The Selective Neuronal NO Synthase Inhibitor 7-Nitro-Indazole Blocks Both Long-Term Potentiation and Depotentiation of Field EPSPs in Rat Hippocampal CA1 In Vivo

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The membrane-permeant gas NO is a putative intercellular messenger that has been proposed on the basis of previous *in vitro* studies to be involved in synaptic plasticity, especially the induction of long-term potentiation (LTP) of excitatory synaptic transmission in the hippocampus and cortex. In the present study, the role of NO in synaptic plasticity has been investigated *in vivo*. In particular, the action of the novel and selective neuronal NO synthase (nNOS) inhibitor 7-nitro-indazole (7-NI) has been investigated on the induction of LTP and depotentiation (DP) of field EPSPs in CA1 of the hippocampus *in vivo*. Unlike previously studied nonselective NOS inhibitors, 7-NI does not increase arterial blood pressure. In vehicle-injected rats, high-frequency stimulation consisting of a series of trains at 200 Hz induced LTP. However, LTP induction was strongly inhibited in 7-NI (30 mg/kg, i.p.)-treated animals. The inhibitory effect of 7-NI on the induction of LTP was prevented by pre-treatment with L-arginine, the substrate amino acid used by NOS. In control animals, low-frequency stimulation consisting of 900 stimuli at 10 Hz induced DP of previously established LTP, whereas in 7-NI-treated animals only a short-term depression was induced. This effect of 7-NI also was prevented by D-arginine. The LTP and DP induced in control animals in this study were NMDA receptor-dependent, the NMDA receptor antagonist 3-(R,S)-2-carboxypiperazin-4-yl-propyl-1-phosphonic acid inhibiting the induction of both forms of synaptic plasticity. The present experiments are the first to demonstrate that an NOS inhibitor blocks the induction of the synaptic component of LTP and DP *in vivo* and, therefore, these results strengthen evidence that the production of NO is necessary for the induction of LTP and DP.

Key words: NO; 7-nitro-indazole; neuronal NO synthase; long-term potentiation; depotentiation; CA1 region; hippocampus

Long-term potentiation (LTP) is a long-lasting enhancement of synaptic transmission induced by high-frequency afferent stimulation that is the basis of certain cellular models of memory (Bliss and Lomo, 1973; for review, see Bliss and Collingridge, 1993). Conversely, depotentiation (DP) is the eradication of previously established LTP and usually is induced by low-frequency afferent stimulation (Barrionuevo et al., 1980; for review, see Linden, 1994). The induction of LTP is known to be postsynaptic in origin via, at least in part, the activation of NMDA receptors (Collingridge et al., 1983), whereas the expression of LTP may be partially mediated presynaptically via an increase in transmitter release (Dolphin et al., 1982; Bliss and Collingridge, 1993). Therefore, it has been hypothesized that a retrograde messenger links the postsynaptic and presynaptic cells. The diffusible molecule NO has been proposed as one such retrograde messenger.

NO was shown to be formed in the cerebellum after activation of NMDA receptors (Garthwaite et al., 1988) and was proposed to be an intercellular messenger (Garthwaite et al., 1988; Bredt and Snyder, 1989; Gally et al., 1990). NO is produced from the enzymatic conversion of L-arginine to L-citrulline by NO synthase (NOS). Experimental evidence consistent with the theory that NO acts as a retrograde messenger in LTP has been presented based on several lines of evidence in the CA1 region of rat hippocampus *in vitro*. First, the NOS inhibitors L-N^G-monomethylarginine (L-

NMMA), L-N^G-nitroarginine (L-NOARG), and its methyl ester derivative (L-NAME) blocked the induction of LTP of the EPSP (Bohme et al., 1991; O'Dell et al., 1991; Schuman and Madison, 1991; Bon et al., 1992; Haley et al., 1992), an effect partially reversed by a precursor of NO, L-arginine (Haley et al., 1992). Second, agents that bind NO, such as hemoglobin, also blocked the induction of LTP (O'Dell et al., 1991; Schuman and Madison, 1991; Bon et al., 1992; Haley et al., 1992). Third, NO donors such as sodium nitroprusside and hydroxylamine produced a long-lasting enhancement of excitatory synaptic transmission that was not additive with LTP (Bohme et al., 1991). Fourth, direct application of NO converted the short-term potentiation (STP) produced by weak, high-frequency stimulation into an LTP (Zhuo et al., 1993). L-NMMA also inhibited LTP in the frontal cortex *in vitro* (Nowicky and Bindman, 1993).

