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Interactions of glutamate receptor agonists with long-term potentiation in the rat hippocampal slice

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Abstract

Previous work has described the apparent desensitisation of neuronal networks in the rat neocortex to amino acid agonists, following prior exposure several minutes earlier. Since long-term potentiation is believed to involve activation of amino acid receptors, we have now sought to determine whether long-term potentiation can modify the sensitivity of neurones to glutamate receptor agonists in rat hippocampal slices. Responses were measured as the change in population spike or postsynaptic potential (e.p.s.p.) size. Two applications of *N*-methyl-D-aspartate (NMDA), quinolinic acid, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) or kainate, 45 min apart, did not exhibit any apparent desensitisation. However, the induction of long-term potentiation produced a marked loss of sensitivity to quinolinic acid, with smaller effects on NMDA, AMPA and kainate responses. No marked changes were obtained of e.p.s.p. size. In order to localise the cellular sites of these changes, agonists were also applied by microiontophoresis to the cell bodies or dendritic regions of CA1 neurones. Responses to quinolinic acid showed apparent desensitisation at both sites, whereas no decrease was observed in responses to NMDA or AMPA application. The induction of long-term potentiation again produced a decrease in the size of responses to NMDA and AMPA. Inhibition of nitric oxide (NO) synthase prevented the long-term potentiation-induced loss of responsiveness to NMDA, but not AMPA, implying a role for NO in the loss of NMDA sensitivity. Recordings of single cell activity during the iontophoretic application of agonists and induction of long-term potentiation showed that responses to NMDA were often suppressed to a greater extent than to quinolinic acid. The results indicate that long-term potentiation can modify the sensitivity of hippocampal neurones to glutamate receptor agonists, and that differences exist in the pharmacology of NMDA and quinolinic acid.

Keywords: NMDA (*N*-methyl-D-aspartate); Quinolinic acid; Kynurenine; Long-term potentiation; Hippocampus; NMDA receptor subunit

1. Introduction

The neuronal receptors for glutamate are now considered to exist as two families — the ionotropic and the metabotropic (Dingledine et al., 1999; Zorumski and Thio, 1992). Of the former family, one member is the receptor sensitive to *N*-methyl-D-aspartate (NMDA), a receptor that promotes the influx of sodium and calcium into neurones through channels which are gated by magnesium ions in a voltage-dependent manner (Mori and Mishina, 1995). This property confers on the NMDA receptor the ability to play a key role in determining the pattern of neuronal activity. On activation, it results in the production of a series of intermittent depolarising shifts, which may be of importance in the initiation of epileptic phenomena (Dingledine et al., 1990). The permeability of the receptor-coupled channels to calcium can also lead to excitotoxicity after repeated or excessive activity (Whetsell, 1996), and is believed to be important for the phenomenon of long-term potentiation in the hippocampus and other areas of the central nervous system (CNS) (Bliss and Collingridge, 1993).

Since the repeated application of agonists can often lead to the desensitisation or down-regulation of receptors (Fagni et al., 1983; Zorumski and Thio, 1992), it was of interest to determine whether any interaction occurred between neuronal responses to exogenously applied amino

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acid agonists and the physiological phenomenon of long-term potentiation. In the previous work, examining the effects of amino acid agonists applied to the surface of the cerebral cortex in vivo, a number of unexpected and surprising interactions were observed, including the fact the cortical evoked potentials recovered during the maintained presence of NMDA, that NMDA prevented the suppression of potentials produced by kainic acid (but not potassium), and that quisqualic acid would prevent the effects of NMDA (Addae and Stone, 1986, 1987, 1988). These studies raised the possibility that the physiological activation of amino acid receptors might also change the long-term sensitivity of neurones to agonists. Several previous studies have attempted to examine the effects of NMDA on physiological functions, such as neuronal sensitivity to long-term potentiation. In the present study, we have sought to determine the opposite, that is, whether long-term potentiation can modify neuronal responsiveness to NMDA. For comparison, we have also examined the influence of long-term potentiation on responses to quinolinic acid.

Quinolinic acid is an analogue of aspartate, which was noted to be an agonist at receptors for NMDA in the neocortex (see Stone and Perkins, 1981; Perkins and Stone, 1983; Stone, 1993 for review). Although quinolinic acid is less potent than NMDA itself, it is an endogenous compound, produced by the metabolism of tryptophan along the kynurenine pathway (Stone, 1989, 1993). The concentration of quinolinic acid in the CNS has been shown to rise several hundred-fold in some circumstances, such as infection by HIV, to levels that could produce a marked activation of NMDA receptors (Heyes et al., 1991). While the pharmacology of quinolinic acid closely parallels that of NMDA, however, differences have been reported in the cellular targets of their excitotoxic actions, their dependence on afferent synaptic terminals, and their sensitivity to blockade by kynurenic acid (Foster and Schwarcz, 1989; Winn et al., 1991; Perkins and Stone, 1983; Stone, 1993). Although neurones in regions such as the spinal cord and cerebellum respond to NMDA, they are almost insensitive to quinolinic acid (Perkins and Stone, 1983). The present study has revealed further differences between NMDA and quinolinic acid in their interactions with long-term potentiation, which may indicate a role for specific receptor subunit combinations in this phenomenon.

