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STIMULUS INTENSITY, CELL EXCITATION AND THE *N*-METHYL-D-ASPARTATE RECEPTOR COMPONENT OF SENSORY RESPONSES IN THE RAT SPINAL CORD *IN VIVO*

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Abstract—The importance of receptors for *N*-methyl-D-aspartate in synaptic plasticity and in triggering long-term pronociceptive changes is explained by their voltage-dependence. This suggests that their contribution to acute nociceptive responses would be determined both by the magnitude of synaptic input and by the level of background excitation. We have now examined the role of *N*-methyl-D-aspartate receptors in acute nociceptive transmission in the spinal cord. Drugs selectively affecting activity mediated by these receptors were tested on responses of dorsal horn neurons to noxious stimuli of different intensities and at different levels of ongoing spike discharge. The drugs used were the *N*-methyl-D-aspartate receptor channel blocker ketamine; the competitive antagonists, 3-((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (D-CPP) and D-2-amino-5-phosphonopentanoic acid (D-AP5), and the positive modulator thyrotropin-releasing hormone. The activity of dorsal horn wide dynamic range neurons was recorded extracellularly in α-chloralose-anaesthetized spinalized rats. Their responses to noxious stimuli (pinch, heat and electrical) were monitored in parallel with responses to iontophoretic *N*-methyl-D-aspartate and (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA). Drugs were given i.v. or (D-AP5) iontophoretically. At doses that selectively inhibited responses to exogenous *N*-methyl-D-aspartate, ketamine (4 or 8, mean 5 mg/kg i.v.) reduced the nociceptive responses of the majority of the cells in deep dorsal horn. Ketamine also reduced wind-up of the responses to repetitive electrical stimulation. Ketamine (4 or 8 mg/kg), D-CPP (2 mg/kg), D-AP5 (iontophoretically) and thyrotrophin-releasing hormone (1 mg/kg) were tested on different magnitude nociceptive responses evoked by alternating intensities of noxious heat or pinch. In percentage terms, the less vigorous responses were affected by all four drugs as much as or more than the more vigorous responses. When background activity of neurones was enhanced by continuous activation of C-fibres with cutaneous application of mustard oil, ketamine was less effective against superimposed noxious pinch responses. Ongoing background activity was affected in parallel with evoked responses. When background discharge of the cells was maintained at a stable level with continuous ejection of kainate, neither the *N*-methyl-D-aspartate antagonists nor thyrotrophin-relasing hormone affected the responses to noxious pinch or heat, although responses to exogenous *N*-methyl-D-aspartate were still blocked. The wind-up of the electrical responses was, however, reduced by ketamine irrespective of the level of background activity.

The results indicate that under these conditions *in vivo*, *N*-methyl-D-aspartate receptors mediate ongoing low-frequency background activity rather than phasic high-frequency nociceptive responses. The effects of *N*-methyl-D-aspartate antagonists and positive modulators on nociceptive responses are evidently indirect, being secondary to changes in background synaptic excitation. These results cannot be explained simply in relation to the voltage-dependence of *N*-methyl-D-aspartate receptor-mediated activity; other factors, such as modulation by neuropeptides, must be involved. © 1997 IBRO. Published by Elsevier Science Ltd.

Key words: nociception, wind-up, ketamine, D-CPP, D-AP5, TRH.

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Abbreviations: AMPA, (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; D-AP5, D-2-amino-5-phosphonopentanoic acid; D-CPP, 3-((R)-2-carboxy-piperazin-4-yl)-propyl-1-phosphonic acid; DH, dorsal horn; EPSP, excitatory postsynaptic potential; NMDA, *N*-methyl-D-aspartate; TRH, thyrotropin-releasing hormone; WDR, wide dynamic range

Binding and hybridization studies indicate that *N*-methyl-D-aspartate (NMDA) receptors are abundant in the spinal cord and are present in both dorsal and ventral horn.[21,22,44,45,49] However, data on their functional role in the spinal cord are inconsistent. NMDA receptor activation has predominantly been regarded as an intermediate event leading to long-term pro-nociceptive changes of sensory transmission. Activation of NMDA receptors caused by prolonged nociceptive stimulation, inflammation, or other factors leading to a barrage of excitatory inputs to spinal sensory neurons, has been proposed to be responsible for triggering long-term changes of neural excitability that underlie hyperalgesia.[14,35,50,53] The principal mechanism underlying

such apparent activity-dependence is believed to be the relief of the magnesium block of the NMDA receptor channel that occurs with membrane depolarization.[34,39]

However, if such stimuli are capable of triggering chronic pro-nociceptive changes via NMDA receptor activation, the acute responses to these stimuli should also involve NMDA receptors. The voltage dependence of NMDA receptor functioning is an immediate phenomenon, and it should, therefore, be possible to reveal an NMDA receptor involvement in phasic nociceptive responses—synaptic events that still persist for tens of seconds.

When the role of NMDA receptors in "acute" nociception has been studied using specific antagonists, attenuation of responses to noxious or innocuous stimuli was observed under some experimental conditions (e.g., Refs 15, 18, 28). However, other authors have reported little effect of NMDA antagonists on nociceptive responses of spinal dorsal horn neurons.[25,26] Furthermore, the NMDA receptor contribution to nociceptive responses appears to be different in the dorsal and ventral horn of the spinal cord when tested under comparable conditions.[26]

A reduction of background activity has often been reported to accompany the effects of NMDA receptor antagonists on synaptically evoked responses of spinal neurons, whether *in vivo*[15,26] or *in vitro*.[1] This implies an NMDA receptor-mediated tonic input to spinal sensory neurons. Any change in tonic discharge will alter the spiking response to superimposed phasic inputs. It therefore remains unclear to what extent the reported changes in nociceptive responses reflect the reduction of the phasic, as against tonic, components of excitatory inputs.

In the light of the voltage-dependence of the NMDA receptor-mediated conductance, one could expect a greater contribution of NMDA receptor-mediated events to the stimulus-evoked phasic than to ongoing activity of sensory neurons, since they presumably involve a greater level of membrane depolarization. In the same vein, the involvement of NMDA receptors in sensory responses should be related to the magnitude of spiking responses as the latter presumably correlates with the degree of stimulus-evoked depolarization. Although well established under *in vitro* conditions (e.g., Refs 28, 47, for review see also Refs 25, 50, 53) these relationships have not yet been clearly demonstrated in fully-developed intact spinal networks with preserved peripheral inputs.

It therefore seemed important to investigate *in vivo* the contribution of NMDA receptor-mediated components to synaptically evoked and background activity of dorsal horn sensory neurons involved in nociceptive transmission. This has been examined by testing the effects of NMDA antagonists and of the peptide NMDA receptor modulator, thyrotrophin-releasing hormone (TRH), on spinal dorsal horn neuron responses to peripheral stimuli of various modalities, whilst monitoring cell responses to exogenous amino acid agonists. These effects were compared on naturally-evoked responses (pinch, heat) and on electrically-elicited wind-up. Since the voltage-dependence of the NMDA receptor-mediated conductances suggests their greater recruitment at higher levels of cell excitation, different intensities of peripheral stimulation were applied in order to evoke graded spiking responses. Since the effects on the synaptic responses were often paralleled by a change in spontaneous activity, the drugs were compared with and without control of background firing. The NMDA receptor component of phasic nociceptive responses was also examined after induction of background activity by continuous C-fibre stimulation. Some of the results have been published in preliminary form.[5,6,8,46]

EXPERIMENTAL PROCEDURES

Experimental preparation and recording procedure

All experiments were performed in accordance with the U.K. Home Office Animals (Scientific Procedures) Act 1986. Adult male Wistar rats (290–390 g, bred in-house) were anaesthetized with halothane in oxygen and cannulae were inserted in the trachea, one carotid artery and one or two jugular veins. A laminectomy was performed (Th9–L2 segments), the dura mater was removed and the cord cut at Th9–10. The animal was placed in a recording frame, and the cord surface was covered with warm (37°C) liquid paraffin. Anaesthesia was switched to, and maintained with, α-chloralose (50 mg/kg i.v. initially, 10–20 mg/kg/h i.v. either in a bolus as required or by infusion via one of the venous cannulae). Animals breathed oxygen-enriched air spontaneously. Fluid therapy with isotonic saline and/or a plasma expander (Haemaccel, Hoechst) was provided throughout experiments at a rate of 3–5 ml/kg/h. Blood pressure was monitored continuously and systolic pressure remained above 100 mmHg. Core temperature was kept at 36.5–37.5°C. An interval of at least 1 h was allowed for recovery from halothane before the beginning of the recording session. A multibarrel glass micropipette was then inserted into the spinal cord (L5–L6 segment). The central barrel, filled with 3.5 M NaCl (1.5–5 MΩ resistance), was used for extracellular recording of single-unit action potentials.

