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The effects of focal *N*-methyl-D-aspartate pretreatment on the parameters of amygdaloid electrical kindling

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Abstract

Evidence is accumulating for a role of glutamate in both the development (epileptogenesis) and spread of epileptic neuronal hyperactivity in the brain. In the present investigation we examined the influence of daily focal pretreatment with the selective glutamate receptor agonist *N*-methyl-D-aspartate (NMDA) on the parameters of amygdaloid electrical kindling, an animal model of human complex partial and secondary generalised focal seizures. Pretreatment with NMDA significantly increased the electrical afterdischarge threshold in this model. With subsequent daily suprathreshold electrical stimulation, however, NMDA pretreatment enhanced the kindling process as shown by both electroencephalographic and motor seizure responses. Marked reductions in the number of stimulations required to reach each distinct stage of kindling development were evident. The number of stimulations required to achieve the fully kindled state was approximately halved by pretreatment with NMDA (6.8 ± 1.6 stimulations) compared with control, buffer-pretreated animals (11.6 ± 1.4 stimulations; mean $\pm$ S.E.M.; $P < 0.05$). Consistent with this, the mean durations of the electrically-evoked afterdischarges on most NMDA pretreatment days were significantly increased compared to those recorded in control animals. Importantly, fully kindled animals showed a markedly enhanced sensitivity to focally applied NMDA. The results of the present experiments provide strong in vivo evidence to support the concept that ion fluxes through NMDA receptor-linked cation channels play a major role in the mechanisms of kindling epileptogenesis. Extracellular glutamate at abnormally raised levels, acting at least in part via NMDA receptors, may be the principal agent triggering many forms of epilepsy.

Keywords: NMDA (*N*-methyl-D-aspartate); Kindling; Amygdala; Seizure; Epilepsy

1. Introduction

The role of excitatory amino acids, and in particular glutamate, in mechanisms of epileptogenesis is presently a major focus of research interest (for reviews see Bradford and Peterson, 1987; Dingledine et al., 1990; Bradford, 1995). Electrical kindling (Goddard et al., 1969) is an animal model of epilepsy widely used for such studies. We, and others, have previously demonstrated that competitive antagonists acting at the *N*-methyl-D-aspartate (NMDA) sub-type of excitatory amino acid receptors, e.g., 2-amino-7-phosphonoheptanoic acid (AP7), 3-((*RS*)-2-carboxy-piperazin-4-yl)-propyl-1-phosphonic acid (CPP) and DL-[*E*]-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 37849) both retard the rate of electrical kindling (Croucher et al., 1988; Cain et al., 1988; Vezzani et al., 1988; Holmes et al., 1990) and raise generalised seizure thresholds in fully electrically kindled animals (Croucher et al., 1992a). In addition, daily intra-amygdaloid microinjections of the endogenous amino acids glutamate and/or aspartate causes kindling of limbic seizures (Mori and Wada, 1987; Croucher and Bradford, 1989) which is blocked by co-administration of an NMDA receptor antagonist (Croucher and Bradford, 1990a) and can transfer to electrical kindling (i.e., reduce the number of electrical stimulations required to reach stage 5) (Mori and Wada, 1987; Croucher and Bradford, 1989). More recently, using intracerebral microdialysis, we have shown that both tissue content and basal extracellular levels of glutamate are elevated in the amygdaloid complex following electrical kindling of this region (Kaura et al., 1995). Others have reported raised extracellular focal glutamate levels in hippocampi of amygdala kindled rats, measured using microdialysis (Minamoto et al., 1992; Ueda and Tsuru, 1994).

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The above observations support the hypothesis that glutamate plays a central role in both the development (epileptogenesis) and spread of epileptic neuronal hyperactivity in the brain. Extracellular glutamate at abnormally raised levels, and acting at least in part via NMDA receptors, may therefore be the principal agent triggering many forms of epilepsy.

