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THE ROLE OF *N*-METHYLASPARTATE RECEPTORS IN MEDIATING RESPONSES OF RAT AND CAT SPINAL NEURONES TO DEFINED SENSORY STIMULI

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SUMMARY

1. Single-cell recordings were made from neurones in various spinal laminae in anaesthetized or decerebrated, spinalized or intact rats and cats. Cells were activated by controlled peripheral sensory stimuli which mimicked natural conditions and with some cells also by micro-electrophoretically administered excitatory amino acid analogues. Such responses were tested with amino acid antagonists administered both micro-electrophoretically and intravenously.

2. With cells in the dorsal horn, the dissociative anaesthetic ketamine, administered either micro-electrophoretically or intravenously at doses which selectively reduce responses to *N*-methylaspartate, had no consistent effect on any of the sensory responses examined.

3. The non-selective amino acid antagonist *cis*-2,3-piperidine dicarboxylate was somewhat more effective at reducing sensory responses.

4. With motoneurones, intravenous *N*-methylaspartate-blocking doses of ketamine consistently reduced nociceptive responses. Non-nociceptive responses were less affected.

5. With ventral horn interneurones, intravenous but not micro-electrophoretic ketamine reduced nociceptive responses on about half the cells tested.

6. These results are interpreted in terms of the physiological role of the *N*-methylaspartate class of excitatory amino acid receptor in mediating responses in the ventral but not dorsal horn of the spinal cord to peripheral somatic stimuli.

INTRODUCTION

There is abundant evidence that receptors for excitatory amino acids are functionally involved in mediating synaptic transmission in the mammalian spinal cord (for review see Watkins & Evans, 1981). There is, however, little evidence as to the roles of the subtypes of amino acid receptors in mediating spinal responses to specified sensory inputs. The present report addresses this question.

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Receptors for excitatory amino acids are at present classified into three subtypes named after the analogues which display the greatest selectivity for them, these being *N*-methylaspartate, quisqualate and kainate (see Watkins & Evans, 1981). To date only the *N*-methylaspartate (NMA) class of receptors is well provided with selective antagonists. Two groups of such antagonists are suitable for use in experiments *in vivo*. The first are amino acid structural analogues such as D-α-aminoadipate and 2-amino, 5-phosphonovalerate (see Watkins & Evans, 1981). The second are the dissociative anaesthetics such as ketamine (Anis, Berry, Burton & Lodge, 1983) and the benzomorphans such as *N*-allylnormetazocine (SKF 10047; Berry, Dawkins & Lodge, 1984).

The structure of the amino acid analogue antagonists implies that they will cross the blood–brain barrier poorly, so that their experimental use *in vivo* has largely been restricted to micro-electrophoretic or intrathecal administration. Although recent publications do suggest that some of these antagonists can penetrate into the brain (Meldrum, Croucher, Czuczwar, Collins, Curry, Joseph & Stone, 1983) the doses required are very high and the efficacy of their actions as amino acid antagonists when administered systemically has not been fully evaluated. Dissociative anaesthetics, on the other hand, cross the blood–brain barrier very quickly and effectively; they can therefore be administered systemically in the confident expectation that all synapses in the pathway under study are subjected to similar concentrations of the antagonist.

To date there is information on NMA receptor involvement at three 'sites' in the spinal cord. The clearest information comes from reports of the selective reduction by NMA antagonists of Renshaw cell responses which followed activation of primary afferents with electrical stimuli which probably excited only larger diameter myelinated fibres (Biscoe, Evans, Francis, Martin, Watkins, Davies & Dray, 1977; Lodge, Headley & Curtis, 1978; Davies & Watkins, 1979; Lodge & Anis, 1984). In contrast, the cholinergic activation of the same cells by ventral root stimulation was not affected. Secondly, the antagonists reduce polysynaptic (more than monosynaptic) reflexes (see Watkins & Evans, 1981; Evans, Francis, Jones, Smith & Watkins, 1982; Lodge & Anis, 1984). Thirdly, the antagonists can reduce synaptic responses of dorsal horn neurones. Some of the reports relate to electrical stimulation of undefined afferent fibres (Davies & Watkins, 1979, 1983; Lodge, Anis, Berry & Burton, 1983) whereas others describe selective reductions of some (Davies & Dray, 1979) but not all (Salt & Hill, 1981) responses of neurones in the spinal or trigeminal dorsal horns to non-noxious somatic stimuli. In addition there are older reports relating to dissociative anaesthetics (see White, Way & Trevor, 1982) but in most cases these reports are hard to relate to the NMA-blocking capacity of the drugs used.