However, many later *in vitro* studies have raised doubts about NO acting in a simple way as a retrograde messenger. First, the ability of NOS inhibitors to block LTP is highly variable. For example, very large differences in potencies of NOS inhibitors in their ability to block LTP were found in one study, despite a relative similarity in the ability to inhibit NOS; L-NMMA, in particular, was only marginally active in blocking LTP (Bon et al., 1992). L-NOARG also was shown to be ineffective in blocking LTP, despite inhibition of NOS (Cummings et al., 1994). Second, the protocols of stimulation used to induce LTP were found to be critical in producing a block of LTP by NOS inhibitors, although no uniformity was found in different studies. Thus, NOS inhibitors were found to block LTP induced by weak, but not intense, high-frequency stimulation in three studies (Chetkovitch et al., 1993; Haley et al., 1993; O'Dell et al., 1994). However, another

study demonstrated that NOS inhibitors were effective in reducing LTP induced by intense, but not weak, high-frequency stimulation (Gribkoff and Lum-Ragan, 1992; Lum-Ragan and Gribkoff, 1993). NOS inhibitors were found in one *in vitro* study to produce an effective block only at room temperature in young animals weighing between 70 and 100 gm (Williams et al., 1993). However, a blocking effect of NOS inhibitors at high temperatures (>30°C) (Bohme et al., 1991; Bon et al., 1992; Gribkoff and Lum-Ragan, 1992; Haley et al., 1993; Lum-Ragan et al., 1993; Nowicky and Bindman, 1993) and in older animals weighing between 100 and 200 gm (Bohme et al., 1991; Bon et al., 1992; Haley et al., 1993) has been observed. Studies on the effects of NO donors also have raised doubts about the role of NO in long-term synaptic plasticity. Thus, Boulton et al. (1994) found that several NO donors induced a transient synaptic depression, but not LTP or LTD, in CA1 *in vitro*.

To date, *in vivo* studies have not clarified the role of NO in the induction of LTP, because although the blocking action of intracerebroventricular L-NAME on LTP of the population spike in the dentate gyrus (Iga et al., 1993; Mitzuni et al., 1993) was confirmed in one study (Bannerman et al., 1994a), neither inhibition of the induction of LTP of the EPSP nor population spike was found when the drug was given systemically in a later study by the same group (Bannerman et al., 1994b). Because there are differences in the sensitivity of different hippocampal pathways to NOS inhibitors even within the same subregion (Nicolarakis et al., 1994), it is important to determine whether NO has a role in synaptic plasticity in CA1 *in vivo*. The NOS inhibitors used previously, such as L-NAME, significantly increased peripheral blood pressure, decreasing cerebral blood flow to brain regions including the hippocampus (Lot et al., 1993; Bannerman et al., 1994b). Some of the older NOS inhibitors have other side effects that complicate the interpretation of their *in vivo* effects; for example, L-NAME antagonizes muscarinic acetylcholine receptors (Buxton et al., 1993). In the present studies, therefore, we have studied the effects of a novel NOS inhibitor, 7-nitro-indazole (7-NI), on the induction of both LTP and DP in CA1 *in vivo*. This compound is selective for neuronal NOS (nNOS) *in vivo* (Babbedge et al., 1993; Moore et al., 1993a) and has no effect on mean arterial blood pressure at doses that produce a marked reduction in NOS activity, i.e., 20–80 mg/kg, i.p. (Moore et al., 1993a,b).

MATERIALS AND METHODS

This study was performed using male Wistar rats weighing 250–350 gm (BioResources Unit, Trinity College, Dublin, Ireland). The rats were anesthetized with urethane (ethyl carbamate, 1.5 gm/kg, i.p.) for the duration of all experiments. All animals were maintained on a halothane/oxygen (1% halothane in pure oxygen; flow rate, 1 l/sec) gas mixture during the implantation procedure only. Recovery from halothane was assessed by measuring electroencephalogram activity, which usually took less than 30 min.

Recordings of field EPSPs were made from the CA1 stratum radiatum of the right hippocampal hemisphere in response to stimulation of the Schaffer collateral–commissural pathway. Burr holes 1.5 mm in diameter were drilled over the unilateral electrode implantation sites, which were identified using stereotaxic coordinates relative to bregma and lambda, with the recording site located 3 mm posterior and 2 mm lateral to the midline and the stimulating electrode 4 mm posterior to bregma and 3 mm lateral to the midline. Bipolar stimulating and monopolar recording electrodes consisted of two pieces of twisted tungsten wire attached to a connecting socket insulated along its length with a Teflon coat except at the tips. The dura mater was pierced, and the electrodes were lowered slowly through the cortex and the upper layers of the hippocampus into the CA1 region until the appearance of a negative deflecting EPSP. The electrodes then were fixed in place with cyanoacrylate glue and acrylic dental cement for the stimulation and recording of evoked field EPSPs.

Stainless steel screws fixed to the skull served as ground (anterior, 7 mm; lateral, 5 mm) and reference (posterior, 8 mm; lateral, 1 mm) electrodes.

