. 1998). Conceivably in these scenarios, endogenous NO generation, combined with the Ca\(^{2+}\) accumulation associated with NMDA receptor stimulation, may be acting in a manner similar to exogenous NO in our experiments. It is notable that the NO concentration within the slices during perfusion with 300 \(\mu\)M DEA/NO is probably in the submicromolar range (Bon and Garthwaite 2001b), whereas micromolar NO concentrations have been reported in the brain in vivo following ischemia (Malinski et al. 1993). This suggests that NO synthase has the capacity to generate NO concentrations capable of inhibiting mitochondrial function.

**Restored rebound potentiation**

That the rebound potentiation should be reinstated in the continued presence of an NMDA antagonist is somewhat surprising, considering that this potentiation is normally NMDA receptor-dependent (Bon and Garthwaite 2001b). Similarly
surprising is that the restored potentiation remained sensitive to NO synthase inhibition because NO synthase activity is classically coupled to NMDA receptor activity (East and Garthwaite 1991). However, there are several examples of LTP-like phenomena being generated in the CA1 hippocampus in an NMDA receptor-independent manner, for example, by very high-frequency tetanic stimulation (Grover and Teyler 1990), the K$^+$ channel blocker tetraethylammonium (Aniksztejn and Ben-Ari 1990), depolarization combined with synaptic stimulation (Kullmann et al. 1992), or perfusion of a metabotropic glutamate receptor agonist (Bortolotto and Collingridge 1993).

There are also several examples of NO synthase activity in neurons being stimulated independently of NMDA receptors, for example, by stimulation of non-NMDA ionotropic glutamate receptors (Marin et al. 1993; Southam et al. 1991), metabotropic glutamate receptors (Okada 1992), and voltage-sensitive Ca$^{2+}$ channels (Rodriguez-Alvarez et al. 1997). With respect to both LTP and NO synthase activity, however, the NMDA receptor pathway appears to be the one with the lowest threshold (Grover and Teyler 1990; Southam et al. 1991). Thus the simplest interpretation is that prolonged NMDA receptor blockade, under 0.2-Hz synaptic stimulation and when A1 receptors are inhibited, reduces the Ca$^{2+}$ influx such that, on application of NO, the relevant Ca$^{2+}$ levels become similar to those normally found when NO is applied at 0.2 Hz in the presence of functional A1 and NMDA receptors. This then leads to endogenous NO formation and the engagement of other mechanisms required for the potentiation to become manifest (Fig. 9C).

Conclusions

The activation of presynaptic A1 receptors by ambient levels of adenosine normally provides a brake on LTP, as A1 antagonism lowers the threshold for LTP (De Mendonça and Ribeiro 1990) whereas increased adenosine inhibits LTP (De Mendonça and Ribeiro 1990; Mitchell et al. 1993). The first conclusion from the present results is that A1 receptor activity plays an additional complementary role: by curtailing synaptic excitation, it helps to protect the LTP mechanism from becoming desensitized through prior NMDA receptor activity. Second, the notion that the NO-triggered rebound potentiation and LTP were interrelated was based previously on evidence that they mutually exclude each other (Böhme et al. 1991; Bon and Garthwaite 2001a,b; Bon et al. 1992) and that they are both dependent on NMDA receptor activity (Bon and Garthwaite 2001b). The demonstration that the two types of plasticity are suppressed by the DPCPX/0.2-Hz stimulation/NO treatment contributes an additional piece of evidence in favor of this hypothesis. Third, the data provide the first example of a non-NMDA receptor-mediated process generating an enduring potentiation of synaptic transmission through an NO synthase-dependent mechanism. This raises the possibility that activation of NO synthase by Ca$^{2+}$-elevating stimuli distinct from NMDA receptor activity has physiological relevance. Finally, we provide a second example of exogenous NO acting to bring about synaptic plasticity through endogenous NO formation; other effects of exogenous NO reported in the literature may have a similarly paradoxical origin.

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ADENOSINE REGULATES NO-INDUCED SYNAPTIC PLASTICITY