We have exploited the selectivity for NMA and the systemic efficacy of the dissociative anaesthetic ketamine in an attempt to further specify the role of NMA receptors in the spinal transmission of sensory information derived from controlled 'natural' peripheral somatic stimuli. We now report our findings on sensory responses of dorsal and ventral horn neurones including motoneurones. Some of these results have been presented elsewhere in preliminary form (Anis, Headley, Lodge & West, 1982; Allen, Dawkins, Headley, Roe & West, 1983; Headley, Parsons & West, 1984).

METHODS

The results presented here were obtained in experiments on twenty-six cats (2·4–3·7 kg) and on sixty-seven rats (210–600 g) of either sex.

Anaesthetic and surgical procedures

With all but one of the cats anaesthesia was induced with alphaxalone–alphadolone (Saffan; Glaxo) I.V. to effect ($\geqslant$ 9 mg/kg) or I.M. 18 mg/kg, supplemented I.V. as required for the duration of surgery. Tracheal, carotid artery and radial vein cannulae were inserted. Decerebration by mid-collicular section was followed by removal of all brain tissue rostral to the section. Dorsal laminectomies were performed both at the thoraco-lumbar junction to give access for spinal section and from lumbar segments 3–5 to give access for micro-electrode penetrations of the dorsal horn in the region receiving hind-leg afferent inputs, or from segments 3–7 so that ventral roots L5–S1 could be isolated and cut distally. Following immobilization in the recording frame, anaesthetic supplementation was ceased and the animals were paralysed using gallamine, pancuronium or succinylcholine. End-tidal CO_2 was maintained close to 4 % by artificial ventilation with humidified air which was often enriched with oxygen. The one remaining cat was anaesthetized throughout with pentobarbitone (35 mg/kg I.P. initially) and was not paralysed; otherwise it was prepared as above.

Fourteen of the rats were anaesthetized throughout with pentobarbitone (60 mg/kg I.P. initially, supplemented I.V. as required). Fifty-one rats were anaesthetized with α-chloralose (100 mg/kg I.P. initially) which was supplemented with either pentobarbitone or halothane in oxygen, as required, for the duration of surgery. On some occasions pentobarbitone, 5 mg/kg, was administered during the recording period so as to prevent the spontaneous 'chloralose jerks' which were often not abolished by supplementary doses of α-chloralose. The remaining two rats were decerebrated under pentobarbitone anaesthesia. Some of the rats received a single large dose (1–2 mg/kg) of betamethasone (Betsolan Soluble; Glaxo) I.V. at the start of surgery. Following tracheal, carotid and jugular cannulations, a laminectomy was performed from thoracic 10 to lumbar 4 segment, for micro-electrode recording, or to lumbar 6 segment, for ventral root recording. In twenty-six experiments the spinal cord was left intact and with seven of these a cold-block device (Headley & West, 1984) was placed on the lower thoracic cord. In all others the cord was sectioned under thoracic 11 lamina; in those animals in which the dorsal spinal vein flowed past this segment the vein was preserved intact together with a small wedge of dorsal column. Respiration was spontaneous but the inspired air was often enriched with humidified oxygen.

All animals received fluid (mostly Haemaccel; Hoechst) at a rate of about 80 ml kg^{-1} 24 h^{-1}. In some experiments rats received blood or plasma transfusions so as to replace blood lost during surgery. The bladder was expressed manually as necessary. In long experiments lactate supplements were sometimes given to counter metabolic acidosis, but blood pH was not monitored. Blood pressure was recorded continuously and experiments were terminated if systolic pressure fell consistently below 100 mmHg (i.e. other than for short periods after drug injections).