In all experiments, control EPSPs were evoked at a frequency of 0.033 Hz and an input–output curve (stimulus intensity vs EPSP amplitude) was plotted for each experiment at this test frequency. For the control EPSPs, the stimulation voltage intensity was adjusted to give an EPSP amplitude of 50% of maximum input–output amplitude. LTP was induced using either one set or three sets of trains of stimuli, each set of trains being 10 trains of 20 stimuli (interstimulus interval, 5 msec (200 Hz); intertrain interval, 2 sec; interset interval, 5 min). The three sets were used in the experiments investigating the effects of 7-NI on LTP, whereas the single set was used in the DP and 3-(R,S)-2-carboxypiperazin-4-yl-propyl-1-phosphonic acid (CPP) studies. The low-frequency stimulation used to induce DP consisted of 900 stimuli at 10 Hz which, in previous studies in this laboratory, was found to evoke maximal DP. The stimulation intensity was increased to give an EPSP of 75% maximum amplitude during the stimulation used to induce LTP.

All recording and stimulation was performed using an on-line computerized oscilloscope/stimulator and data analysis interface system (MacLab/2e). Unless otherwise stated, all data are expressed as mean ± SEM percent baseline EPSP amplitude. Statistical tests were carried out using nonparametric Wilcoxon and parametric t tests, and the significance level in all cases was $p < 0.05$.

All drugs used in this study were applied intraperitoneally at the times indicated in the text. 7-NI (Affiniti Research Products) was administered as an emulsified suspension in the fat-soluble vehicle sesame oil (Tesco), prepared in a sonicator immediately before use. 7-NI (9 mg) was prepared in 2 ml of sesame oil to give a final concentration of 30 mg/ml and was administered intraperitoneally to the animal after a 10 min control recording period. Previous studies have shown that 30 mg/kg, i.p. inhibited NOS in the hippocampus by 85 and 45% at 30 min and 4 hr, respectively, after injection (Mackenzie et al., 1994). L-Arginine was obtained from Sigma Ltd. (Poole, UK). CPP was obtained from Tocris Cookson Ltd.

RESULTS

The effect of urethane and 7-NI on baseline EPSP amplitude

EPSPs were elicited at the control test frequency of 0.033 Hz for 2 hr to determine the effect of the anesthetic urethane (1.5 gm/kg) on the test EPSP amplitude. Figure 1a (*top*) shows that the amplitude of the EPSPs did not change significantly over the 90–100 min recording period. For example, the EPSP amplitude at 10, 60, and 100 min from the start of the recording period measured 101 ± 2, 103 ± 2, and 99 ± 5%, respectively ($n = 4, p > 0.05$). Similarly, 7-NI (30 mg/kg) had no significant effect on the test EPSP amplitude over an 80 min period, the EPSP measuring 100 ± 4% for the 5 min preceding drug administration, 102 ± 5% at 25–30 min, 96 ± 8% at 55–60 min, and 103 ± 10% at 75–80 min after injection ($n = 3, p > 0.05$; Fig. 1a, *bottom*).