Experimental protocols and drug administration

Wide dynamic range (WDR) neurons in deep dorsal horn (DH, depth 450–1390 μm, mean 920 μm from the pial surface) were selected on the basis of their receptive field characteristics and consistent responsiveness to noxious natural (mechanical or thermal) and electrical stimulation of the receptive field on a hind paw. Spikes were monitored continuously on a digital oscilloscope in order to ensure that the spike configuration remained consistent. Spike firing rate was displayed on a pen recorder and counts of action potentials during stimulus epochs were logged into a computer and used for on-line analysis. Four protocols were used in which neurons were activated by different combinations of iontophoretic ejections of NMDA, (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and kainate, and by activation of receptive fields by noxious heat, pinch or electric stimuli. At least 30 s intervals were allowed between the stimuli in each cycle (which was normally 3–4 min long), and a 60 s interval between stimulus cycles was used for assessing the level of background activity.

Microiontophoretic ejections of NMDA (Tocris Cookson, 100 mM in 100 mM NaCl), AMPA (Tocris Cookson, 5 mM in 200 mM NaCl), kainic acid (Sigma, 5 mM in 200 mM NaCl) and D-2-amino-5-phosphonovaleric acid (D-AP5, Tocris Cookson, 50 mM in 150 mM NaCl) were made via the outer barrels of a multibarrel glass electrode. All excitatory amino acids and D-AP5 were at pH 7.5–8 and were ejected as anions. Positive retaining current was applied to prevent drug leakage; a standard of 0.5 V was used to compensate for variability of barrel resistance. Automated current balancing via one of the outer barrels (150 mM NaCl) was used routinely. Electrode position within the spinal cord was estimated on the basis of micromanipulator readings of the depth relative to the dorsal pial surface. Ejection currents for excitatory amino acids were adjusted so as to evoke firing approximately matching at least one of the synaptically-mediated responses. Responses were always submaximal.

Noxious pinch stimuli (15 s) were applied to one toe using pneumatically driven forceps (0.4–1.8 N over 28 mm^2). Noxious heat stimulation was applied to the foot by a contact thermode with a ramp of 5°C/s from the baseline (35°C) to test temperature (45.5–49.7°C), which was held for 10–15 s. The two modalities of noxious stimulation were used because they activate different balances of A- and C-fibres. Data for the two types of response were always analysed separately, but where no differences were evident, data have sometimes been pooled. Only the last 10 s of the response to noxious stimuli was analysed, so as to select for the slowly-adapting nociceptive component and minimize the contribution from rapidly adapting low-threshold receptor discharge.

Electrical stimuli (trains of 16 square pulses of 1 ms duration at 1 Hz) were delivered via needle electrodes inserted percutaneously within the receptive field. The intensity was in the range of 5–15 times threshold for eliciting a short latency (<10 ms) response (T_r) and was sufficient for activation of A- and C-fibres, as the responses were divisible into two components with a latency for the second of around 200 ms (see Ref. 23). The response to the train of stimuli was subdivided into five epochs corresponding to stimulus no. 1, 2–4, 5–8, 9–12 and 13–16. The electrical stimulation resulted in progressive increase of the spike responses during the train ("wind-up",[36]). This was quantified as $WU=R_{tot}-16R_1$, where R_{tot} was the spike count over the whole train and R_1 was the spike count to the first stimulus of the train.

With each protocol the effects were assessed of intravenous administration of one or more of the following drugs: ketamine (2–8 mg/kg, Vetalar, Parke-Davies), 3-((R)-2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid (D-CPP, 2 mg/kg, Tocris Cookson) and pGlu-His-Pro amide (thyrotropin-releasing hormone, TRH, 0.5–1 mg/kg, Sigma). Iontophoretic D-AP5 (10–80 nA) was tested with Protocol 2 only (see below). Drugs were usually administered in a dose (or current)-doubling cumulative regime. The convenient pharmacokinetics of the NMDA channel blocker ketamine ($t_{1/2}$ 9–15 min, see Ref. 26) and of the positive NMDA receptor modulator TRH ($t_{1/2}$ 12–15 min, see Ref. 7) permitted multiple tests on each cell. The effects of the competitive NMDA receptor blocker D-CPP, however, were long-lasting, as no recovery was observed within the 50–60 min allowed; this test was therefore necessarily the last in the experiment.

The four experimental protocols used in the present work were as follows.

Protocol 1

Iontophoretic ejection of NMDA (40–45 s, 5–70 nA, mean 19 nA) was cycled with two of the following: AMPA (40–45 s, 3–22 nA, mean 9 nA), noxious pinch, noxious heat or noxious electrical stimulation.

Protocol 2

Iontophoretic ejection of NMDA was cycled with noxious pinch or heat stimuli of two alternating intensities. The intensities were set so that the higher intensity stimulus evoked substantially greater responses than the lower intensity one (usually twice the spiking frequency). Although the difference of absolute stimulus intensity was often rather small to achieve this different level of activity, the stimuli are for convenience referred to as "high" or "low" heat or pinch. With iontophoretic D-AP5, non-noxious tap stimuli (15 s, 12–30 Hz) were also sometimes applied to the receptive field using a solenoid-driven device.

Protocol 3

Ketamine was tested twice on responses to noxious pinch before and after inducing ongoing discharge by "neurogenic inflammation". Continuous activation of C-fibres was achieved by application of mustard oil (10% or 20% in liquid paraffin) to the hindpaw cutaneous receptive field. The second test with ketamine was performed during the plateau phase of the response to mustard oil, i.e. 15–25 min after the application.

Protocol 4

Drug tests using a protocol similar to Protocols 1 or 2 were repeated on the same cells during control of background firing achieved by a continuous iontophoretic ejection of kainate (as used by Cumberbatch *et al.*[11]). For tests with NMDA antagonists on quiescent cells, background firing was induced and maintained as constant as possible at around 20 spikes/s. This rather high level of ongoing discharge was chosen with the intent to relieve the Mg^{2+} block presumably operating on such cells when at rest. With spontaneously-active cells, kainate was applied to compensate for the reduction in background activity produced by the antagonist. With TRH (which often increased firing rates), background spiking discharge was maintained with kainate at about 20 spikes/s throughout the test, the ejection current being reduced as required following TRH administration.

Data processing and statistical analysis

In all cases counts of spikes during stimulus-related epochs were adjusted by subtraction of ongoing activity so as to allow for a separate analysis of drug effects on background and on evoked discharges. Control values were taken as the average of the three stable responses immediately prior to the drug test. Data were rejected where responses failed to recover by more than 50% of the drug-induced effects (except with D-CPP, see above). Test vs control comparisons were performed on spike count data using the Wilcoxon matched pairs test or, when multiple comparisons were needed, by analysis of variance (Friedman's or two-way repeated measures ANOVA) with Student–Newman–Keuls *post hoc* tests. Between-group comparisons and correlation analysis (linear regression) were performed on data normalized as percentages of control. All analyses were performed using GraphPad Instat® software. A probability of less than 0.05 was deemed significant. Data are presented as mean ± S.E.M.

RESULTS

Data from 53 dorsal horn wide dynamic range neurones from 45 rats are described in the present work.

Table 1. Effects of ketamine on dorsal horn wide dynamic range neuron background activity and responses to excitatory amino acids and noxious stimuli

	Background activity	NMDA	AMPA	Pinch	Heat	Wind-up
% Control	39 ± 7***	14 ± 3***	90 ± 5	63 ± 7***	66 ± 6***	72 ± 7**

Ketamine was given i.v. in a dose-doubling regime (up to 4 or 8, mean 5 mg/kg) so as to substantially reduce cell responses to iontophoretic NMDA (40–45 s, 5–70 nA, mean 19 nA). Of 31 cells, 17 were also tested with AMPA (40–45 s, 3–22 nA, mean 9 nA), 16 with noxious pinch (15 s, 0.6–1.8 N, mean 1.4 N), 15 with noxious heat stimuli (15–20 s, 46.5–49.7°C, mean 48.5°C), and 10 with electrical stimulation at 1 Hz evoking wind-up (see Experimental Procedures). 27 cells were spontaneously active (>0.5 spikes/s); the other four were quiescent and were therefore excluded from the analysis of background activity. With noxious heat and pinch stimuli, only the last 10 s of the response was analysed. In this table and all subsequent figures counts have been adjusted by subtraction of background activity; data are shown as mean ± S.E.M.; and significant differences from pre-ketamine control are shown as $*P<0.05$, $**P<0.01$, $***P<0.001$ (Wilcoxon matched pairs test).