Peripheral stimuli

In some animals reflexes were evoked by electrical stimulation of dorsal roots (in rats) or of the posterior biceps–semitendinosus nerve (in rats and cats). In most experiments, however, responses were elicited by peripheral somatic stimuli which were designed to mimic natural conditions as closely as is feasible. These stimuli were noxious heat or pinch and non-noxious deflexion of hair, skin or distal joints. The noxious heat was either radiant over a 3 mm diameter area or contact over most or all of the glabrous skin of the foot. In either case surface skin temperature was monitored and maintained constant using feed-back circuitry, a point of importance when blood pressure changes are encountered as a result of systemic injection of drugs; see Duggan, Griersmith, Headley & Maher (1978). The noxious pinch device utilized modified Allis tissue forceps driven by a pneumatic system (Brown, Headley & West, 1984). Hair deflexion was achieved using an intermittent and/or moving airjet; low-frequency skin or joint deflexion using the armature of a relay; and 50 Hz skin vibration using a modified commercially available skin massage unit.

All of these stimuli were controlled electronically for intensity, duration and repetition rate. The duration of each peripheral stimulus was 10–20 (usually 15) s, although heat stimuli were longer because of the delay in ramping from base-line (35–38 °C) to noxious ($\geqslant$ 45 °C) temperatures; the

duration at noxious temperatures was not longer than 20 s. The repetition interval was between 2 and 4 min depending on cell responsiveness and on the number of stimuli being cycled.

Synaptic responsiveness

It was predictably easy to find dorsal horn cells which were responsive to the peripheral stimuli used. In general noxious stimuli (heat or pinch) could be adjusted so that the evoked firing was well maintained for each stimulus period, although responses to pinch most often showed an initial adaptive phase, lasting generally less than 5 s. This adaptation is likely to be related at least in part to the inevitable activation by the pinch stimulus of peripheral rapidly adapting low-threshold receptors. We would therefore predict that this initial part of each pinch response is likely to contain appreciably less nociceptive content than the later phase, although this cannot be quantified. In most experiments we therefore reset the spike counter so as to display separate counts of initial (usually the first 5 s) and subsequent phases of the response. The phases will be referred to as early and late pinch, and when combined counts are considered, as total pinch.

With low-intensity peripheral stimuli (indenting the skin, deflecting hairs, moving toes) it was less easy to elicit firing rates which remained stable throughout the stimulus application. Even with repetitive stimuli (tapping the limb or pulsing an airjet at 3–15 Hz) it was often difficult to evoke mean firing rates which were comparable to those of nociceptive responses.

A separate problem is adaptation of responses over the period of recording, which in these experiments ranged up to 8 h per cell. Nociceptive responses often remained stable to stimuli repeated every 2–3 min over 3 or more hours. Changes were evident with altering anaesthetic depth; the use of decerebrated cats and α-chloralose-anaesthetized rats ameliorated this problem. None the less, small changes in stimulus intensity were required with some cells so as to maintain an approximately constant firing rate pattern per response; consistency of evoked firing rate of responses during the various pre-drug control periods of any one cell was considered more important than absolute consistency of peripheral stimulus intensity. Between cells there was of course considerable variation of evoked firing rates. When cell responsiveness changed over longer periods it was generally downwards, necessitating an increase of stimulus intensity; it was rare to see any signs of sensitization to the stimulus, even on those few occasions when frank tissue damage had been caused by excessive stimulus intensities.

Long-term stability of evoked firing rates was more of a problem with responses to low-intensity stimuli, particularly in rats. Occasionally it was not possible to compensate by altering the peripheral stimulus; that stimulus would therefore be dropped from the cycle. These combined problems with non-noxious stimuli explain the small proportion of cells in our sample on which non-nociceptive responses have been analysed concurrently with nociceptive responses.