2. Methods

Male Wistar or Sprague–Dawley rats were anaesthetised with urethane at a dose of 1.3 g/kg and placed on ice until the rectal temperature was reduced to 30° as recommended by Newman et al. (1992). The rats were killed by dislocation of the cervical vertebrae and decapitated. The brains were taken out, placed in ice-cold artificial cerebrospinal fluid (ACSF), the hippocampi removed, and cut transversely into 450-μm-thick slices using a McIlwain tissue chopper. The slices were transferred into an incubation chamber where they were exposed to a gas mixture of 95% O_2 and 5% CO_2 at room temperature. After leaving the slices in this chamber for at least 1 h, a slice was transferred to a 1-ml capacity submerged recording chamber and superfused with pre-gassed (95% O_2/5% CO_2) ACSF at a temperature of 26–28°C and a rate of approximately 4 ml/min.

The Schaffer collateral and commissural fibres in the stratum radiatum were stimulated at 0.1 Hz with a concentric bipolar electrode (Clark Electromedical, Reading, UK) using parameters of 100–300 μs duration and 80–300 μA amplitude via a constant current stimulator. For the recording of orthodromic extracellular field potentials, a glass microelectrode filled with 2 M NaCl (resistance 2–5 MΩ) was placed with its tip in the CA1 pyramidal cell layer. Field e.p.s.p.s were recorded with the electrode tip placed in the stratum radiatum. The population spikes used for analysis were at least 2.0 mV in size and the e.p.s.p.s were at least 1.0 mV in size. Potentials were displayed on digital oscilloscopes and plotted on-line on a *Y–t* chart recorder. Changes in the peak-to-peak size of the population spike and in the initial slope of the e.p.s.p. were used to assess the effects of applied agents. When stable field potentials were attained, solutions of drugs, which had been made in ACSF and pre-gassed, were introduced into the perfusion line. The concentration and duration of drug application was chosen so as to produce a submaximal decrease in the size of the field potentials. In order to achieve such a partial effect, it proved to be easier to employ a given drug concentration and to vary the duration of application, depending on the sensitivity of a particular slice to the drug, until an approximately 50% decrease in potential size had been obtained. Thereafter, the duration of application was maintained constant for each agonist in each slice.

For the recording of single units and drug microiontophoresis, single glass recording microelectrodes were glued alongside a seven-barrelled microiontophoresis pipette (Clark Electromedical) as described and illustrated previously (Stone, 1973, 1985). The iontophoresis pipettes were filled immediately before use with solutions of some or all of the following: NMDA, 10 mM in 165 mM NaCl, pH 7.0; quinolinic acid, 100 mM in 65 mM NaCl, pH 7.5; α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) hydrobromide, 10 mM in 165 mM NaCl, pH 7.5. The solution pH was adjusted using 1 M NaOH or HCl. Drug ejection was achieved by a Medical Systems Neurophore Iontophoresis System using currents in the range of 10–100 nA, balanced by a barrel containing a solution of 165 mM NaCl, pH 7.5.

Long-term potentiation was induced by applying a 100 Hz tetanus for 1 s to the Schaffer collateral and commissural fibres. The population potential was always monitored at this time to ensure that long-term potentiation was induced. During the single cell studies, a separate single

recording electrode was used for this purpose, the tip of which was sited approximately 100 μM distant from the iontophoresis assembly. The criterion for the establishment of long-term potentiation was that the population spikes or e.p.s.p.s were at least 20% greater than the control potential 30 min after the induction of long-term potentiation.

2.1. Data analysis

Results were analysed by paired *t*-test or repeated-measures ANOVA as appropriate. The tests used are indicated in the text.

3. Results

3.1. Responses to agonists

3.1.1. Population spikes

Quinolinic acid was tested at a concentration of 100 or 150 μM for 5–10 min, since this protocol caused an approximately 50% reduction of population spike size (46.3% ± 5.3; $n = 15$) (Fig. 1). Recovery of population spikes occurred within 5–10 min. In the control paradigm, a second application of quinolinic acid was subsequently made to the same slice, at the same concentration and for the same duration, 45 min following the first application. This second application produced a decrease in the field potential size of 43.1% + 4.1 ($n = 15$). The difference between the effects of the first and second applications of quinolinic acid were not significant (paired *t*-test) (Fig. 2A).

NMDA was tested at concentrations of 5 and 7.5 μM since this, when applied for a period of 4–7 min, caused a 42.8% + 4.4 (mean + s.e.m., $n = 18$) decrease in the field potentials (Fig. 2A). The second application, 45 min after recovery, produced a decrease in the field potential size of 43.1% + 3.5 ($n = 18$). The difference between the effects of the first and second applications of NMDA was not significant (paired *t*-test).

AMPA was superfused at 1 μM for 3–6 min and kainate at 500 nM for 2–6 min. As with the effects of NMDA, there was no change detectable in the size of the response to a second application 45 min after the first. The results are summarised in Fig. 2A.

3.1.2. Field e.p.s.p.s

All four agonists depressed the size of the field e.p.s.p.s, but there was no difference between the size of the response to the first and second applications (Fig. 2C).

3.2. Interactions with long-term potentiation

To examine the effects of long-term potentiation on the population spike responses of the slice to NMDA or quinolinic acid, long-term potentiation was induced twice with a 10-min interval. The first period of long-term potentiation was induced 25 min following the first application of the agonist. As in the control experiments, the drug was applied a second time to the same slice, at the same concentration and for the same duration, 10 min following the second long-term potentiation, thus maintaining the same 45-min interval between the two agonist applications.