Effects of N-*methyl*-D-*aspartate antagonists on nociceptive responses of dorsal horn neurons*

In the first protocol, NMDA antagonists were used to assess the relative NMDA receptor-mediated component of the ongoing discharge and of evoked nociceptive responses of DH neurons. The NMDA receptor channel blocker ketamine was used routinely in most experiments (see Experimental Procedures).

The majority of the cells studied were located in the deep dorsal horn (570–1390 μm, average 920 μm from the pial surface). More superficial cells were occasionally tested with ketamine, but it appeared difficult with them to obtain any consistent effect of the NMDA antagonist on nociceptive responses. There was a significant correlation between the inhibition by ketamine of the nociceptive responses (in percentage terms) and the depth of the cell in the spinal cord relative to the pial surface ($r=0.55$, $P=0.001$, $n=31$, data not shown). For these reasons, deeper cells were selected in most of the experiments.

On 31 cells, ketamine was administered i.v. in a dose-doubling regime so as to reduce substantially each cell's response to iontophoretic NMDA (range 0–48%, mean 14 ± 3% control). Table 1 shows pooled data of ketamine effects on background (spontaneous) activity and on the responses to iontophoretic NMDA and AMPA, to noxious pinch and noxious heat, and on wind-up. The ratemeter trace in Fig. 4A exemplifies the protocol used to generate these data. Ketamine administration, at doses (mean 5 mg/kg) that virtually blocked the response to exogenous NMDA, significantly reduced the mean value for responses to noxious heat and pinch stimuli whilst having only marginal effects on AMPA-induced firing. The wind-up of the responses to a train of 1 Hz electrical stimulation was also reduced, although by no means abolished, by ketamine. The response to the first stimulus in the train was also reduced (from 14 ± 4 to 10 ± 2 spikes, or to 71 ± 7% control, $P<0.01$, $n=10$). Importantly, ketamine also significantly depressed the background firing of cells showing any such activity.

These data were subjected to linear regression analysis to quantify the relationships between the reductions of background activity, of responses to NMDA and of responses to noxious stimuli. Figures 1A and B show that even when NMDA receptors were blocked (as judged by the response to exogenous NMDA), the degree of reduction of nociceptive responses (Fig. 1A) or background activity (Fig. 1B) was highly variable; there was a proportion of cells with which the activity was scarcely affected. The correlation between the degree of block of the response to NMDA and the reduction of the nociceptive responses by ketamine was poor ($r=0.34$, $P=0.06$). There was a better correlation of the NMDA blocking effect of ketamine with the reduction of background firing ($r=0.48$, $P=0.01$). There was also a significant correlation between the reduction of background activity and the depression of nociceptive responses (Fig. 1C: $r=0.56$, $P=0.004$). Similar trends were observed with the competitive NMDA receptor antagonist D-CPP (2 mg/kg, i.v.; see Fig. 1). These results indicate that NMDA receptors contribute to the overall discharge of most of these neurons and account for a highly variable proportion of both background firing and evoked responses.

Since ongoing activity was subtracted from all responses before analysis, the correlation between reduction of background and of net evoked activity is not due to loss of the spontaneous component from evoked responses. Three possibilities remain. First, as background activity decreases, the soma is less depolarized, and fewer excitatory postsynaptic potentials (EPSPs) reach spiking threshold, so that fewer spikes are generated even if EPSP amplitude is not affected. Second, and in addition to this, any NMDA receptor-mediated component of evoked responses would be reduced with decreasing background activity because of increasing channel block by Mg^{2+}. Third, some form of modulation by maintained nociceptive input may be required for full expression of NMDA receptor-mediated activity. To distinguish between these possibilities three further protocols were performed.

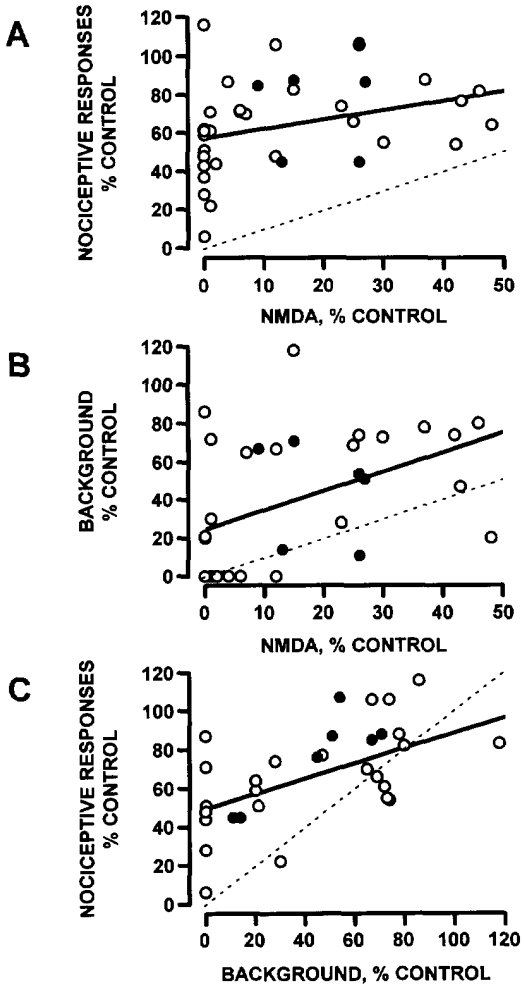

Fig. 1. Variable NMDA receptor-mediated components of nociceptive responses and background activity of DH WDR neurons. Linear regression analysis of the data shown in Table 1. Reduction of nociceptive responses (A) and of background activity (B) of dorsal horn neurones by the NMDA antagonist ketamine (open circles), plotted against its effect on the responses to iontophoretic NMDA (% control values). A) linear regression r=0.34, P=0.06, slope 0.5, n=31; B) r=0.48, P=0.01, slope 1.1, n=27. C) Percentage reduction by ketamine (open circles) of nociceptive responses plotted vs the reduction in background firing: r=0.56, P=0.004, slope 0.4, n=27. Closed circles in A, B and C represent analogous results obtained with the competitive NMDA antagonist D-CPP (2 mg/kg, i.v.; full analysis not presented). In A and C, data have been pooled for responses to noxious heat (n=14–16) and pinch (n=13–15). Dotted lines indicate a slope of 1.

Effects of N-*methyl*-D-*aspartate antagonists, and of the positive* N-*methyl*-D-*aspartate receptor modulator thyrotrophin-releasing hormone, on dorsal horn neuron responses to noxious stimulation of different intensities*

Sensory stimuli of alternating intensities, applied with the same devices, and at identical sites in the receptive field, were used to vary the discharge in a relatively homogenous population of afferents, and hence to produce varying levels of subsynaptic depolarization. This protocol is illustrated in Fig. 2, which shows a ratemeter record of the activity of a DH WDR neuron evoked by iontophoretic NMDA and by two different intensities of noxious heat. The alternating intensities of stimuli evoked responses of different amplitudes, in terms of both peak frequency and spike counts (average firing rates during the last 10 s were 21 spikes/s for the lower intensity stimulus and 62 spikes/s for the higher intensity one). When ketamine was administered i.v. (Fig. 2A), the response to NMDA was completely abolished, and the responses to both lower- and higher-intensity heat were clearly reduced (to mean firing rates of 5 and 37 spikes/s, respectively). In percentage terms, the smaller response was inhibited more than the bigger one (to 23% vs 59% control).

A similar trend was observed in all 15 cells on which i.v. ketamine was tested with two alternating intensities of noxious stimulus. Pooled data (Fig. 3A) show that although noxious heat stimuli of two different intensities evoked significantly different spiking responses of each cell (P<0.05), they were reduced by ketamine to a similar degree at the doses that virtually blocked responses to NMDA. This applied to the absolute reduction of the average firing rates during responses (Fig. 3A). In percentage terms, the effect of ketamine on low intensity heat responses was somewhat greater than that on the higher intensity ones (Fig. 3D), although the difference was not significant. Likewise, there was no significant difference between the effects of ketamine on pinch responses of different magnitude (Fig. 3A and D). Importantly, when in a separate group of experiments ketamine was tested on more vigorous responses to noxious pinch (average firing rate during the late phase 78 ± 12 spikes/s), they were still only reduced to a similar extent (Table 2).

Because the actions of channel blockers such as ketamine are themselves variably voltage-dependent,[32,40] we also examined two competitive NMDA receptor antagonists, D-AP5 and D-CPP, which should not display any voltage dependence.