Recording procedures and administration of NMA antagonists

In those experiments in which reflexes were elicited by peripheral electrical stimuli, recordings were made from entire ventral roots either as averages of sixteen to thirty-two consecutive reflexes or as integrals of individual reflexes. In the majority of reflex experiments, however, the responses were evoked by 'natural' stimuli and single-unit monopolar recordings were made from unidentified motoneurone axons using silver-wire hook electrodes on which dissected ventral root filaments were placed. Administration of antagonists in these tests was necessarily systemic (intravenous).

With all other neurones extracellular recordings were made using glass micropipettes which were either single or seven barrelled, the latter enabling micro-electrophoretic administrations of agonists and antagonists to be made to the cell under study. Recording barrels were filled with 3·5 M-NaCl and the side barrels of multibarrel pipettes with combinations of the following compounds: the excitatory amino acid analogues sodium *N*-methylaspartate (NMA; D-isomer, 100 mM in 100 mM-NaCl; DL-racemate, 200 mM), sodium quisqualate and sodium kainate (both 5 mM in 200 mM-NaCl), sodium *cis*-2,3-piperidine dicarboxylate (PDA) and sodium γ-D-glutamylglycine (both 200 mM) all at PH 7–8; ketamine HCl (50 mM in 150 mM-NaCl); and the dye Pontamine Sky Blue (2 % in 0·5 M-sodium acetate, pH 7·7).

Single-cell action potentials were recorded by conventional means. Because the natural stimuli used often resulted in synchronized activation of closely spaced neurones, great care had to be taken to ensure that the recorded spike configuration was consistent, thereby indicating that the recording was indeed unitary. This monitoring of spike configuration on an oscilloscope was performed by triggering an analogue delay circuit from the spike discriminator output which was used for all

further analysis. Spike firing rate was displayed continuously on a pen recorder. Counts of the number of action potentials evoked during preset periods relating to the sensory stimuli were, in most experiments, also displayed on the pen recorder, and these stimulus-related counts have been used for calculating percentage changes in response amplitude. In later experiments these calculations were performed 'on line' by an RML 380Z microcomputer (Headley, Parsons & West, 1985; Headley & West, 1985).

The antagonists were administered during an ongoing cycle of one or two somatic stimuli, sometimes with added micro-electrophoretic stimuli (i.e. the ejection of one or two of the amino acids NMA, quisqualate or kainate). Administration of antagonists was either micro-electrophoretic or intravenous; both routes were tested on many cells. In intravenous tests ketamine (Ketalar; Parke-Davis) was given at a starting dose usually of 1 or 2 mg/kg; when required, further doses (producing a logarithmic progression of the cumulative dose) were injected over 30–60 s at 3–5 min intervals, corresponding to one to two stimulus cycles.

The technical problems associated with testing drugs by intravenous administration in such experiments include alterations in blood pressure and the possibility of progressive changes in cell recording conditions, or of cell excitability, during the period of drug metabolism. In this study we have made careful note of blood pressure changes and have excluded tests in which changes of neuronal responsiveness paralleled the inital drug-induced fluctuations in blood pressure. We have also excluded any tests in which responses did not recover by at least 50 % of the drug-induced change; with the majority of tests recovery was considerably more than this.

Neuronal identification

Motoneurones were clearly distinguished from other spinal neurones because recordings were made from their axons in ventral roots. We have not, however, further identified motoneuronal cell types. This distinction could only be made by recording from peripheral motor nerves, thereby entailing surgery close to or even within the receptive field of the recorded neurone.

Other spinal neurones were characterized anatomically by their laminar location. Dye was ejected at some recording sites, allowing retrospective verification of these sites by histological examination of 50 μm frozen sections. This procedure, together with micromanipulator readings of other cell recording sites, allowed the majority of cells to be apportioned to a specific lamina. The rostral projection of recorded neurones was not examined.

All neurones were also characterized by their receptive field properties, with emphasis being given to the responsiveness of the cell to the various somatic peripheral stimuli which could be cycled (see above).