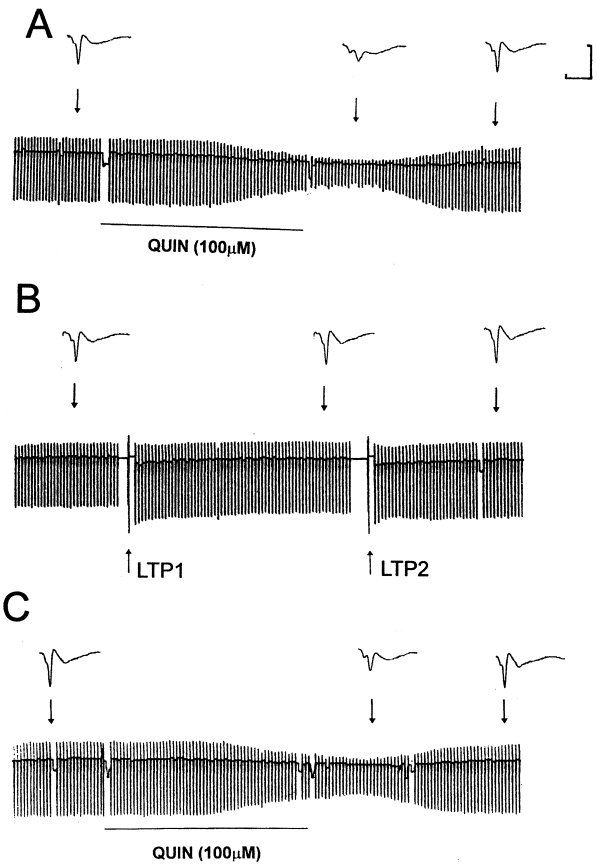

Fig. 1. Records of the population spike recorded in a hippocampal slice. The records above the continuous tracing show representative potentials recorded at the times indicated by the downward arrows. In A, quinolinic acid is superfused at a concentration of 100 μM, producing a decrease of potential size. During B, two periods of long-term potentiation are induced, which have the effect of reducing the subsequent response to quinolinic acid (C). Calibration bars: 2 mV and 10 ms for sample potentials; 1 mV and 75 s for the continuous tracing.

A first application of quinolinic acid (100 or 150 μM for 5–10 min) decreased the population spike size in this series of slices by 46.5% + 6.3 ($n = 15$), whereas the second application, following the two periods of long-term potentiation induction, reduced the potential size by only 22.9% + 4.7 ($n = 15$) (Figs. 1 and 2B). The difference between the first and second applications of quinolinic acid was highly significant ($p = 0.0017$; paired *t*-test). Similar experiments using applications of the other glutamate receptor agonists yielded a similar result with the