In the present study we examined the influence of daily focal pretreatment with the selective excitatory amino acid agonist NMDA on the parameters of amygdaloid electrical kindling. Pretreatment with NMDA is shown to *increase* the electrical afterdischarge threshold. With subsequent daily suprathreshold stimulation, however, NMDA pretreatment enhanced electrical kindling, as shown by both electroencephalographic and motor seizure responses, with marked reductions in the number of stimulations required to reach each stage of kindling development. Importantly, fully kindled animals also showed a markedly enhanced sensitivity to locally applied NMDA. Preliminary accounts of some of this work have been published (Croucher et al., 1992b,c).

2. Materials and methods

2.1. Surgical procedures

Details of the surgical procedures used in the present study are described in detail elsewhere (Croucher et al., 1992a). Briefly, male Sprague-Dawley rats (290–320 g) were implanted with a guide cannula/bipolar electrode assembly into the right basolateral amygdala under halothane/nitrous oxide anaesthesia. The construction of these assemblies has been documented previously (Croucher and Bradford, 1989). The stereotaxic coordinates for the tips of the bipolar electrodes were: AP = −0.8, L = 3.8, V = −8.8 from the skull surface (incisor bar 2.5 mm above the interaural line). The unit was secured to the skull with the aid of two stainless steel anchor screws, using cyanoacrylate cement and zinc powder. Animals were subsequently allowed at least one week for recovery from surgery before kindling commenced. Food and water were available ad libitum.

2.2. Afterdischarge threshold estimation and electrical kindling

Electrical kindling was performed according to a standard protocol used in this laboratory (Croucher and Bradford, 1991). Briefly, constant current stimulations (1 s train of 1 ms biphasic square-wave pulses at 60 Hz frequency) were applied daily through the bipolar electrodes and evoked afterdischarges were recorded on a polygraph (Grass Model 79D). An afterdischarge was defined as post-stimulus amygdaloid spiking on the electroencephalogram which showed a frequency exceeding 1 Hz with an amplitude of at least twice that of the pre-stimulus recording. The afterdischarge threshold was defined as the minimum current required to elicit an afterdischarge of at least 6 s. This threshold was estimated in each animal using a method of ascending limits based on that of Freeman and Jarvis (1981). Essentially, constant current stimulations (parameters as above) were applied to the amygdala at 2 min intervals, using an initial stimulating current of 100 μA and with progressive increments of 50 μA until an afterdischarge was evoked. Twenty-four hours later the procedure was repeated using an initial stimulating current of 50 μA less than the maximum current required the previous day and stimulating increments of 25 μA. The current required to elicit an afterdischarge on the second day was taken as the afterdischarge threshold.

Animals were then kindled by daily stimulation with 125% of their individual afterdischarge threshold currents. The durations of the evoked afterdischarges and the severity of the accompanying motor seizures were monitored. Motor seizure activity was rated on a scale of 0–5 based on that of Racine (1972), as follows: 0 = no behavioural response; 1 = facial myoclonus and vibrissae twitching; 2 = jaw myoclonus and/or head bobbing; 3 = 2 plus unilateral forelimb myoclonus; 4 = 3 plus rearing with bilateral forelimb myoclonus; 5 = 4 plus repeated rearing and falling.

2.3. Generalised seizure threshold estimation

The generalised seizure threshold was defined as the minimum current required to elicit a fully kindled stage 5 seizure. Generalised seizure thresholds were estimated using the method of ascending limits described above (Section 2.2), with stimulation commencing 100 μA below the afterdischarge threshold for each animal and increasing every 2 min, in 25 μA increments, until a stage 5 seizure response was evoked.

2.4. Focal injection procedure

Buffer or NMDA was delivered focally into the amygdala through a 28-gauge stainless steel injection cannula inserted into the indwelling guide cannula, 15 min before electrical stimulation was applied. Injections were made in a total volume of 0.5 μl phosphate buffer (50 mM, pH 7.4) delivered over a period of 90 s. The injection cannula was left in position for a further 30 s to allow diffusion of the injectate away from the cannula tip, after which a stylette was re-inserted into the guide cannula.