The effect of 7-NI on the induction of LTP

7-NI was found to inhibit significantly LTP induced by strong, high-frequency stimulation consisting of three sets of high-frequency trains. In these experiments, recordings first were made from two control groups of rats. In one set of control rats injected with the vehicle sesame oil, the amplitude of LTP 20 min after stimulation measured 156 ± 14% ($n = 6$; Fig. 1b). This value was not significantly different from noninjected control rats (155 ± 11%, $n = 5$; data not shown). It can be seen from Figure 1b that the second and third sets, but not the first set, of high-frequency trains induced a short-term depression lasting up to 5 min in the control rats. In the experimental group of rats, 7-NI was administered after a 10 min control recording period, and test EPSPs were monitored for 30 min to determine whether there was an effect of the drug on the baseline amplitude. There was no significant difference between the EPSP amplitude measured 30 min after 7-NI was applied and the EPSP amplitude measured
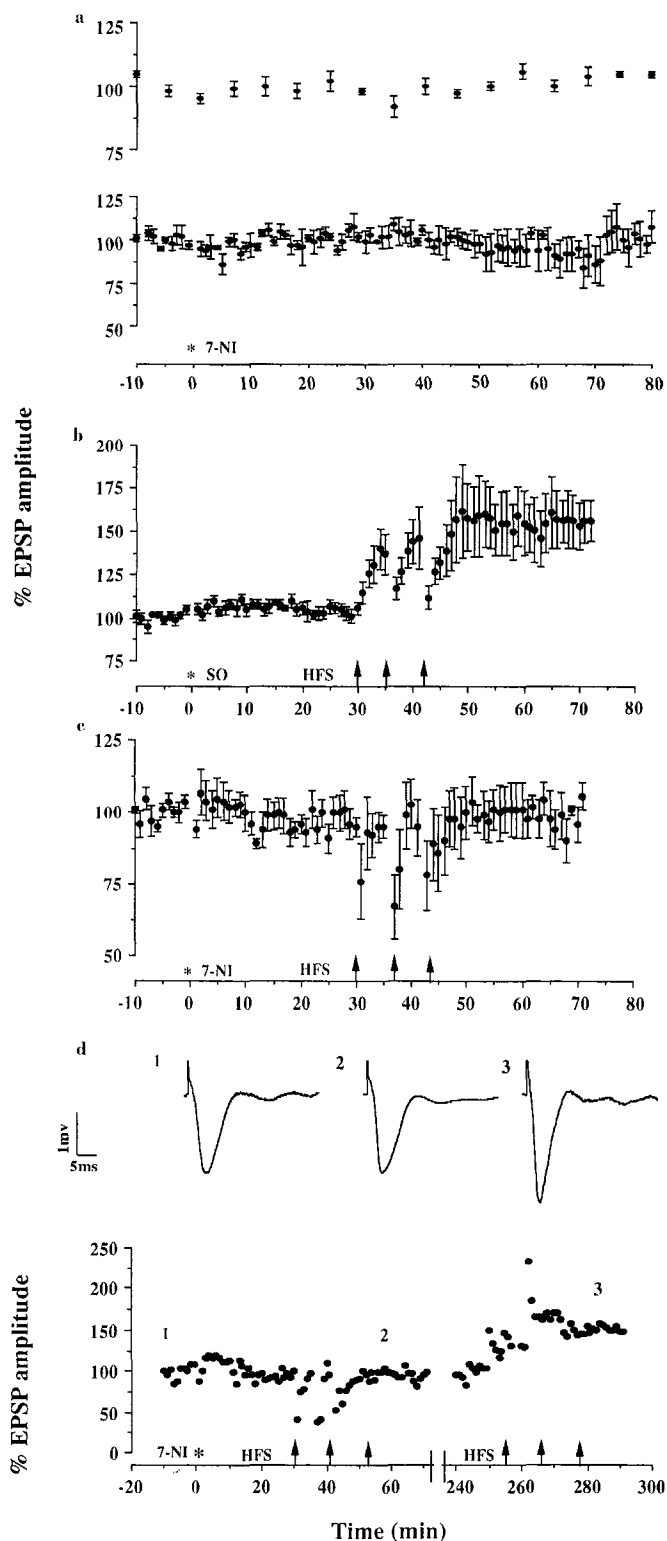
during the preinjection control period (n = 6, p > 0.05). In this experimental group, 7-NI completely blocked the induction of LTP, the value of the EPSP amplitude at 20 min after stimulus measuring 97 ± 13% (n = 6). This was a significant reduction in LTP (p < 0.05; Fig. 1c) compared with the vehicle control group. It can be seen from Figure 1c that all three sets of high-frequency trains of stimuli induced a short-term depression lasting up to 5 min in animals injected with 7-NI.

In three experiments, high-frequency stimulation was applied at 30 min and 4 hr after the injection of 7-NI. 7-NI inhibited the induction of LTP when the high-frequency stimulation was given at 30 min after injection (mean, 101%). However, robust LTP averaging 158% was elicited by the high-frequency stimulation when applied at 4 hr after 7-NI injection (Fig. 1d).

The effect of L-arginine on the action of 7-NI on LTP induction

L-Arginine, the substrate amino acid used by NOS for NO production, was found to reverse partially the inhibitory effect of 7-NI on the induction of LTP. In these experiments, L-arginine (225 mg/kg) was given intraperitoneally 60 sec before application of the drug or the vehicle. In the control group of rats, the effect of L-arginine was investigated on baseline responses and on the induction of LTP. There was no significant effect of the amino acid on the test EPSP amplitude (100 ± 2% before administration of L-arginine vs 100 ± 3% 30 min later; n = 6, p > 0.05). High-frequency stimulation induced LTP measuring 140 ± 7% (n = 4, Fig. 2a). In the experimental group of rats, high-frequency stimulation in the presence of L-arginine and 7-NI (30 mg/kg) induced LTP of 134 ± 6% (n = 5, p > 0.05 compared with L-arginine alone; Fig. 2b). This was a significantly greater amplitude of the EPSP than that observed in the group of rats injected only with 7-NI (97 ± 13%; n = 6, p < 0.05).