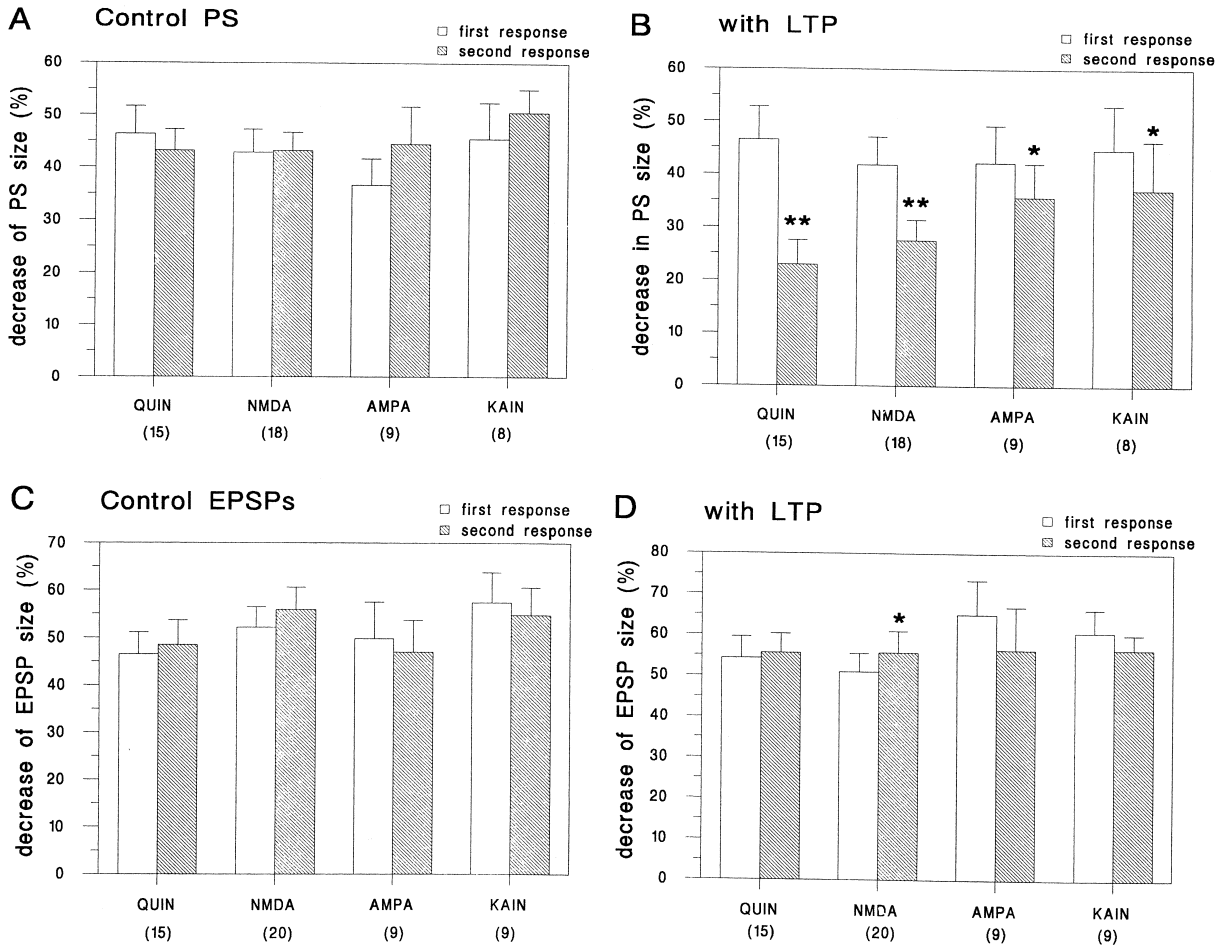

Fig. 2. Histograms summarising the decrease in potential size produced by quinolinic acid (QUIN), NMDA, AMPA and kainate (KAIN). A and B refer to population spikes. Panel A indicates the absence of change when two periods of superfusion are applied to control slices. Panel B shows the decrease in the amino acid responses when long-term potentiation has been induced. C and D refer to population EPSPs and show only a small change in response to NMDA following long-term potentiation. $^{*}P < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Numbers of slices are indicated in parentheses below the abscissa.

second response, following the two periods of long-term potentiation, being significantly smaller than the first responses. The data are summarised in Fig. 2B.

In contrast, the effects of quinolinic acid, AMPA and kainate on the field e.p.s.p.s were essentially unchanged by long-term potentiation, although a small increase was noted in the response to NMDA, which changed slightly from $50.9\% \pm 4.6$ to 55.6 ± 5.3 ($p < 0.05$; $n = 20$).

Control experiments were done to examine the possibility that a change in the initial size of the potential size could subsequently affect receptor sensitivity. The potential size was increase by at least 20% prior to the second application of 7.5 μM NMDA. There was no significant difference between the effects of the first and second applications of NMDA, $p = 0.47$, paired t-test ($n = 6$).

3.3. Cellular localisation of the long-term potentiation-induced change in sensitivity

In order to determine whether the effects of long-term potentiation on agonist sensitivity were exerted at the soma or dendrites, the agonists were applied by microiontophoresis to these two sites, with responses being measured from the surrounding population of neurones as changes in the size of the population spike. When two applications were made by iontophoresis into the cell body region, 15 min apart, there was no difference between the two responses to NMDA or AMPA. However, the second response to quinolinic acid was significantly smaller than the first (Fig. 3A; Table 1). The second response remained smaller even when the interval between the applications was increased to 30 or 60 min (Table 1). When applied to the dendritic region, a smaller second response was obtained to all the agonists tested with a 15-min interval. However, when the interval was increased to 30 min, the second response to AMPA was significantly larger than the first, and only quinolinic acid continued to induce second responses which were smaller than the first (Table 1; Fig. 3C).

The effect of long-term potentiation, applied 10 min before the second agonist application, on these responses to localised applications was to reduce the size of the