2.5. Histology

At the end of the study animals were deeply anaesthetised with pentobarbitone (Sagittal) and perfused transcardially with phosphate buffered saline (200 mM, pH 7.4). The brains were removed and rapidly frozen in

cooled isopentane. Serial 20 μm coronal sections were cut using a cryotome, mounted on gelatine-coated microscope slides, and stained with Toluidine Blue. Sections were examined by light microscopy for histological changes and to confirm the positioning of the injection cannula and electrode tips.

2.6. Drugs

NMDA was supplied by Tocris Cookson (Bristol, UK). It was dissolved in a minimum volume of 1 M NaOH prior to dilution with phosphate buffer (50 mM, final pH 7.4). All other chemicals were purchased from approved commercial suppliers.

2.7. Statistical analysis

Student's *t*-test for independent groups was used for analysis of all data, as approved by the Statistics Department of Imperial College London for this purpose.

3. Results

3.1. Sensitivity of animals to focally injected NMDA

Ten rats were randomly assigned to one of two groups: Group A (controls: buffer injected) or Group B (NMDA pretreated). The sensitivity of the animals in Group B to focally administered NMDA was first established in order to identify an appropriate dose of the agonist for subsequent pretreatments, i.e., a dose which, when administered alone, produced no significant changes in electrical or behavioural activity. Increasing doses of NMDA were given on consecutive days and behavioural and electrographic responses were monitored for 30 min post-injection. NMDA, 1–2 nmol, produced no behavioural or electrographic signs of seizure activity. NMDA, 5 nmol, on the other hand, evoked mild focal seizure activity (stage 1–2 seizures) and spiking on the electroencephalogram in 2/5 animals in the group. A dose of 2 nmol of NMDA (just sub-threshold) was therefore selected for subsequent use and was administered focally, 15 min prior to each daily electrical stimulation, during the kindling procedure.

3.2. Effects of NMDA pretreatment on afterdischarge threshold and initial seizure response

Afterdischarge thresholds were estimated in all animals prior to commencing buffer (Group A) or NMDA pretreatment (Group B), as described above. The mean afterdischarge thresholds for the 2 groups (115.0 ± 29.2 μA vs. 90.0 ± 17.0 μA, mean ± S.E.M. for Groups A and B, respectively) did not differ significantly (Table 1). The influence of focally injected buffer or NMDA on the mean afterdischarge threshold was assessed on the following day again using the method of ascending limits but starting with a stimulating current 50 μA below the initial afterdischarge threshold and using 25 μA increments at 2-min intervals.

Focal pretreatment with NMDA, 2 nmol, produced a significant (77.8%) increase in mean afterdischarge threshold (Table 1). Buffer pretreated animals showed no significant change in this parameter (Table 1). In addition, the seizure activity evoked by the first threshold current following NMDA pretreatment was markedly enhanced compared to that seen in the buffer treated group. Thus, both the mean afterdischarge duration (51.6 ± 8.9 s vs. 24.0 ± 7.6 s) and motor seizure rating (3.0 ± 0.3 vs. 1.4 ± 0.4) were significantly greater in Group B compared to Group A (Table 1).

3.3. Effects of NMDA pretreatment on the development of electrical kindling

Daily pretreatment with NMDA, 2 nmol, significantly enhanced the rate of electrical kindling as shown by the marked reductions in the number of stimulations required to reach each stage of kindling development (Table 1 and Fig. 1). The mean number of daily stimulations required to attain the fully kindled state was significantly less in the NMDA pretreated (6.8 ± 1.6 stimulations) compared to the control, buffer pretreated group (11.6 ± 1.4 stimulations; $P < 0.05$). The mean durations of the evoked afterdis-

Table 1
The effect of NMDA pretreatment on electrical kindling of the rat amygdala