RESULTS

Dorsal horn

Tests with antagonists administered micro-electrophoretically. Ketamine was tested electrophoretically on seventy-three occasions, fifty-nine tests being on twenty-nine cells in nine cats, and fourteen on eight cells in six rats. Pinch responses were tested on fifteen cells in cats and seven in rats; heat responses on twenty-seven cells in cats but none in rats; non-nociceptive responses on four cat cells and one rat cell. Of these tests, electrophoretic NMA (sometimes with one or two other amino acids) was included in the cycle of stimuli on sixteen cat cells and six rat cells.

On no occasion was any synaptic response decreased by electrophoretic ketamine in parallel with the consistent reductions of responses to NMA. Although synaptic responses were sometimes slightly reduced, this only occurred with higher currents of ketamine which, on those cells tested, also affected responses to either quisqualate (cycled with eighteen cells) or kainate (six cells). This indicates that these higher currents of ketamine were having non-specific actions so that any effects on synaptic inputs cannot be interpreted in terms of antagonism of NMA-receptor-mediated

events. Fig. 1 illustrates an experiment in which ketamine at eight times the NMA blocking current for this cell was already blocking responses to quisqualate yet was still without effect on the responses to noxious pinch.

The non-selective amino acid antagonist *cis*-2,3-piperidine dicarboxylate (PDA) was compared with ketamine on seven of the above cells. On four of these it was clearly more effective at reducing synaptic responses than was ketamine. An example of a particularly marked case is shown in Fig. 2.

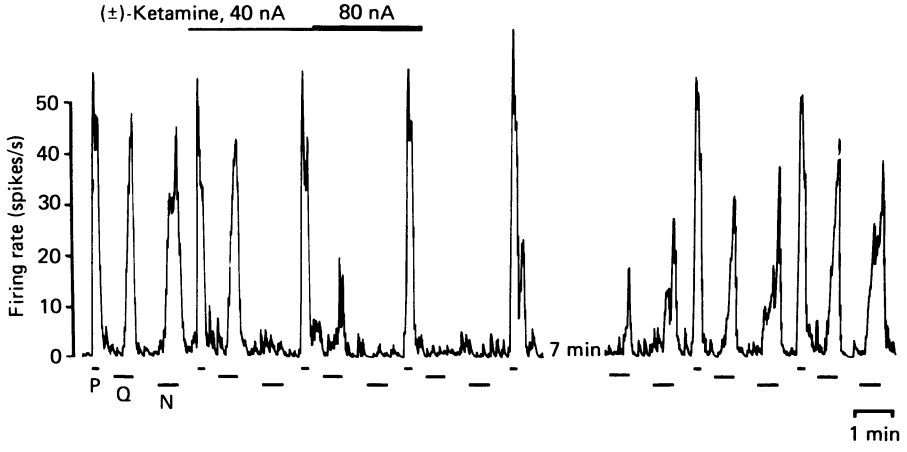

Fig. 1. Lack of effect of micro-electrophoretically administered ketamine on nociceptive responses of a dorsal horn neurone. The record shows the firing rate (spikes/s) of a multireceptive lamina IV cell which was activated in a regular cycle by a noxious pinch stimulus (P) of the skin over the gastrocnemius muscle and by the electrophoretically administered excitatory amino acids quisqualate (Q, 32 nA) and *N*-methylaspartate (N, 32 nA). Racemic ketamine at 10 nA (not shown) and subsequently at 40 nA ejection current (indicated by the bar over the trace) selectively blocked responses to NMA. Further doubling the dose of ketamine caused non-selective amino acid reduction but had minimal effects on the synaptic response. Decerebrate spinalized cat.

Despite this example, on other cells tested with PDA (but not always with ketamine) it was generally the case that synaptic responses were reduced by PDA less than were responses to NMA or quisqualate. PDA was tested fifty-seven times at various currents on ten cells in cats and eleven cells in rats; with forty-nine of these tests either or both of NMA and quisqualate were cycled alternately with one or two peripheral stimuli. At a dose of 40 nA, PDA reduced responses to NMA to a mean of 23 % control (ten cells) and quisqualate to 30 % (ten cells). At the same dose, total pinch responses were reduced to only 60 % control (eleven cells), those to heat to 61 % (four cells) and those to low-intensity stimuli to 95 % (two cells). Increasing the current of PDA to 80–100 nA reduced responses to pinch to 50 % (fifteen cells), to heat to 41 % (five cells) and to low-intensity stimuli to 52 % (seven cells).