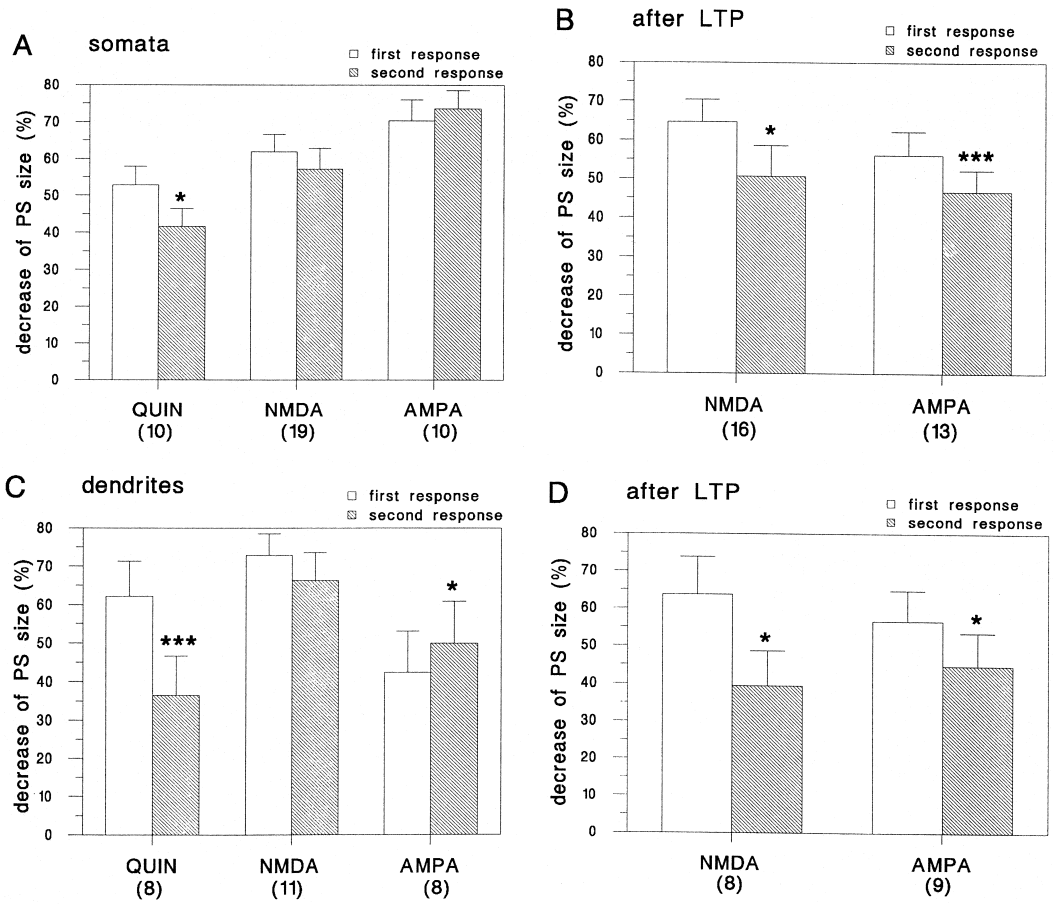

Fig. 3. Histograms summarising the decrease in potential size produced by quinolinic acid (QUIN), NMDA, and AMPA. A and B refer to applications of the agonists by microiontophoresis to the cell soma region (stratum pyramidale). Panel A shows that only quinolinic acid responses were reduced during a second application. Panel B shows that responses to NMDA and AMPA were reduced by the induction of long-term potentiation between the first and second responses. C and D related to the application of agonists to the dendritic region (stratum radiatum). Panel C indicates that again only quinolinic acid responses were reduced on a second application, although AMPA responses were slightly enhanced. After the induction of long-term potentiation, both NMDA and AMPA responses were significantly decreased. $^{*}P < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Numbers of slices are indicated in parentheses below the abscissa.

second responses to both NMDA and AMPA in conditions under which the control second responses had been unchanged in size. Thus, the second responses to somatic application after a 15-min interval, and the second responses to dendritic applications after a 30-min interval were all diminished substantially by the intervening period of long-term potentiation (Fig. 3B,D). The effects of long-term potentiation on localised applications of quinolinic acid could not be performed as all the second responses to iontophoretically applied quinolinic acid were already reduced in size significantly compared to the first responses.

3.4. Involvement of nitric oxide (NO) in agonist sensitivity changes

To determine the possible role of NO in the observed changes of amino acid sensitivity, selected experiments were repeated in the presence of the NO synthase inhibitor L-nitroarginine, 100 μM. As indicated in Fig. 4A, this compound prevented the reduction in the second response to bath applied NMDA induced by long-term potentiation. The second response to AMPA, however, was still depressed by long-term potentiation. Similarly, nitroarginine prevented long-term potentiation from reducing the second response to NMDA, but not AMPA, when applications of these were restricted to the somatic region by microiontophoresis (Fig. 4B).

3.5. Cyclothiazide

To determine whether receptor desensitisation was responsible for any of the long-term potentiation-induced loss of agonist sensitivity, cyclothiazide was superfused at concentrations of 1 and 10 μM during the iontophoretic application of AMPA to the cell soma. Cyclothiazide

Table 1
Responses to amino acid receptor agonists in hippocampal slices

	Location	First response	Interval	Second response	*n*	Significance
Control responses to pairs of agonist applications						
NMDA	soma	61.8 ± 4.8	15 min	57.2 ± 5.6	19	n.s.; $p = 0.17$
AMPA	soma	70.3 ± 5.7	15 min	73.5 ± 5.0	10	n.s.; $p = 0.39$
Quinolinic acid	soma	52.8 ± 5.1	15 min	41.6 ± 4.8	10	$p < 0.05$
	soma	67.6 ± 5.4	30 min	54.4 ± 7.9	10	$p < 0.05$
	soma	46.17 ±	60 min	24.82	8	$p < 0.05$
NMDA	dendrites	67.7 ± 6.1	15 min	55.5 ± 8.1	12	$p < 0.01$
	dendrites	72.8 ± 5.6	30 min	66.2 ± 7.3	11	n.s.; $p = 0.26$
AMPA	dendrites	62.7 ± 5.2	15 min	54.8 ± 5.6	12	$p < 0.05$
	dendrites	42.3 ± 10.7	30 min	49.8 ± 10.9	8	$p < 0.05$
Quinolinic acid	dendrites	54.4 ± 8.3	15 min	25.3 ± 6.9	8	$p < 0.01$
	dendrites	62.1 ± 9.2	30 min	36.5 ± 10.1	8	$p < 0.001$
Responses with intervening induction of long term potentiation						
NMDA	soma	64.6 ± 5.7	15 min	50.7 ± 7.9	16	$p < 0.05$
AMPA	soma	56.2 ± 6.0	15 min	46.8 ± 5.5	13	$p < 0.001$
NMDA	dendrites	63.7 ± 10.0	30 min	39.4 ± 9.2	8	$p < 0.05$
AMPA	dendrites	56.5 ± 8.2	30 min	44.7 ± 8.8	9	$p < 0.05$

prevented the decline of AMPA sensitivity produced by the induction of long-term potentiation, with the glutamate analogue producing a control decrease in size of 60.0% ± 9.5 ($n = 9$) and 66.5% ± 7.4 ($n = 9$) at the two concentrations, and 55.3% ± 8.2 and 61.8% ± 10.2, respectively, after long-term potentiation.