Pretreatment	Initial ADT	Parameters (post-injection)			ADs to first:			
	(μA)	ADT (μA)	ADD [a] (s)	SS [a]	Stage 2	Stage 3	Stage 4	Stage 5
Buffer (controls)	115.0 ± 29.2	90.0 ± 40.8	24.0 ± 7.6	1.4 ± 0.4	3.6 ± 0.4	8.6 ± 1.1	10.2 ± 1.0	11.6 ± 1.4
NMDA	90.0 ± 17.0	160.0 ± 10.0 [e]	51.6 ± 8.9 [c]	3.0 ± 0.3 [d]	(3.0) [b]	3.2 ± 0.2 [d]	4.6 ± 0.7 [d]	6.8 ± 1.6 [c]

Values are mean ± S.E.M., $n = 5$. AD, afterdischarge; ADD, afterdischarge duration; ADT, afterdischarge threshold; SS, seizure score (see text). [a] Measured following ADT estimation; [b] $n = 1$ only as 4/5 animals progressed immediately from stage 1 to stage 3 seizure activity in this group. Significance of differences was determined using Student's *t*-test for independent groups: [c] $P < 0.05$, [d] $P < 0.01$ (compared with control group) or Student's *t*-test for matched pairs; [e] $P < 0.05$ (compared with preceding response in the same animals).

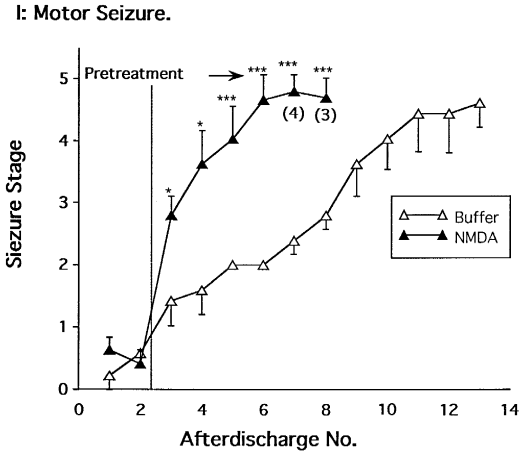

Fig. 1. The effect of NMDA pretreatment on electrical kindling of the rat amygdala. I: Motor seizure. *N*-Methyl-D-aspartate (2 nmol) or buffer was focally administered 15 min prior to daily electrical kindling stimulation and the development of the motor seizure response was monitored (see text for rating scale). Values shown are mean ± S.E.M., $n = 5$ except where otherwise indicated. Significance of differences between the mean responses seen following NMDA compared with buffer pretreatment were calculated using Student's *t*-test for independent groups: * $P < 0.05$; *** $P < 0.005$.

charges were also significantly increased after most kindling stimulations in the NMDA-pretreated animals (Fig. 2).

3.4. Enhanced sensitivity of fully kindled animals to focally injected NMDA

The sensitivity of the animals in Group B, to focally administered NMDA following full kindling, was reassessed and compared to the sensitivity prior to kindling. Buffer (0.5 μl) or NMDA (0.5–1.0 nmol in 0.5 μl buffer)

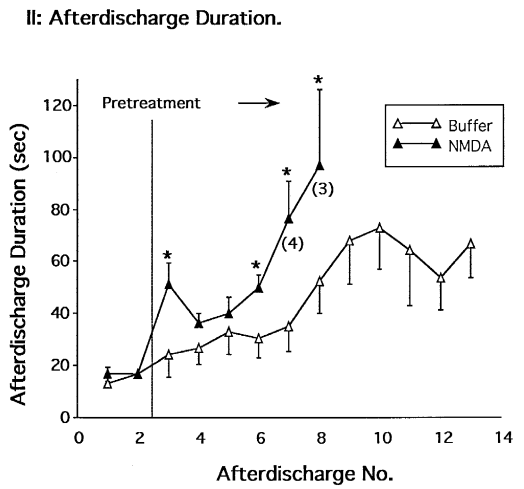

Fig. 2. The effect of NMDA pretreatment on electrical kindling of the rat amygdala. II: Afterdischarge duration. Legend as for Fig. 1 except that the development of the afterdischarge duration in response to electrical kindling stimulation in NMDA (2 nmol) or buffer pretreated animals is shown.