Thus, as with ketamine, none of the types of synaptic response tested was consistently reduced by PDA in parallel with electrophoretically administered transmitter analogues. One possible interpretation of this relative lack of effect on synaptic responses is that the antagonists are reaching only some of the sites of synaptic contact on the cells under study. If antagonists with appropriate phar-

macokinetics exist, the problem can be overcome by administering the antagonists systemically. Ketamine fulfils this criterion and has therefore been tested intravenously in otherwise similar tests to those described above.

Tests with intravenous ketamine. Ketamine was administered intravenously at NMA-blocking doses (2–5 mg/kg; see Anis *et al.* 1983 and Fig. 3) on fifty-five occasions; of these, thirty-two were on twenty cells in eleven cats and twenty-three

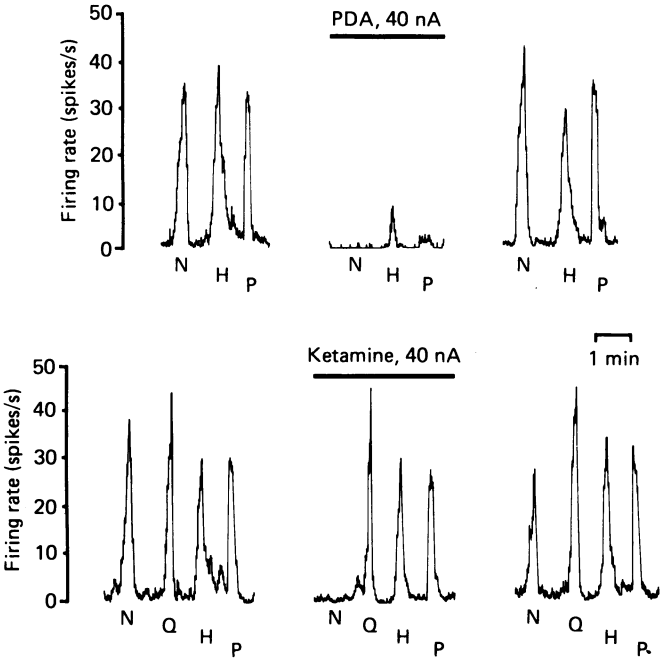

Fig. 2. Comparison of the effects of the non-selective amino acid antagonist PDA and the selective NMA antagonist ketamine on responses of a lamina VI multireceptive neurone. In this record of cell firing rate the cycled stimuli were NMA (N, 80 nA), noxious heat to toe pad 4 (H, 37–49 °C ramp over 32 s, held for 8 s) and noxious pinch to the main pad (P, 10 s duration) for the PDA test shown in the upper trace; subsequently quisqualate (Q, 34 nA) was included in the cycle (lower trace). On this cell PDA was particularly effective at reducing synaptic responses at low ejection currents whereas ketamine reduced only responses to NMA (see also Fig. 1). Decerebrate spinalized cat.

on sixteen cells in thirteen rats. NMA (and sometimes a second amino acid) was included in the cycle with one or two peripheral stimuli with twelve of the cat and five of the rat cells. The synaptic inputs tested were noxious pinch on thirteen cat cells and twelve rat cells; noxious heat on nine cat and three rat cells; non-noxious tapping, toe deflexion or vibration on twelve cat and eight rat cells.

At these doses, intravenous ketamine never blocked any synaptic response. An example of total block of responses to NMA with no effect on synaptic responses to peripheral pinch and tap stimuli is illustrated in Fig. 3.

Taking those tests in which ketamine was administered at 4–5 mg/kg, responses to NMA were reduced to a mean of 17 % (fifteen cells), those to heat to 94 % (ten cells), to late pinch to 82 % (twenty cells) and to tap or toe deflexion to 84 % (sixteen cells).