Iontophoretic D-AP5 (10–80, mean 58 nA) was tested on nociceptive responses at currents that were found to reduce selectively the responses of a similar group of cells to exogenous NMDA (to 20 ± 10% control compared to 80 ± 13% for AMPA, n=5). Both in terms of average firing rates and in percentage terms, the reductions by D-AP5 of responses to noxious heat stimuli of low (45.5–48.8, mean 47.5°C) and high intensity (47.2–49.5, mean 49.1°C) were significant vs control (P<0.05 for both, n=7). However, no significant difference was found between the effects on responses to the stimuli of two intensities (to 65 ± 6% and 68 ± 8% control, respectively). Analogous results were obtained with responses to pinch stimuli of low (0.1–0.8, mean 0.5 N) and high intensity (0.5–1.6, mean 1.3 N). The smaller

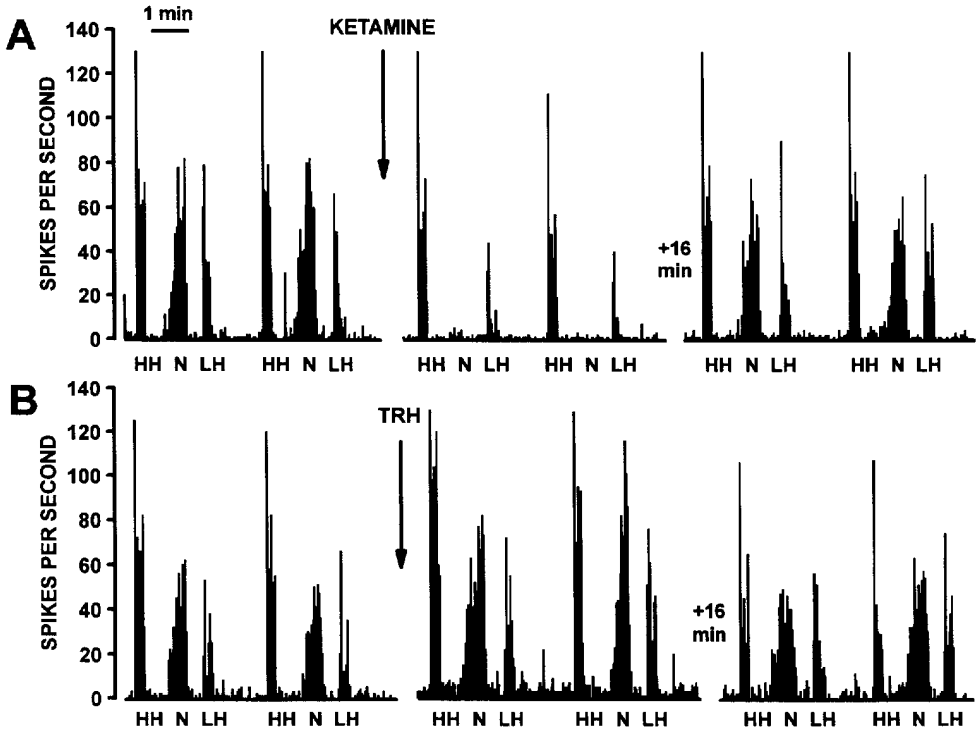

Fig. 2. Effects of ketamine and TRH on responses to higher- and lower-intensity noxious heat stimuli. A DH WDR neurone (860 μm depth) was excited in cycles by noxious stimuli of different intensities (high heat, HH, 46.5°C; low heat, LH, 45.5°C) and by iontophoretic NMDA (55 nA). A) Effects of ketamine (4 mg/kg, i.v.); B) effects of TRH (1 mg/kg, i.v.). In A and B, left hand panels show two cycles of responses during the control period. The gaps between the left and middle panels in A and B represent 3 min intervals (duration of one cycle) over which maximum effects of ketamine and TRH developed. The right hand panels show recovery 25 min after drug administration. A and B are from the same cell.

responses were reduced to 70 ± 8% control, whilst the larger ones were inhibited to 82 ± 8% control (no significant difference, n=8). Responses to innocuous tap stimuli were also reduced to a degree similar to that for nociceptive responses (to 70 ± 10% control, n=13, P<0.05 vs control). Background activity was also significantly reduced by D-AP5 (to 50 ± 7% control, n=15, P<0.01).

Since the access of an iontophoretically administered drug to the relevant synaptic sites is questionable, a series of tests was performed with the systemically active competitive antagonist D-CPP (2 mg/kg, i.v.). Results from seven tests were similar to those observed with the channel blocker, ketamine (Fig. 3B). D-CPP was very selective with respect to NMDA-evoked activity, although the effect developed slowly, taking 15–25 min to reach a plateau. The responses to iontophoretic NMDA were largely blocked by this dose of D-CPP, whereas those to AMPA were not changed. Figure 3B also shows the changes in the background firing and in responses to noxious pinch stimuli of lower and higher intensity (see also single-cell activity in Fig. 6). As with ketamine, the smaller responses evoked by weaker stimuli and the more vigorous ones to higher intensity stimuli were reduced to a similar extent. In percentage terms D-CPP reduced responses to low intensity stimuli somewhat more than those to high intensity ones, although the difference was not statistically significant (Fig. 3D).

The positive NMDA receptor modulator, TRH, was tested with this protocol at the doses that have been shown to be selective in potentiating NMDA-, but not AMPA- or kainate-induced activity of DH WDR neurons under similar experimental conditions.[7] A representative ratemeter trace (Fig. 2B) illustrates potentiation of the response to iontophoretic NMDA and facilitation of the nociceptive responses to both the higher and lower intensity heat stimuli. Note that these effects were reciprocal to those of ketamine tested on the same cell (Fig. 2A). Pooled data from seven cells (Fig. 3C and D) show a trend analogous to that observed with the NMDA antagonists; in absolute terms, TRH potentiated the nociceptive responses of different magnitude to a similar degree. Consequently, in percentage terms, TRH had a smaller, rather than larger, effect on the higher frequency spiking response (Fig. 3D). It was notable that TRH potentiated, or in some cases induced, spontaneous activity of the cells.

These results with two levels of afferent input showed that the NMDA receptor-mediated

component of evoked responses failed to correlate with the magnitude of spiking responses, which presumably reflects the degree of synaptic depolarization. It was possible that the phasic activation of neurons examined in the present protocol was of insufficient duration to permit full expression of NMDA receptor activity, or that there was insufficient C-fibre activation, which generates slow and asynchronised EPSPs. A protocol was therefore needed that involved selective and maintained activation of C-fibre nociceptors.

Effects of N-*methyl*-D-*aspartate antagonists on nociceptive responses before vs during maintained C-fibre activation induced by mustard oil*

The 13 cells tested with this protocol (Table 2) had a rather high mean level of spontaneous activity and noxious pinch stimuli evoked vigorous firing. Ketamine significantly reduced both the background discharge and the evoked activity (Table 2). Following recovery, mustard oil was applied to the cell receptive field. After the initial peak response, the ongoing firing stabilized at a level higher than control (Table 2) and ketamine was re-tested. It was less effective both against background activity and against nociceptive responses; in percentage terms, the difference was significant (Table 2). This was also true for the absolute reduction by ketamine of the mean spiking frequency during pinch responses, although the mean reduction of ongoing activity was similar. This latter finding indicates that the mustard oil evoked firing was not reduced by the NMDA antagonist.