The effect of 7-NI on the induction of DP

7-NI was found to inhibit the induction of DP. In a control group of rats, high-frequency stimulation resulted in stable LTP measuring 163 ± 16% at 25–30 min after the tetanus and 160 ± 16% at 55–60 min after the tetanus (n = 5, p > 0.05). In a separate group of rats, DP of 77% was induced by applying low-frequency stimulation, consisting of 900 stimuli at 10 Hz, 30 min after inducing LTP. Thus, the EPSP measured 135 ± 5% at 25–30 min after the tetanus and 108 ± 3% at 25–30 min after low-frequency stimulation (n = 5, p < 0.05 compared with the potentiated level; Fig. 3a). In the experimental group of rats, 7-NI (30 mg/kg) was administered 60 sec after high-frequency stimulation, and the EPSP amplitude was monitored as before for 30 min. LTP at this time measured 152 ± 12% (n = 6). Low-frequency stimulation (900 pulses at 10 Hz) induced a short-term depression lasting 10–15 min with a maximum decrease to 111 ± 20% at 60 sec after stimulus, but induced no DP, the EPSP amplitude fully recovering by 25–30 min after low-frequency stimulation to a value not

Figure 1. The effect of 7-NI and its vehicle on the field EPSP amplitude and LTP in the CA1 of the hippocampus. *a, Top:* urethane (1.5 g/kg, i.p.) does not affect the baseline EPSP significantly over the duration of the experiments, the EPSP measuring 101 ± 2, 103 ± 2, and 99 ± 5% at 10, 60, and 100 min, respectively, from the start of recording (n = 4). *Bottom:* 7-NI (30 mg/kg, i.p.) does not affect the baseline EPSP over the duration of the experiment, the EPSP measuring 100 ± 4% over the 5 min preceding 7-NI injection and 102 ± 5, 96 ± 8, and 103 ± 10% (n = 3) at 25–30, 55–60, and 75–80 min, respectively, after injection. *b,* Sesame oil did not affect EPSP amplitude or the induction of LTP by three high-frequency trains [high-frequency stimulation (*HFS*) indicated by *arrows*],

the amplitude of LTP at 20 min after stimulation measuring 156 ± 14% (n = 6). *c,* 7-NI inhibited the production of LTP by three high-frequency trains, with LTP at 20 min after high-frequency stimulation measuring 97 ± 13% (n = 6, p < 0.05). *d,* Recovery of LTP in a single experiment 4 hr after the administration of 7-NI. Three high-frequency trains failed to induce significant LTP in this experiment after injection of 7-NI, whereas 3.5 hr later robust LTP was induced. *Insets* show representative traces at the points indicated on the graph. An *asterisk* indicates when the drug or vehicle was administered. All data points are mean ± SEM.
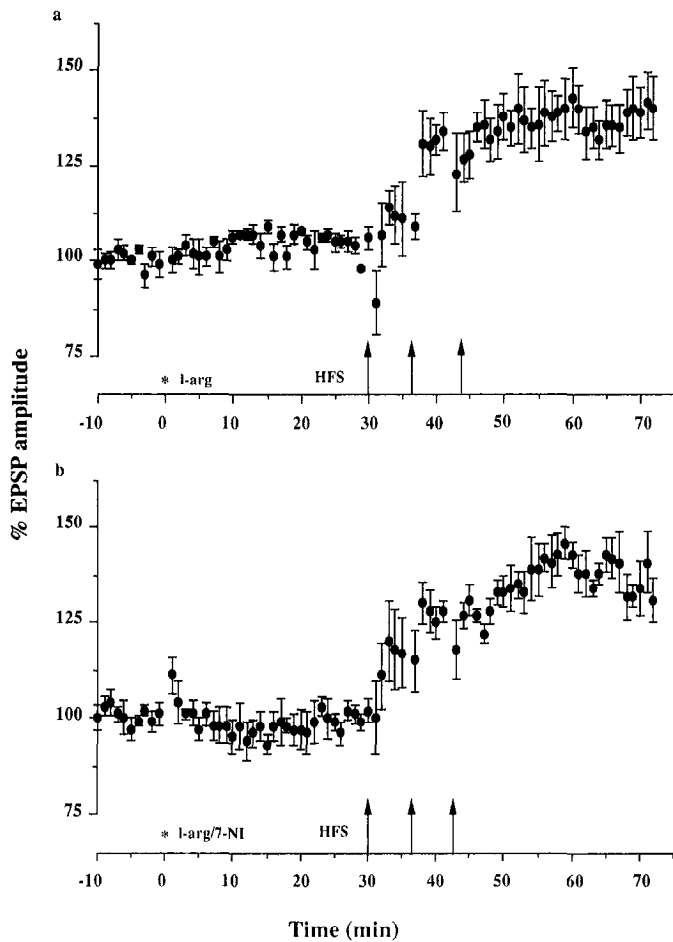
Figure 2. L-Arginine prevented the effect of 7-NI on LTP. *a*, L-Arginine (225 mg/kg, i.p.) had no significant effect on the test EPSP amplitude or on the induction of LTP (LTP 140 ± 7%, *n* = 4). *b*. L-Arginine (225 mg/kg, i.p.) administered 60 sec before 7-NI (30 mg/kg, i.p.) partially overcame the inhibition of induction of LTP by 7-NI (LTP 134 ± 6% 25–30 min after tetanus, *n* = 5). All drug administrations are indicated by *asterisks*; sets of high-frequency stimulations are indicated by *arrows*.
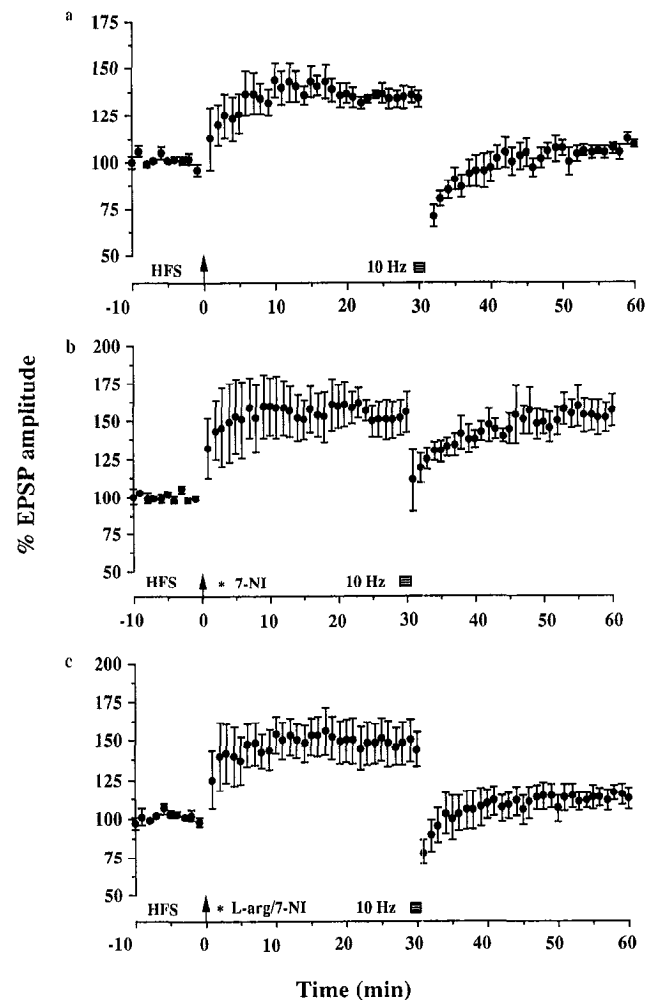