3.6. Single cell studies

Neurones in the CA1 layer are normally silent in a normoxic slice. Cells were, therefore, excited by periodic application of NMDA, quinolinic acid or AMPA by microiontophoresis. Applications were temporally spaced so that neurones did not show any evidence of overdepolarisation or progressive desensitisation.

Long-term potentiation was successfully induced while recording amino acid responses on 26 neurones. Twenty of these exhibited changes in sensitivity to NMDA or quinolinic acid. In most cases (16 cells), the reduction in sensitivity was greater for NMDA than for quinolinic acid as reflected in the total number of action potentials induced by the agonist (Fig. 5). In the remaining four cells, the responses to NMDA and quinolinic acid declined and recovered in parallel.

There was some variation in the time course of the changes. Most (17) of the cells showed a loss of sensitivity, which was maximal only after a period of 2–5 min following long-term potentiation. Of these, four cells showed an increase in sensitivity to NMDA and quinolinic acid immediately after the induction of long-term potentiation, which gave way to the more slowly developing

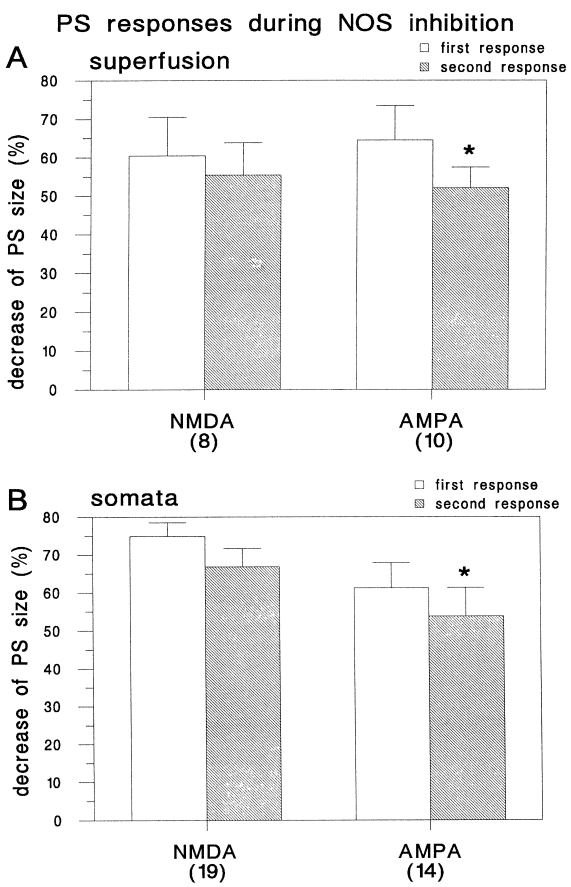

Fig. 4. Histograms summarising the effects of NOS inhibition on the decrease of population spike size produced by NMDA and AMPA. The response to NMDA is unchanged, whether induced by bath superfusion or localised application to the cell somata, whereas responses to AMPA are decreased in both cases. * $P < 0.05$. Numbers of slices are indicated in parentheses below the abscissa.

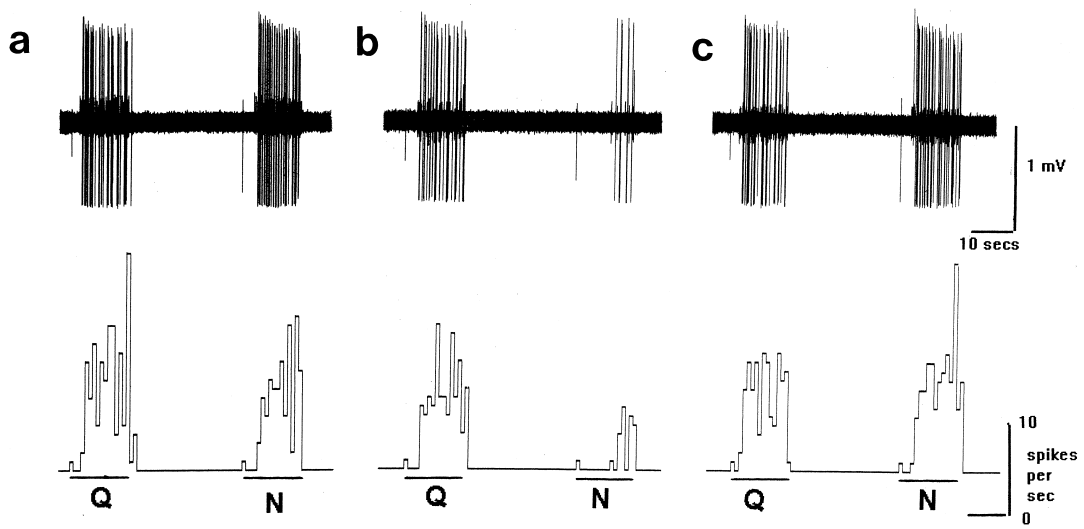

Fig. 5. Records of action potential firing in the hippocampal slice in response to the localised application of agonists by microiontophoresis. The upper tracing shows the raw recording of action potentials, while the lower record shows the spike frequency. Panel (a) illustrated control responses to quinolinic acid (Q, 32 nA) and NMDA (N, 6 nA). Panel (b) shows responses 4 min after the induction of long-term potentiation, with a greater reduction in sensitivity to NMDA than to quinolinic acid. Panel (c) shows recovery towards the control levels, taken 20 min later. Calibration bars: −1 mV and 10 s for the spike record, and 10 spikes/s and 10 s for the frequency record.

decrease in responses. The other three cells exhibited a loss of responsiveness to NMDA immediately after the induction of long-term potentiation. In all cases, cells showed recovery from the effects of long-term potentiation, with normal sensitivity being attained within 15 min.