was administered focally and all animals were carefully observed for any signs of seizure activity. All fully kindled animals showed greatly enhanced sensitivity to focally administered NMDA. Thus, although NMDA, 0.5 nmol, evoked no response in any animal, NMDA, 1 nmol, either reduced the generalised seizure threshold (mean reduction 53.3%, 2/5 animals) or induced focal or generalised seizure activity alone (3/5 animals). In these same animals prior to kindling, initial administration of NMDA, 1 nmol, failed to evoke any motor or electroencephalographic signs of seizure response (see Section 3.1).

3.5. Histology

Histological examination confirmed the positioning of the electrode tips in the basolateral amygdala of all animals. There was no evidence of neuronal loss or glial cell proliferation within the amydaloid complex of either the buffer pretreated or NMDA pretreated animals.

4. Discussion

The results of the present study demonstrate that focal administration of a low dose of the selective glutamate receptor agonist NMDA, prior to electrical kindling stimulation, significantly enhances the seizure kindling process as judged by both electroencephalographic and motor manifestations. Thus, even following the first NMDA pretreatment, both the duration of the subsequent electrically evoked afterdischarge and the magnitude of the evoked motor seizure response were significantly increased compared to control, vehicle-pretreated animals (Table 1). Subsequently, the number of electrical stimulations required to reach each stage of the kindling process was markedly reduced by daily pretreatment with the agonist (Table 1 and Fig. 1). The number of stimulations required to achieve full kindling was approximately halved (mean of 6.8 compared to 11.6 stimulations) by the NMDA pretreatment (Table 1). Consistent with this, the mean durations of the electrically evoked afterdischarges on most NMDA pretreatment days were significantly increased compared to those recorded following vehicle administration (Fig. 2). Although the durability of this enhanced kindling process was not examined in the present study, previous 'long-term' studies in our laboratory have shown that kindling induced by either NMDA (Croucher et al., 1995) or glutamate alone (Croucher and Bradford, 1989) is fully maintained for at least several months.

Earlier studies concluded that physiological levels of extracellular Mg^{2+} ions may provide a voltage-dependant blockade of NMDA receptor-linked ion channels in vivo under resting conditions (Mayer et al., 1984; Nowak et al., 1984). However, subsequent work by our own group, including studies of hippocampal Ca^{2+} ion fluxes in response to stimulation by NMDA (Crowder et al., 1987)

and the demonstration that repeated focal administration of NMDA alone (2 nmol in a different group of male Sprague-Dawley rats) causes kindling of full limbic seizures (Croucher et al., 1995), strongly suggests that a significant proportion of these receptors, at least in the hippocampus and basolateral amygdala, are not blocked by Mg^{2+} and are responsive to NMDA receptor agonists in the absence of additional depolarising influences. Indeed, electrophysiological data have also demonstrated the occurrence of NMDA receptor activation in the basolateral amygdala under normal physiological conditions (Rainnie et al., 1991). In the present study the observation of electroencephalographic and motor seizure activity in response to intra-amygdaloid injection of NMDA (5 nmol) (Section 3.1) provides further evidence for the existence of significant numbers of fully responsive (i.e., non-Mg^{2+}-blocked) NMDA receptor populations under resting conditions.