Effects of the N-*methyl*-D-*aspartate antagonists, and of the positive* N-*methyl*-D-*aspartate receptor modulator thyrotrophin-releasing hormone, on dorsal horn neuron responses to noxious stimulation compared before and during controlled levels of background excitation*

An alternative strategy for examining the relationship between ongoing and evoked activity was to

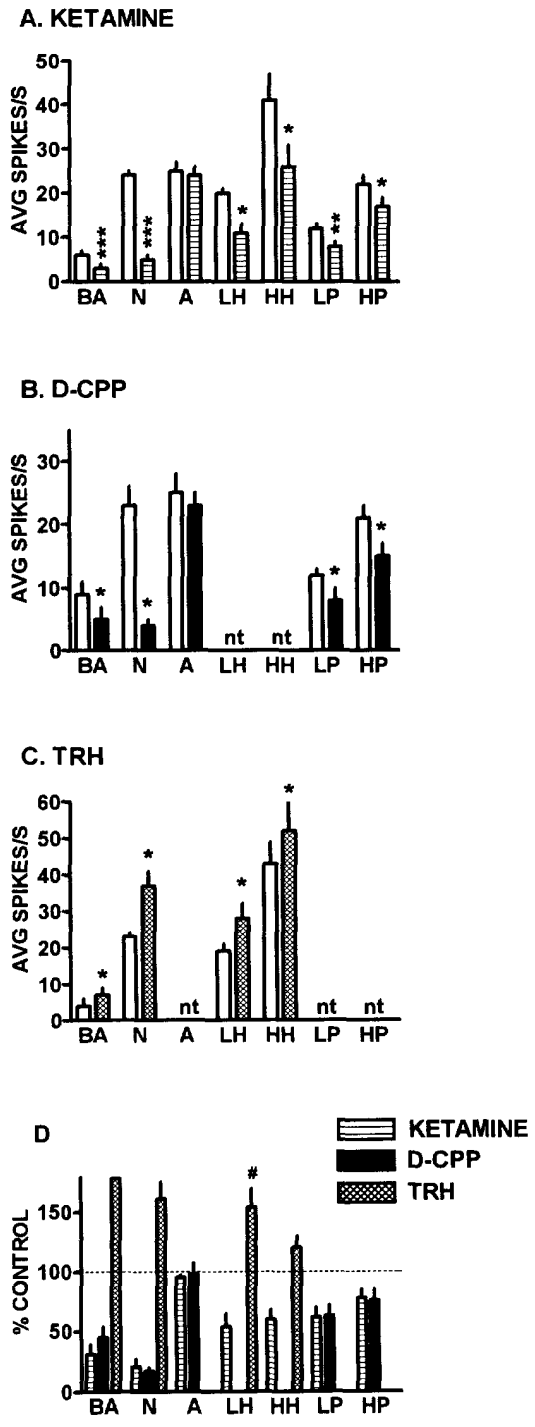

Fig. 3. Effects of ketamine, D-CPP and TRH on spontaneous activity and responses of DH WDR neurons to excitatory amino acids and noxious stimulation. In A–C open bars show pre-drug control responses, and filled bars post-drug responses, expressed as mean discharge rate (spike count corrected for ongoing activity/epoch duration). In D the same data are shown as mean percentages of control. From left to right: background activity (BA), responses to NMDA (N), AMPA (A), low- and high-intensity heat (LH and HH) or pinch (LP and HP) stimuli. A) Ketamine (4 or 8 mg/kg, mean 5 mg/kg, i.v.): of 15 cells, seven were tested with two intensities of heat stimulus (LH, mean 47.2°C; HH, mean 48.0°C), the other eight with noxious pinch stimuli (LP, mean 0.7 N; HP, mean 1.6 N); responses to AMPA were tested in nine cases. B) D-CPP (2 mg/kg, i.v.): all seven neurones were tested with two intensities of pinch stimulus (LP, mean 0.7 N; HP, mean 1.5 N) and AMPA; responses to heat were not tested (nt). C) TRH (1 mg/kg, i.v.): all seven cells were tested with two intensities of heat stimuli (LH, mean 47.4°C; HH, mean 48.2°C); responses to AMPA and noxious pinch were not tested (nt). D) Pooled percentage data. With TRH, the mean of percentage enhancements of background activity was to 526±290% control; this large value reflects very large percentage increases with some cells having low initial firing rates. For this reason, the firing rates were averaged for all cells (as shown in Fig. 3C) and the percentage change of this mean caused by TRH is shown. #Significantly different from the effect on the response to the corresponding high-intensity stimulus, $P<0.05$, Wilcoxon matched pairs test. Other significance symbols as in Table 1.

Table 2. *N*-methyl-D-aspartate receptor-mediated component of the background activity and nociceptive responses during continuous stimulation of C-fibres by mustard oil

	Before mustard oil			After mustard oil		
	Control, spikes/s	Ketamine, spikes/s	% Control	Control, spikes/s	Ketamine, spikes/s	% Control
Background activity	14±3	6±2**	36±9	23±3	15±3***	64±7$^{\#}$
Pinch	78±12	58±9**	76±5	83±13	73±11*	96±8$^{\#\#\#}$

Effects of ketamine (4 mg/kg, i.v.) on background activity and on responses to noxious pinch stimuli (0.5–1.5, mean 1.2 N) tested on DH WDR neurons. Ketamine was compared on each cell before and 15–25 min after application of mustard oil to the cutaneous receptive field on the hind paw. Significant differences from control are shown by asterisks, as in Table 1. Significant difference from the tests before mustard oil, $^{\#}P<0.05$; $^{\#\#\#}P<0.001$.

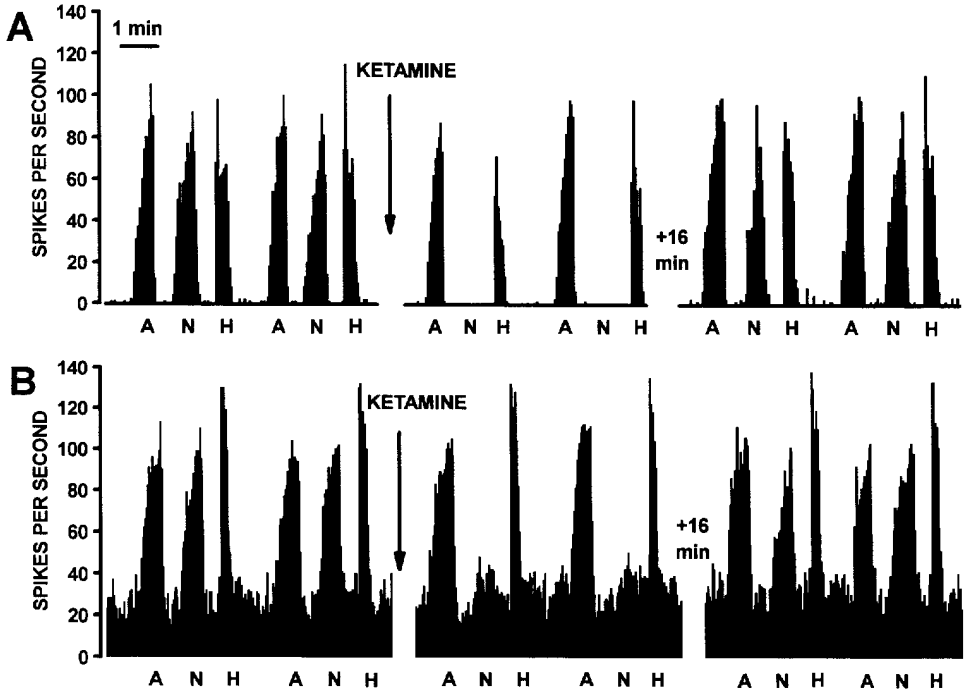

Fig. 4. Different effects of ketamine on nociceptive responses depending on the level of background firing. A) i.v. ketamine (4 mg/kg) was tested on responses of a DH WDR neuron to NMDA (26 nA), AMPA (20 nA) and to noxious heat (48.5°C). B) After recovery from ketamine (in 45 min), background firing was induced with continuous iontophoretic ejection of kainate and was maintained at a stable level with intermittent adjustments of the kainate ejection current. A test with the same dose of ketamine was repeated. In A and B, the gaps between the left-hand and middle panels represent 3 min intervals (one cycle duration) over which the maximum effect of ketamine on responses to NMDA developed.

clamp the former by varying the continuous iontophoretic ejection of an excitatory amino acid. Figure 4 illustrates one of the two approaches used. In this case, the cell did not have any spontaneous activity; i.v. administered ketamine completely blocked NMDA-evoked firing and reduced the response to noxious heat (to 62% control) and to AMPA (to 71% control; Fig. 4A). After complete recovery, a stable background firing was evoked by continuous iontophoretic ejection of kainate, and the ketamine test was repeated. Any alteration of background firing caused by ketamine was compensated by adjusting the kainate ejection current. Under this condition, neither the nociceptive heat response (100% control) nor the response to AMPA (102% control) were affected, whilst the response to NMDA was still almost completely blocked (13% control, Fig. 4B). Pooled data shown in Fig. 5 demonstrate that this pattern was seen in all 15 cells that were tested with this protocol.