4. Discussion

While several studies have reported the effects of amino acid agonists on the induction or maintenance of long-term potentiation, there are relatively few which have examined the effects of long-term potentiation on amino acid sensitivity. An early report by Lynch et al. (1976) found a long-lasting reduction in the sensitivity of neurons to glutamate after long-term potentiation, but selective agonists were not then available for testing. In the present study, we have used evoked population potentials and single cells to examine the interactions of several glutamate receptor agonists and long-term potentiation.

When superfused over the slices, all the agonists tested induced a decrease of potential size. We have assumed that this reflects the depolarisation of the neuronal population responsible for the evoked spike. This view is supported by the fact that the same concentrations as used here are also able to decrease the size of antidromically induced field potentials in the hippocampal slice or to produce depolarisation in intracellular studies. However, we cannot fully exclude the possibility that a presynaptic action to reduce transmitter release could contribute to the decrease in evoked potential size (Shew et al., 1995; Kato et al., 1999; MacDermott et al., 1999). When applied 45 min apart in the medium superfusing the whole slice, no loss of sensitivity was seen to any of the four agonists, whether tested on the population spike size or the field e.p.s.p.s. When long-term potentiation was induced electrically between the two agonist applications, changes were seen in the second responses to all compounds, presumably reflecting some consequence of the long-term potentiation process and activation of amino acid receptors during long-term potentiation. The effect of long-term potentiation, however, was only exhibited towards the changes of population spike size, no change being found in the amino acid effects on e.p.s.p. slope. This strongly suggests that the effect of long-term potentiation is being exerted at a postsynaptic site, since a presynaptic action would also modify e.p.s.p. size. Quantal analysis of transmission also led Yamamoto et al. (1992) to the conclusion that long-term potentiation was associated with postsynaptic but not presynaptic changes of amino acid sensitivity.

There are at least two possible mechanisms, which could contribute to the non-specific loss of amino acid sensitivity after long-term potentiation and account for the preferential change of postsynaptic (population spike) rather than presynaptic (EPSP) effects. One is the increase of neuronal excitability associated with EPSP–spike coupling (E–S potentiation) (Chavez-Noriega et al., 1990). Since the amino acid responses are a direct reflection of neuronal excitability, the enhanced E–S coupling, which accompanies long-term potentiation, could result in a greater depolarisation by the exogenous compounds. How-

ever, this explanation would probably not account for the fact that amino acid sensitivity was depressed after both somatic or dendritic applications, since E–S coupling is believed to occur at the level of the cell bodies.

An alternative explanation is that of changes in GABA activity. Several groups have demonstrated changes of GABAergic activity, which may play a role in paired-pulse facilitation and/or long-term potentiation and which may involve $GABA_A$ (Stelzer et al., 1987; Pitler and Alger, 1992) or $GABA_B$ (Chavez-Noriega et al., 1989, 1990; Nathan et al., 1990; Davies et al., 1991; Mott and Lewis, 1991) receptor mechanisms. It is not clear that a change in GABA receptor activation could account for the differential changes of amino acid sensitivity, with greatest effects being seen on quinolinic acid and NMDA responses. However, this differentiation was only apparent when the agonists were superfused over the slices, no clear difference being seen with localised applications. Changes in GABAergic processes could conceivably be involved, therefore, if they can result in alterations of glutamate agonist sensitivity by actions at distant sites within the slices.

Finally, it should be recognised that the application of compounds by superfusion or by microiontophoresis may still result in the activation of many neurons and interneurons within the local hippocampal network, so that it is not yet possible to localise further the site of the changes observed. Nevertheless, the importance of these results lies in the demonstration of changes within the hippocampal network, which could have significant effects on overall hippocampal function.

The results are consistent with the conclusions of Davies et al. (1989) and Fedorov et al. (1997), both of whom reported an increased sensitivity to AMPA after long-term potentiation and deduced that the predominant change after long-term potentiation was postsynaptic in nature. It is, however, more difficult to reconcile their increase of AMPA responses with the decrease of sensitivity to agonists observed here. Since the essential difference between the experimental design is that Davies et al. (1989) examined single cell responses to iontophoretically applied AMPA, whereas the present work examined population spikes, we proceeded to try and resolve the difference, and clarify the locus of the postsynaptic change, by applying agonists by microiontophoresis to the cell soma or dendritic regions. These experiments not only confirmed that long-term potentiation could induce a loss of sensitivity to NMDA and AMPA after localised applications, but also revealed that the effects could be demonstrated at both somatic and dendritic sites. One possibility explored here is that NO, generated by the induction of long-term potentiation, is responsible for the change of amino acid sensitivity. Inhibition of NO synthase was able to prevent the loss of sensitivity to NMDA, while responses to AMPA were not affected. Conversely cyclothiazide, which blocks the desensitisation of the AMPA receptors (Arai et al., 1996; Boxall and Garthwaite, 1995; Hoyt et al., 1995) prevented the loss of AMPA sensitivity. The mechanisms of long-term potentiation-induced change may therefore be different for the two agonists, with desensitisation accounting for the loss of AMPA responses and the generation of NO contributing to the inhibition of NMDA receptor function.