A considerable body of evidence now supports a central role for glutamate, acting at least in part via NMDA receptor-mediated mechanisms, in epileptogenesis. Thus, NMDA receptor antagonists, acting either at the NMDA recognition site or at other sites on the receptor complex, e.g., the receptor-linked cation channel or the strychnine-insensitive glycine binding site, are potent inhibitors of electrical kindling (Croucher et al., 1988; Cain et al., 1988; Vezzani et al., 1988; Holmes et al., 1990; Croucher and Bradford, 1990b). In addition, daily focal injection of glutamate into the basolateral amygdala has been shown to cause kindling of full limbic seizures (Mori and Wada, 1987; Croucher and Bradford, 1989; Mori et al., 1989), a process that is also inhibited by co-administration of NMDA receptor antagonists (Croucher and Bradford, 1990a). Moreover, as alluded to earlier, seizure kindling can be induced by focal injection of NMDA itself (Croucher et al., 1995). The hypothesis that endogenously released glutamate is the agonist that normally stimulates the NMDA receptors during seizure kindling has been considerably strengthened by our recent studies, both in vitro and in vivo, showing chronic increases in tissue content as well as raised basal extracellular levels of this amino acid after electrical kindling (Kaura et al., 1995). Others have also reported enhanced in vivo extracellular levels of glutamate prior to seizure onset in kindled animal brain regions and in human temporal lobe epilepsy (Minamoto et al., 1992; Ueda and Tsuru, 1994; During and Spencer, 1993). The results of the present experiments concur with the above observations and further support the concept that ion fluxes through NMDA receptor-linked cation channels may play a crucial role in the mechanisms of kindling epileptogenesis (see below).

The mechanism by which NMDA foreshortens the development of electrical kindling remains obscure. However, if a chronic rise in extracellular glutamate levels is a trigger for inducing the kindled state, as suggested above, then daily focal microinjections of NMDA may be inducing sustained local glutamate release which leads, over a period, to chronically raised basal glutamate levels. Similar rises in these levels induced by subsequent daily electrical stimulations (see above) would further enhance this effect and thereby accelerate the rate at which full kindling (i.e., stage 5) is achieved. Consistent with this hypothesis, local application of NMDA into striatum in vivo, at 300–1000 μM for 15 min, has been shown by microdialysis to cause a doubling in basal levels of glutamate (Young and Bradford, 1991). In the present experiments local application of 2 nmol of NMDA in 0.5 μl is equivalent to applying 4 mM NMDA, i.e., 4-fold the level used by Young and Bradford (1991).

Further evidence of a common mode of action (i.e., via NMDA receptors) of electrical and NMDA-induced kindling is the transference between these two modes. Thus, each foreshortens the development of fully kindled epileptogenesis (i.e., to stage 5) by the other, when applied on a daily basis to give, for example, 50% kindling (e.g., stage 2–3) (Mori and Wada, 1987; Croucher and Bradford, 1989).

A further important finding in the present study is that fully kindled animals show greatly enhanced sensitivity to focally applied NMDA. Thus, prior to kindling, intra-amygdaloid NMDA (2 nmol) failed to evoke either electroencephalographic or motor signs of seizure activity. Following kindling, however, in the same group of animals, focally applied NMDA, at a dose of only 1 nmol, evoked either a proconvulsant response, i.e., a reduction in the generalised seizure threshold, or induced focal or generalised motor seizure activity (Section 3.4). This NMDA 'supersensitivity' parallels the increase in sensitivity to electrical stimulation clearly apparent following electrical kindling and is consistent with an increase in the NMDA receptor-mediated component of excitatory synaptic potentials originally recorded in dentate gyrus granule cells following hippocampal kindling (Mody and Heinemann, 1987; Mody et al., 1988). However, the mechanism or mechanisms responsible for this enhanced excitability are at present elusive. It does not clearly appear to be an increase in NMDA receptor population size. Thus, while some authors, using a range of different radioligands, have reported increased NMDA receptor binding following electrical kindling (Yeh et al., 1989; Wu et al., 1990; Cincotta et al., 1991) others have reported decreased binding (Sircar et al., 1987; Akiyama et al., 1992) or no significant changes (Jones and Johnson, 1989; Okazaki et al., 1989; Vezzani et al., 1990; Chaudieu et al., 1991). Interestingly, Kraus et al. (1994) have recently demonstrated a selective increase in the number of binding sites for the competitive NMDA receptor antagonist 3-[(±)-2-(carboxypiperazine-4-yl)] [1,2-^{3}H-]propyl-1-phosphonic acid ([^{3}H]CPP) in hippocampal region CA3 28 days after electrical amygdaloid kindling. No changes in the binding of another competitive NMDA receptor antagonist, *cis*-4-(phosphonomethyl)-2-^{3}H-piperidinecarboxylate ([^{3}H]CGS-19755),