In a further group of seven cells that had a relatively high and stable level of spontaneous background firing (7–14, mean 10±1 spikes/s), the second test with ketamine was performed a little differently. The reduction of the spontaneous activity brought about by ketamine (4 or 8, average 5 mg/kg) was counterbalanced by a continuous ejection of kainate so that the ongoing firing was maintained close to the

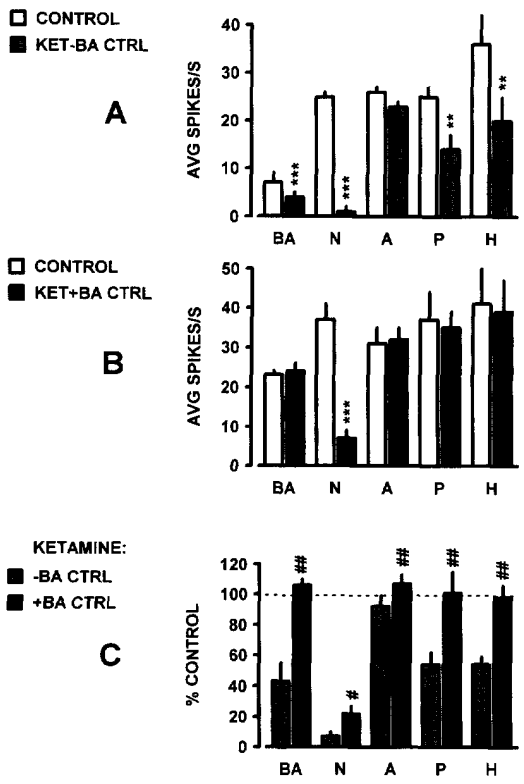

Fig. 5. Pooled data on the effects of ketamine (4 or 8 mg/kg, i.v.) on DH WDR neuron nociceptive responses tested without or with control of background excitation performed as shown in Fig. 4. In A and B, spontaneous or evoked background activity (BA, n=15), responses to NMDA (N, n=15), AMPA (A, n=9), noxious pinch (P, n=6) or heat stimuli (n=9) are shown in terms of average spiking rates before (open bars) and after (filled bars) administration of the NMDA antagonist. A) Tests without control of background activity (KET-BA CTRL); B) with such control (KET+BA CTRL). In C, the data have been replotted to show the effects of ketamine as percentages of control before (hatched) and during (solid columns) control of background firing. Significant difference from control, $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$. Significant difference from the tests with normal (uncontrolled) background firing rate, $^{\#}P<0.05$; $^{\#\#}P<0.01$. Wilcoxon matched pairs test.

pre-drug level (12 ± 1 spikes/s). In this situation, the nociceptive responses were again not affected by the NMDA antagonist (responses to pinch 105 ± 4% control, cf. 77 ± 8% control during the first test).

Results equivalent to those with ketamine were obtained with the competitive NMDA antagonist, D-CPP. Tests with this long-lasting agent required a third variant of this protocol; kainate was used to restore the depressed background firing to control for three to four cycles once the D-CPP effect had reached a plateau. A typical ratemeter record is shown in Fig. 6. Injection of D-CPP (2 mg/kg, i.v.) abolished the response to iontophoretic NMDA (but not to AMPA) and reduced the response to noxious pinch (to 76% control) in parallel with reduction in spontaneous firing. When the ongoing activity was brought back to the pre-drug level with continuous kainate application, the response to pinch was restored, although the response to NMDA was still blocked. As the effects of this NMDA antagonist were long-lasting, the nociceptive responses became depressed again as soon as the kainate ejection current was switched off. Figure 7 shows pooled data from the seven cells on which D-CPP was tested using this experimental protocol; these results are very similar to the equivalent data for ketamine shown in Fig. 5.

Similarly, no significant changes of nociceptive responses were observed when the positive NMDA receptor modulator, TRH, was tested on six DH cells during control of background firing (Fig. 8). In the first test, TRH increased the NMDA-evoked firing; responses to AMPA remained little changed, and the responses to noxious heat were significantly potentiated. The spontaneous activity was also increased by TRH. Following complete recovery from TRH (normally in 60–90 min), background firing was induced and maintained at a stable level using a continuous iontophoretic application of kainate. When TRH was given i.v. at the same dose as in the first test, the kainate ejection current was reduced so as to keep the ongoing discharge close to the pre-drug rate. Under such controlled conditions, the response to noxious heat was not increased by TRH, whilst the response to NMDA was still potentiated.

These results with ketamine, D-CPP and TRH are consistent in indicating that under these conditions NMDA receptors contribute to background but not to phasic activity evoked by short duration mechanical or thermal noxious stimuli.

Effects of the N-*methyl*-D-*aspartate antagonists on the wind-up of responses to electrical stimulation tested without and with control of background discharge*

As illustrated by Fig. 9A, repetitive electrical stimulation of the receptive field evoked wind-up of the response in all seven DH WDR neurones tested with this protocol. Ketamine at NMDA-blocking doses reduced all phases of the response to the train of electrical stimuli, i.e. the wind-up and the initial response (Fig. 9A). The spontaneous activity of the cells was also substantially reduced by ketamine (from 10 ± 4 to 4 ± 2 spikes/s, or to 49 ± 22% control, Fig. 9C). Following complete recovery, the ketamine test was repeated whilst the background firing of the cell was controlled with iontophoretic kainate. During the second test, there was no reduction of the response to the first stimulus, although the wind-up of the response was still reduced (Fig. 9B and C). Although the reduction by ketamine of the wind-up index was greater during the second test, this difference was not statistically significant. The response to iontophoretic NMDA was still almost completely blocked during the test with controlled background firing (Fig. 9C).

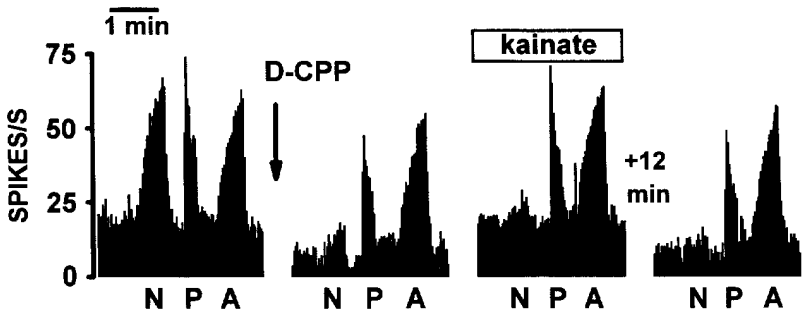

Fig. 6. Different effects of the competitive NMDA receptor antagonist D-CPP on nociceptive responses depending on the level of background discharge. The cell was a DH WDR neuron (800 μm from the dorsal pial surface) activated by NMDA (N, 10 nA), noxious pinch (P, 1.6 N) and AMPA (A, 4 nA). First panel before D-CPP; second panel 25 min after D-CPP 2 mg/kg i.v.; third panel 4 min after restoring background firing to the pre-drug level by continuous ejection of kainate (indicated by box); fourth panel after switching the kainate current off.

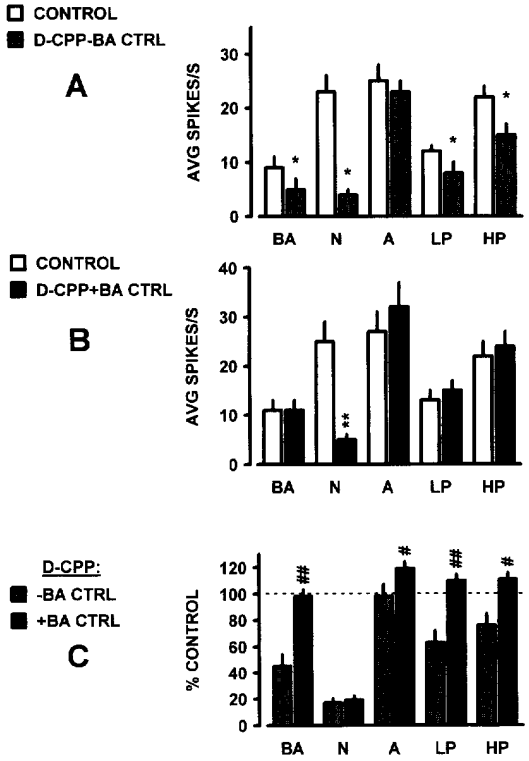

Fig. 7. Pooled data on the effects of D-CPP (2 mg/kg, i.v.) on nociceptive responses tested without or with control of background excitation. Data from seven DH WDR neurons. LP, low intensity; HP, high-intensity pinch stimuli. Format, other abbreviations and significance symbols are as in Fig. 5.