Another possible explanation of these results involves the trafficking of receptors during long-term potentiation. Synapses that contain only NMDA receptors have been identified in the CA1 region of the hippocampus. During normal synaptic transmission, these synapses are functionally ''silent'', but following long-term potentiation, such synapses become active as a result of AMPA receptor redistribution (Malenka and Nicoll, 1997). Although the location of these AMPA receptors prior to long-term potentiation has not been clearly identified, it is possible that they could come from intracellular pools, or cycled from extrasynaptic to synaptic sites (Luscher et al., 1999; Morales and Goda, 1999). Such a scenario could contribute to long-term potentiation and also to the decreased sensitivity to the exogenous AMPA, which is more likely to be acting at extrasynaptic sites than at synaptic ones.

While most studies of desensitisation to NMDA have been performed on isolated cells, the phenomenon has been shown previously in neuronal networks, being seen both as a recovery upon maintained, continuous application of NMDA, and the failure to induce a response to a second application of the agent (Addae and Stone, 1986, 1987, 1988). Such interactions raise the possibility of functionally important long-term changes to amino acid sensitivity, both as a result of repeated physiological stimulation and, more especially, under pathological conditions such as ischaemia, when the levels of endogenous glutamate may increase substantially. The concept of using glutamate receptor desensitisation as a means of affording neuroprotection has been supported by work showing a neuroprotective action of prior exposure to glutamate receptor agonists against neuronal overactivity (Ballerini et al., 1995) and excitotoxicity (Brorson et al., 1995; Strahlendorf et al., 1996) and in preparations as diverse as cerebellar cultures (Sei et al., 1999) and the retina (Rocha et al., 1999).

The long-term potentiation-induced loss of sensitivity to exogenous glutamate receptor agonists observed here may reflect a physiological mechanism for achieving neuroprotection, and it is interesting to reflect on the possible relevance of our data for problems associated with ageing in humans. For example, the Rotterdam study (Ott et al., 1995), which involved 7528 participants showed an inverse relation between education level and dementia. This correlation remained after correcting for a number of confounding factors, which relate the level of education to various illnesses and behaviour patterns that are known to influence the development of dementia. If long-term potentiation is involved in learning and education, the present

results would indeed predict that the greater the amount of long-term potentiation in the brain, the lower the functional activation of glutamate receptors and, thus, the lower the potential of excitotoxicity-induced neurodegeneration and dementia.

4.1. NMDA and quinolinic acid

In addition to the main finding of this work, the results have revealed interesting differences in the behaviour of NMDA and quinolinic acid. The latter has been regarded as a selective agonist at NMDA receptors since the first report of this action on cortical neurones (Stone and Perkins, 1981). Its activity has been confirmed many times using selective antagonists and detailed comparisons of the nature of its effects on the cell membrane (Peet et al., 1986; Herrling et al., 1983; McLarnon and Currry, 1990). Nevertheless, it is also clear that some differences exist in their mechanism or site of action since, for example, the two compounds exert their neurotoxic actions towards different populations of hippocampal neurones, their toxicity differs in its dependence on the presence of synaptic input, and they show a differential sensitivity to blockade by kynurenic acid and other amino acid antagonists (Pawley et al., 1996; Foster et al., 1984; Winn et al., 1991). Clear differences have also emerged in the behaviour of these agonists after their application by microiontophoresis and the recording of field potentials. No difference was observed between the first and second responses to NMDA and AMPA when applied to the cell soma region, and responses to dendritic applications recovered to control size within 30 min. The second response to quinolinic acid, in contrast, was reduced after applications to the cell soma, and did not show any recovery for at least 60 min following applications to the dendrites. In addition, the recordings of single cell firing rates, revealed that long-term potentiation caused a reduction in responses to NMDA to a greater extent than to quinolinic acid.

One possible reason for this different behaviour of NMDA and quinolinic acid is that they are acting on different combinations of receptor subunits. Soon after the discovery of quinolinic acid's activity at NMDA receptors, it was noted that cerebellar and spinal neurones were relatively insensitive to quinolinic acid (Perkins and Stone, 1983). This difference is probably due to the fact that quinolinic acid is not able to activate the combination of NR1 with NR2C subunits (Decarvalho et al., 1996) found in the cerebellum (Farrant et al., 1994; Wenzel et al., 1995) and parts of the spinal cord, indicating differences in molecular conformation and/or stringent structural requirements of the various subunit combinations, which are known to determine the variations of NMDA receptor pharmacology in different regions of brain (Buller et al., 1994; Bresink et al., 1995; Porter and Greenamyre, 1995). Since combinations of NR1 with each of the NR2A, B, C and D subunits have been identified in the hippocampus (Monyer et al., 1994), we propose that the different behaviour of NMDA and quinolinic acid may reflect their differential activation of those combinations.

The different qualitative changes in responses to NMDA and quinolinic acid seen with bath superfusion and microiontophoresis are probably due to the different types of recording. When superfusing the slices, agonists have access to all parts of the cell surface including the whole of the dendritic membrane. In the single cell experiments, however, the site of action is restricted to the immediate region of the recording and iontophoresis electrode, i.e. the cell soma. The observed differences between NMDA and quinolinic acid may therefore be the result of a difference in the distribution of NMDA receptor subunit combinations on the somatic and dendritic surfaces.

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