Figure 3. The effect of 7-NI on induction of DP of the field EPSP. *a*, In control experiments, LTP was induced by high-frequency stimulation (LTP 135 ± 5%, *n* = 5) and low-frequency stimulation (900 stimuli at 10 Hz) induced DP to 108 ± 3% (*n* = 5). *b*, 7-NI (30 mg/kg, i.p.) applied 1 min after LTP induction inhibited induction of DP of the EPSP. In these experiments, high-frequency stimulation induced LTP of 152 ± 12% (*n* = 6). Low-frequency stimulation applied 30 min after the induction of LTP induced only a short-term depression, and induced no DP with the EPSP fully recovering to a value not significantly different from the previous potentiated level (154 ± 11%). *c*, L-Arginine (225 mg/kg, i.p.) prevented the 7-NI block of DP. LTP of 147 ± 13% (*n* = 7) was induced by high-frequency stimulation. L-Arginine injected 1 min before injection of 7-NI resulted in a large DP to 113 ± 6, being induced by low-frequency stimulation, a DP of 72%, which is a value significantly different from that of the animals injected with 7-NI alone. All drug administrations are indicated by *asterisks*. Low-frequency stimulation is indicated by the *hatched box*; high-frequency stimulation (*HFS*) is indicated by *arrows*.

significantly different from the previous potentiated level (154 ± 11%; Fig. 3*b*).

The effect of L-arginine on the action of 7-NI on the induction of DP

L-Arginine was found to prevent fully the inhibitory effect of 7-NI on the induction of DP. LTP was induced by high-frequency stimulation in a group of rats (147 ± 13%, *n* = 7), with L-arginine injected 2–3 min later followed by a 7-NI (30 mg/kg) injection. After another 30 min, low-frequency stimulation induced DP, the amplitude of the EPSP measuring 113 ± 6% 25–30 min after low-frequency stimulation. This is a reduction of 72% in the potentiated EPSP amplitude, a value that was significantly different from that of rats injected with 7-NI alone in which no DP occurred (*p* < 0.05; Fig. 3*c*).

The effect of CPP on LTP and DP

Because previous studies have shown that NMDA receptor activation leads to stimulation of NO production, the action of the NMDA receptor antagonist CPP was investigated on the induction of LTP and DP. CPP (7 mg/kg) inhibited the induction of LTP (Fig. 4*a*). Thus, the EPSP amplitude 20 min after tetanus was 106 ± 4% in rats that had been injected with CPP at 30 min before

a single, high-frequency stimulation compared with a pretetanus baseline of 101 ± 2% (*n* = 4).