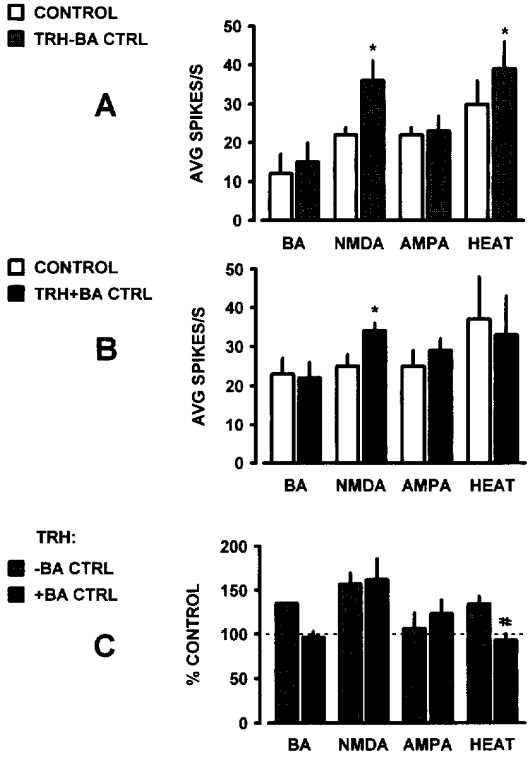

Fig. 8. Effects of TRH (0.5 or 1, mean 0.7 mg/kg) on nociceptive responses of six DH WDR neurons tested without or with control of background discharge. Format, abbreviations and significance symbols are as in Fig. 5. TRH induced highly variable percentage changes in background firing (317 ± 208% control; see Fig. 3 legend); the effect is shown instead in panel C as the percentage increase of the mean firing rate shown in A.

DISCUSSION

The well-established voltage-dependence of NMDA receptor-mediated events implies their greater contribution to high frequency as against low frequency spike discharges. In the current context this would suggest greater involvement in high frequency nociceptive responses than in low frequency discharges, whether ongoing or evoked. The surprising finding of the present work is that this was not the case.

The higher of the two intensities of noxious heat or pinch stimuli used here evidently caused greater depolarization of the cell under study than the lower intensity stimuli, since they evoked higher frequency

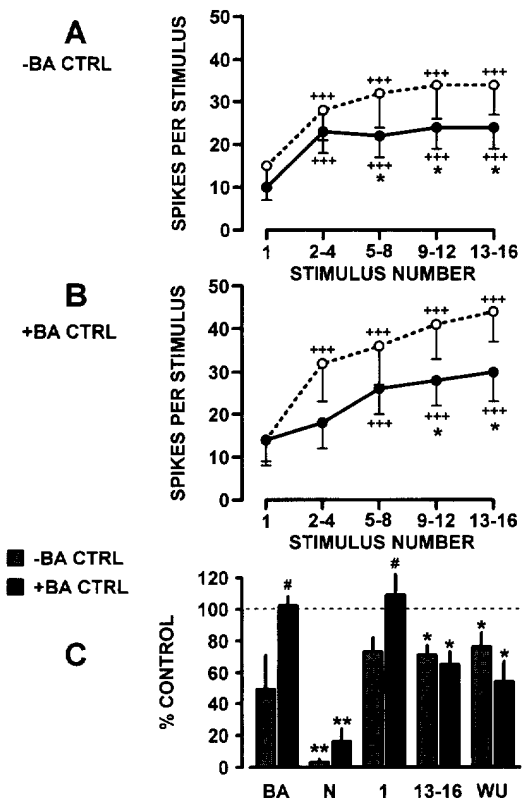

Fig. 9. Reduction of wind-up by ketamine under conditions of uncontrolled and controlled background firing. Spiking responses of DH WDR neurons were evoked by trains of 16 electrical stimuli (1 ms width, $10T_r$, 1 Hz) applied by needles to the cutaneous receptive field. In A and B data are shown as the mean number of spikes per stimulus during epochs corresponding to stimulus 1, 2–4, 5–8, 9–12 and 13–16 of the train. Responses before ketamine are shown as open circles; closed circles represent the values after ketamine administration (4 or 8, mean 6 mg/kg, i.v.). A) Effect of ketamine in the absence of any control of background excitation (−BA CTRL). The reductions of the spiking responses were significant (two-way repeated measures ANOVA, $P<0.01$); the spontaneous activity of the cells was also reduced (from 10 ± 4 to 4 ± 2 spikes/s). B) Background activity was maintained at 24–25 spikes/s (+BA CTRL). The reduction of the wind-up by ketamine was significant (ANOVA $P<0.01$). C) Pooled percentage data on the effect of ketamine on background activity (BA), and on responses to NMDA (N) and the first (1) and the last four (13–16) stimuli of a train of 16 electrical pulses at 1 Hz, and on wind-up (WU; see Experimental Procedures) tested with uncontrolled (−BA CTRL) and controlled (+BA CTRL) background discharge rates. Significant difference from the response to stimulus 1: $^{+++}P<0.001$, repeated measures ANOVA with Student's–Newman–Keuls tests. Significant difference from corresponding pre-ketamine control value, $^{*}P<0.05$, $^{**}P<0.01$; Wilcoxon matched pairs test. Significant difference from the tests with uncontrolled background firing, $^{\#}P<0.05$, Wilcoxon matched pairs test. Data from seven DH WDR neurons.

firing. Yet the effects of NMDA antagonists, and of the positive NMDA receptor modulator TRH, did not correlate with the magnitude of the nociceptive responses. Furthermore, when continuous C-fibre-mediated excitation of DH neurons was evoked by application of mustard oil to the receptive field, we still did not observe any greater NMDA receptor-mediated component of the superimposed nociceptive responses to noxious pinch. Moreover, the discharge induced by mustard oil was evidently not affected by NMDA antagonists.

Rather, there was a trend in the opposite direction, that is, lower frequency discharges were more susceptible to NMDA antagonists than were higher frequency responses. The low frequency background discharge of DH neurons was substantially affected both by NMDA antagonists and by the positive NMDA receptor modulator TRH. On evoked responses, the NMDA antagonists and TRH had a greater effect in percentage terms on weaker than on stronger nociceptive responses. When background firing of DH neurons was increased after mustard oil application, ketamine was less effective with respect to both ongoing and evoked activity.

An important point in experiments such as these is the relative effect of the test agent on the synaptic input generated by the test stimulus, as compared with effects on the background (ongoing, or spontaneous) synaptic activity, which, if reaching threshold, is reflected in the background spike discharge. From first principles, any change in such background activity will alter the spike discharge elicited by low amplitude depolarization to a greater extent than those elicited by higher amplitude depolarization. This was the pattern seen in the present experiments. Likewise, Evans,[19] examining the monosynaptic component of population spike responses in ventral roots evoked by dorsal root stimulation, reported that the competitive NMDA antagonist D-AP5 reduced small amplitude responses more than large amplitude ones. This would be compatible with a reduction of background excitation by NMDA antagonists.

In these, as in previous experiments,[26] any marked reduction of evoked responses of DH neurons by NMDA antagonists was associated with a clear decrease in the background spike activity (whenever this was present). An equivalent effect has also been seen in patch-clamp recordings of DH neurons in isolated hemisected preparations of spinal cord.[1] Furthermore, the NMDA blocking action of ketamine was better correlated with the reduction of background discharge (Fig. 1B) than with its effect on nociceptive responses (Fig. 1A). There was a significant correlation between the reductions of spontaneous activity and of the nociceptive responses (Fig. 1C). These results suggested that the altered background excitation following the administration either of NMDA antagonists or of TRH is the indirect cause of the changes in evoked responses. In the same vein, one could expect that the changed level of excitation would non-selectively affect all types of evoked activity. In fact, the NMDA antagonists affected responses to iontophoretic AMPA less

than those to noxious stimuli. Although the exact reason for this difference is not obvious, it is likely that topical application of AMPA produces a pattern of somatic depolarization different from that caused by nociceptive synaptic input.

The most conclusive evidence in favour of an indirect effect comes from the results of tests in which the level of background excitation of DH cells was controlled by ejection of kainate. Under these conditions, neither the NMDA antagonists nor the positive NMDA receptor modulator TRH had any effect on nociceptive responses. In other words, the depolarization achieved by topical ejection of kainate counteracted the reduction of NMDA receptor-mediated synaptic excitation, and completely restored the superimposed nociceptive responses. This indicates that all of the reduction of the phasic nociceptive responses by the NMDA antagonists was due to a decrease of the level of ongoing synaptic excitation, and, conversely, that there was no involvement of NMDA receptors in nociceptive responses, even when the spike discharge was sufficient to cause spike height attenuation, implying a considerable degree of soma membrane depolarization. The fact that it was possible to compensate for the change of NMDA-mediated transmission caused by a systemically-administered drug by controlling the discharge of only the cell under study suggests that the NMDA receptor component was only prominent on the recorded and/or immediately neighbouring cells.