however, were detected at this time in any hippocampal region. In addition, this group and others have reported an inability to detect any changes in the expression of mRNA for NMDA receptors in the hippocampus at 1 day or 1 month after the last seizure induced by amygdala kindling (Kraus et al., 1994; Hikiji et al., 1993). Other studies have produced contradictory results concerning the effects of kindling on NMDA receptor-mediated biochemical processes, including the modulation of release of central monoamine neurotransmitters (Morrisett et al., 1989; Jones and Johnson, 1989; Ohmori et al., 1992).

Changes in intracellular processes associated with NMDA receptor activity, e.g., calcium-dependent protein phosphorylation, may also be important in kindling. In this respect, Patel et al. (1984) have demonstrated an increase in phosphorylation of a 45-kDa protein in amygdaloid kindled tissue, whilst Wasterlain and Farber (1984) have shown a long-term decrease in Ca^{2+}/calmodulin-stimulated phosphorylation of three intracellular proteins, including calcium/calmodulin-dependent protein kinase II, following electrical kindling. Thus, whilst our own results support the concept of development of NMDA receptor 'supersensitivity' during kindling epileptogenesis, whether this results from an increase in NMDA receptor population size or affinity, or changes in related 'downstream' intracellular processes, has yet to be established. Moreover, our recent report of chronically increased extracellular glutamate levels in vivo following amygdaloid kindling (Kaura et al., 1995), together with earlier in vitro studies reporting long-term, kindling-induced enhancement of stimulated glutamate release (Leach et al., 1985; Guela et al., 1988; Jarvie et al., 1990), strongly suggest the additional presence of a presynaptic component in the mechanism of development of the kindled hyperexcitable state. Our own further recent studies have demonstrated that blockade of glutamate release by stimulation of presynaptic metabotropic glutamate receptors can prevent kindled epileptogenesis (Attwell et al., 1995). Thus, alteration in the properties of these same presynaptic receptors which leads to poor responsiveness to their endogenous agonist (i.e., glutamate) could lead to chronically enhanced presynaptic glutamate release with parallel lowered threshold to glutamate-induced seizures.

It is notable that focal pretreatment with NMDA (2 nmol; $n = 5$) caused an increase in electrical afterdischarge threshold when compared with the control, vehicle pretreated group ($n = 5$; Table 1). This was an unexpected observation and the mechanism involved is unclear. It may, however, be related to reported increases in firing thresholds in in vitro studies following low level NMDA receptor activation (Gilbert, 1991; Chernevskaya et al., 1991; Craig and White, 1992). In this respect, Craig and White (1992) showed that NMDA receptor activation causes the release of adenosine from rat cortical slices and suggested that this may provide an inhibitory threshold against further NMDA receptor-mediated neurotransmission. However, as shown in the present study, with sufficient (suprathreshold) electrical stimulation, kindling in the presence of exogenously applied NMDA clearly occurs at a faster rate than in its absence. This may be due to a change in the balance of stimulation of ionotropic and metabotropic glutamate receptors in the presence of NMDA compared to that normally evoked by endogenous glutamate released by the electrical kindling stimulations alone (Kaura et al., 1995). Although the cellular mechanisms require further study, the present results certainly provide strong in vivo evidence to support a major role for enhancement of NMDA receptor-mediated processes in kindling epileptogenesis.

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