CPP (7 mg/kg, i.p.) also blocked the induction of DP (Fig. 4*b*). In these experiments, LTP was induced measuring 147 ± 6% at 30 min after high-frequency stimulation. CPP was injected at 2–3 min after LTP induction, and 10 Hz stimulation (900 pulses) was given at 30 min after the induction of LTP. LTP at 15–20 min after the 10 Hz stimulation measured 144 ± 7% (*n* = 5), a value that was not significantly different from that before 10 Hz stimulation, i.e., no DP had been induced in the presence of CPP.
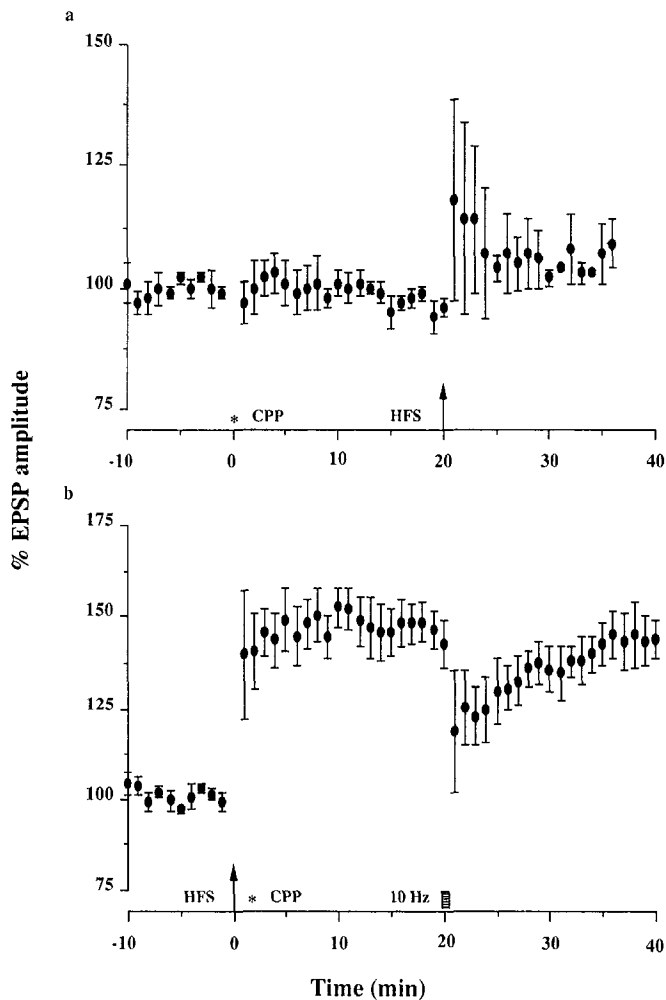
Figure 4. The NMDA antagonist CPP blocked the induction of LTP and DP. *a,* CPP (7 mg/kg, i.p.) inhibited the production of LTP, the EPSP measuring only 106 ± 4% at 20 min after high-frequency stimulation in rats injected with CPP (n = 4). *b,* CPP (7 mg/kg, i.p.) inhibited the induction of DP [LTP 144 ± 6% (n = 5) before low-frequency stimulation vs 144 ± 7% at 20 min after low-frequency stimulation]. CPP was applied at the point indicated by an *asterisk.*

DISCUSSION

The results of the present study show clearly that 7-NI prevents the induction of LTP and that L-arginine, the substrate amino acid used by NOS for NO production, prevented this effect. Thus, these studies support the theory that stimulation of NO production is necessary for the induction of LTP *in vivo* in the CA1 region of the hippocampus. The action of 7-NI was particularly effective in the present studies, with LTP induction being completely abolished by 7-NI. This is the first demonstration *in vivo* that an NOS inhibitor blocks the induction of LTP of the EPSP. Previous studies by Saito's group (Iga et al., 1993; Mitzuni et al., 1993) have reported that the NOS inhibitors L-NAME and L-NMMA blocked LTP induction of the population spike by ~60%, but in such studies the effects of NO could have been exerted via a change in spike threshold rather than a change in excitatory synaptic transmission. Although many previous *in vitro* studies have found a residual STP lasting 15–45 min in the presence of NOS inhibitors (Schuman and Madison, 1991; Haley et al., 1992), others have shown a complete block of STP and LTP (Bohme et al., 1991; Bon et al., 1992). The present conclusions contrast with