The NMDA receptor-mediated component of the evoked and ongoing activity varied considerably between cells (Fig. 1). There was a population of cells in which there was no reduction of nociceptive responses when their responses to exogenous NMDA were virtually blocked. On the other hand, there was a significant positive correlation between cell depth and the magnitude of the depression of nociceptive responses by NMDA antagonists. It therefore seems likely that NMDA receptor-mediated transmission only becomes prominent on deeper cells, which are known to receive excitatory input with considerable spatial/temporal convergence. The latter would predispose to increased tonic EPSP activity.

It therefore appears that NMDA receptors, surprisingly, contribute to low-frequency background activity of DH neurons more than to the high-frequency phasic activity associated with acute nociceptive responses.

Why are N-methyl-D-aspartate receptors not involved in phasic nociceptive responses of dorsal horn neurons?

The degree of depolarization of dorsal horn sensory cells was not sufficient for relief of the Mg^{2+} block of N-methyl-D-aspartate receptor channels

This is unlikely for several reasons. First, firing rates evoked by noxious stimuli in many of the cells in this study were very high (up to 150 spikes/s in some cases). Second, in this as in other studies, iontophoretically-applied NMDA was capable of exciting DH sensory cells, even those with no background activity (see, for example, Fig. 4A). Third, the spontaneous activity was sensitive to selective NMDA antagonists. Therefore, NMDA receptor channels on DH sensory cells are not fully blocked under these conditions.

One could also argue that NMDA receptors may not show voltage-dependence under the conditions of these experiments because the membrane potential (resting or induced by nociceptive stimuli) was more positive than the range of membrane potential displaying negative slope conductance, i.e. that over which the magnesium block develops. A diffuse literature on intracellular records from deep dorsal horn and ventral horn neurons, when compared with the known range of negative slope conductance, does not support such a notion. In addition, recent studies have demonstrated that the non-conventional voltage-dependence of NMDA receptor-mediated events may extend as far as to positive potentials and may be associated with positive modulation by Mg^{2+} of the glycine site of the NMDA receptor complex.[52]

The block by ketamine of N-methyl-D-aspartate receptor-mediated conductances is relieved at higher levels of membrane depolarizations because of its own voltage dependence

In the majority of the experiments in this study, ketamine was used as the NMDA antagonist. It was chosen because it readily crosses the blood–brain barrier and is rapidly metabolized, thus allowing multiple tests on the same cell. As ketamine is a channel blocker, its interaction with the NMDA receptor channel is dependent upon the membrane potential, being less prominent at a more depolarized level.[32,40] Such a relief of ketamine block might contribute to the fact that ketamine was less effective against more vigorous responses (to high pinch/heat as opposed to low) or when cell ongoing discharge was kept at a constant, sometimes rather high, rate. However, results very similar to those with ketamine were obtained with two competitive NMDA receptor antagonists, D-CPP (used systemically) and D-AP5 (used iontophoretically). The actions of these receptor blockers should be devoid of voltage-dependence.

TRH in this system acts as a positive NMDA receptor modulator,[7,9] and its effects would therefore be expected to be greater as NMDA currents increase, i.e. with depolarization. However, TRH enhanced weaker and more vigorous nociceptive responses to a similar degree, and did not increase the synaptic responses when the background excitability was controlled, despite the fact that it still augmented the responses to exogenous NMDA.

N-methyl-D-aspartate receptor-mediated conductances may inactivate at the level of membrane depolarization achieved during phasic nociceptive responses

Local increases in the concentration of intracellular Ca^{2+}, which arise during NMDA receptor activation, may inactivate the receptor.[29,31] However, this seems unlikely to be the case in our experiments; first, firing responses of the neurons in this study evoked by natural stimuli were still submaximal; second, it was possible to demonstrate an NMDA receptor-mediated component of wind-up responses, which were comparable in magnitude with the naturally-evoked ones; and third, exogenous NMDA could readily drive cells to similar rates of firing.

N-methyl-D-aspartate receptors mediating nociceptive responses do not display voltage dependence

Data on the functional diversity of NMDA receptors,[20,37] and on their heterogeneity in the spinal cord,[49] imply that functionally different NMDA receptors might be differently distributed at the cellular level. It is possible that the distribution of the high-conductance NMDA receptor channels that display profound sensitivity to extracellular Mg^{2+} (and, therefore, marked voltage dependence) is such that they are not involved in the synaptic transmission mediating nociceptive responses of DH neurons. If the NMDA receptor channels subserving the nociceptive response pathway in this study were the low-conductance ones, their contribution to phasic nociceptive responses should be independent of the level of cell depolarization; in percentage terms, smaller responses and low-frequency ongoing activity should therefore be more affected by NMDA antagonists. This was seen in experiments with naturally-evoked nociceptive responses. This does not, however, alter the conclusion from tests with controlled background excitation, which clearly demonstrated that there was *no* NMDA receptor-mediated component of phasic responses to noxious heat or pinch stimuli.

More than just cell depolarization is needed for N-methyl-D-aspartate receptor-mediated contribution to nociceptive responses

NMDA receptors were indeed involved in wind-up of the responses of DH cells to repetitive electrical stimulation (irrespective of the level of background discharge), consistent with previous work.[12,14,30] This fact indicates that there are additional factors that can lead to activation of these receptors following peripheral stimulation. One such mechanism could be modulation by endogenous neuropeptides. Repetitive electrical stimulation of unmyelinated fibres can induce a co-release of glutamate and of tachykinins and/or other endogenous peptides. Glutamate and tachykinins co-exist in fine primary afferent terminals in the spinal cord.[13] Release of substance P has been shown to occur in spinal dorsal horn after electrical stimulation of afferent fibres,[17] and neurokinin receptors have been suggested to be involved in wind-up.[54] Very slow voltage-dependent postsynaptic currents (of several minutes duration) evoked by Aδ- and C-fibre stimulation have been described in DH neurons in the spinal cord[47] and may be mediated by neuropeptides.[51] Furthermore, activation of some of the neurokinin receptors has been shown to potentiate responses to NMDA under *in vitro* and *in vivo* conditions.[10,16,42] NMDA receptor-mediated events in the spinal cord are also enhanced by TRH,[7,9] a peptide abundantly present in the spinal cord.[2]

Another possible mechanism that is likely to occur specifically during wind-up, but not during responses to less synchronized, "natural" inputs, is an influx of Ca^{2+} via voltage-gated calcium channels. Involvement of L-type calcium channels in wind-up in dorsal horn neurons has been demonstrated recently in turtles.[43] These mechanisms could positively modulate NMDA receptors via Ca^{2+}-dependent intracellular events.[33,41]

In contrast to phasic responses, low-frequency ongoing discharge of dorsal horn convergent neurons is largely mediated by N-methyl-D-aspartate receptors

Although NMDA receptor activation is typically regarded as a consequence of high-frequency synaptic excitation,[3] their involvement in low-frequency background activity is not surprising. Several authors have reported involvement of NMDA receptors in low level spontaneous[1,26] or evoked activity of spinal neurons.[28,55] Low level EPSP activity can even be mediated preferentially by NMDA receptors, as has been shown in various cortical regions (e.g., Refs 38, 48).

As indicated above, one likely explanation for enhanced involvement of NMDA receptors is their positive modulation by tonically-released endogenous neuropeptides. Tonic release of endogenous tachykinins has been demonstrated to modulate responses to exogenous excitatory amino acids in the spinal cord under conditions very similar to those of the current experiments.[4] The peptide release may be associated with preparative surgery.[27] The question therefore arises as to how much of the NMDA receptor-mediated component of background synaptic excitation can be attributed to maintained sensory input from the sites of the preparative surgery. The neurokinin release caused by this input could be the mechanism to enhance NMDA receptor-mediated activity. Consistent with this hypothesis, the NMDA receptor contribution to spinal nociceptive reflexes is increased when surgical trauma is greater.[24]

CONCLUSIONS

The present work has demonstrated that NMDA receptors are not involved in phasic nociceptive

transmission in the spinal dorsal horn, even when cells are driven to very fast discharge rates. These receptors are, however, involved in low-frequency background synaptic excitation of DH WDR neurons, thus establishing the level of neural excitability in the spinal cord. We hypothesize that several endogenous neuropeptides that are capable of modulating NMDA receptor-mediated transmission may play an important role in setting the level of activation of these receptors. Assuming that NMDA receptors are indeed involved in triggering chronic pro-nociceptive changes in the spinal cord,[35,50,53] it may be that the *in vivo* effectiveness of NMDA receptor activation in spinal nociceptive processing is governed more by co-release of neuropeptides than by well-described voltage-dependence mechanisms.

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