those of Bannerman et al. (1994a,b), who failed to show that L-NAME inhibited the induction of LTP of the EPSP in the dentate gyrus. The effect of L-NAME in the study of Bannerman et al. (1994b) could not be assessed easily because it produced a large decrease in baseline synaptic transmission. L-NAME is unsatisfactory for systemic use, because it produces a significant increase in arterial blood pressure that results in a decreased blood flow to many brain regions, including the hippocampus (Bannerman et al., 1994b), and probably leads to a reduction in synaptic transmission. In comparison, 7-NI appears to be selective in its antagonism of nNOS when given systemically, having no effect on arterial blood pressure in the dose range used (Moore et al., 1993a,b) and no significant effect on baseline transmission in the present experiments. Thus, the lack of change in blood pressure, which has been confirmed by Connop et al. (1994) using a dose of 80 mg/kg, i.p., may have been responsible for the difference with the Bannerman et al. (1994b) study. Alternatively, because intracerebroventricular L-NAME did not decrease either baseline EPSPs or LTP (Bannerman et al., 1994a), there may be a difference in the role of NO between the dentate gyrus and CA1. As summarized in the introductory remarks, most previous *in vitro* studies that found a significant effect of NOS antagonists on LTP in the hippocampus were carried out in CA1. The observation that L-arginine prevented the inhibitory action of 7-NI on LTP induction is in agreement with *in vitro* studies in which the blocking action of the NOS inhibitors L-NAME and L-NMMA on LTP induction was opposed by L-arginine (Schuman and Madison, 1991; Haley et al., 1992; Nowicky and Bindman, 1993). Furthermore, these findings provide confirmation that the site of action of 7-NI, indeed, was NOS. It is interesting to note that the effect of 7-NI appeared to be rapidly reversible, robust LTP being generated 4 hr after the injection. This indicates that a critical level of NO can be generated even at a time when there still should be considerable enzyme inhibition (~45%) (Mackenzie et al., 1994).

7-NI has been shown to be specific for neuronal NOS after systemic injection (Babbedge et al., 1993; Moore et al., 1993a,b) and, therefore, the results of the present paper demonstrate that neuronal NOS generates NO during the induction of LTP. Such a conclusion differs from that of the study of O'Dell et al. (1994), in which it was shown that LTP induced by either weak or strong high-frequency stimulation, and the inhibition of such LTP by nonselective NOS antagonism, was very similar in both wild-type and mutant mice lacking nNOS. These authors suggested that endothelial NOS rather than neuronal NOS generates NO during the induction of LTP. However, because both neuronal and endothelial NOS isoforms have been found to be expressed in CA1 pyramidal cells (Chiang et al., 1994; O'Dell et al., 1994), perhaps neuronal and endothelial isoforms generate NO during LTP induction. Several studies have shown that NOS can be induced in neurons in a use-dependent manner (Kato et al., 1994; Lerner-Natoli et al., 1994; Al-Ghoul et al., 1995). The presence of LTP in the nNOS knock-out mice, therefore, may be attributable to compensatory induction of the endothelial form during development. Other adaptive changes in LTP induction mechanisms and a possible species difference may provide an explanation for this apparent discrepancy.

7-NI also was found in the present studies to inhibit the induction of DP. The action of 7-NI was very effective, resulting in a complete block of DP, and the block was prevented by L-arginine. This is the first demonstration that the induction of DP is blocked *in vivo* by an NOS inhibitor. The NOS inhibitors L-NMMA and L-NOARG were found previously to inhibit long-term depression

(LTD) and DP *in vitro* in one study (Izumi and Zorumski, 1993), but L-NOARG was ineffective in blocking LTD in two other studies (Lum-Ragan and Gribkoff, 1993; Cummings et al., 1994).

As detailed above, many studies either have been unable to demonstrate a blocking action of NOS on the induction of changes in synaptic strength in CA1 *in vitro* or have found that the blocking action occurred only under specific conditions of temperature, age of animal, or stimulation protocol. Such variation in the action of NOS inhibitors may be attributable to at least three factors. First, NO appears to have directly opposing actions on LTP induction, one that facilitates LTP induction and one that inhibits LTP induction. Evidence for the latter has come from studies showing that in CA1 *in vitro*, NOS inhibitors facilitated LTP induced by weak stimulation (Kato and Zorumski, 1993), and NOS inhibitors reversed the "untimely" NMDA receptor-activated induced block of LTP (Izumi et al., 1992). Second, the inconsistency of studies on NOS inhibitors and synaptic plasticity may be attributable to NO inducing LTD/DP, which could occlude LTP. Third, because NO is a relatively unstable gas, the *in vitro* conditions required to show that it has a role in synaptic plasticity may be difficult to reproduce.

The finding that very similar results were obtained with the NMDA receptor antagonists CPP and 7-NI on LTP/DP is consistent with the view that NOS activation by the high-frequency/low-frequency stimulation is NMDA receptor-mediated. To our knowledge, this is the first report of NMDA receptor dependence of DP *in vivo*. The discrepancy with regard to the presence of a transient depression after high-frequency stimulation in the presence of 7-NI but not when CPP was used may be dose-related. NMDA receptors allow calcium entry, which is an essential factor for nNOS activity. NMDA receptors are believed to be essential for certain hippocampal-dependent forms of learning. In a related article, we report that 7-NI impaired rats in spatial tasks (our unpublished observations). The block of LTP and DP by NMDA receptor antagonists and an NOS inhibitor in the present article suggests that a role for LTP and DP in learning and memory should be considered.

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