There was, however, variation between cells. The most convincing reduction we have seen is shown in Fig. 4 which shows that at 4 mg/kg, ketamine reduced both early and late phases of the response to pinch to around 50 % of control and that increasing the dose to 8 mg/kg caused a further marked reduction of the response. 8 mg/kg is greater than the NMA-blocking dose for the great majority of cells, though NMA was not tested on this occasion. It was notable that the short-acting barbiturate anaesthetic, methohexitone, given at 4 mg/kg (about half the induction dose), caused a similar potent reduction of this pinch response. The response of this cell to pinch may therefore have been particularly sensitive to any generalized reduction of cell excitability.

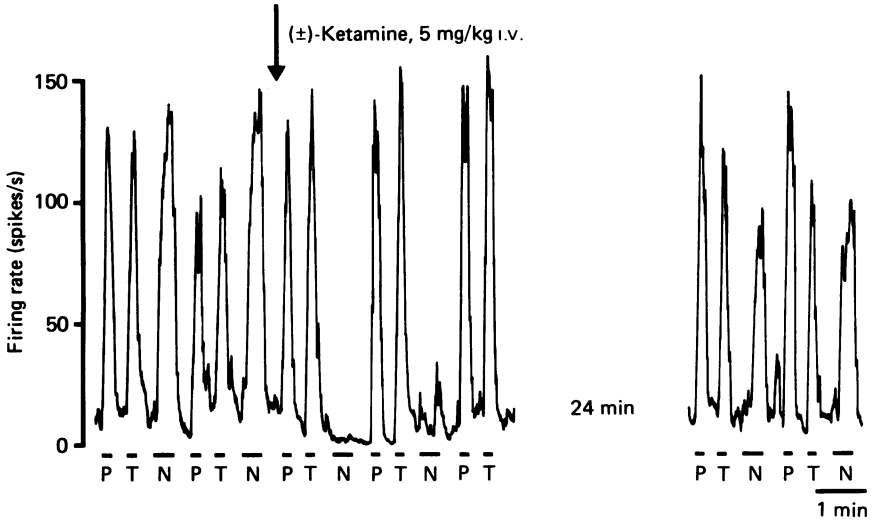

Fig. 3. Intravenous, like micro-electrophoretic, ketamine fails to affect synaptic responses of most dorsal horn neurones. On this lamina VI multireceptive neurone the cycle of stimuli was noxious pinch to the skin over the lateral metatarsal (P), non-noxious repetitive tapping—touching of the skin over toe 3 (T) and electrophoretic NMA (N, 35 nA). Despite exposing the entire synaptic pathway to an NMA-blocking dose of intravenous ketamine, the synaptic responses were unaffected. Decerebrate spinalized cat.

Thus ketamine, whether administered micro-electrophoretically or intravenously, failed to reduce any of the synaptic responses tested in parallel with the clear reductions of responses to *N*-methylaspartate.

Ventral horn

Our finding that ketamine failed to have clear effects on synaptic responses of dorsal horn neurones contrasts with previous reports that ketamine does reduce polysynaptic (more than monosynaptic) reflexes in cats (Tang & Schroeder, 1973; Lodge & Anis, 1984). We therefore wished to extend these observations and examine how ketamine would affect various aspects of spinal reflexes to peripheral stimuli.

Electrically evoked reflexes. We have found it difficult, under our experimental conditions, to evoke reproducible monosynaptic reflexes in rats. In initial experiments in this series we examined ventral root reflexes elicited by electrical stimuli to dorsal roots (3–50 × threshold, 0·1 ms, 0·2–1 Hz; two pentobarbitone-anaesthetized rats, one

spinally intact and one spinalized at T11; and one decerebrate spinalized rat). In none of these animals were the reflexes as predictable and consistent as with cats, even though a wide variety of parameters of dorsal root conditioning stimulus were examined. Ketamine was tested at 5, 10 or 20 mg/kg on five occasions and reduced the reflexes in each case. However, the degree of reduction as well as the selectivity between mono- and di- or polysynaptic reflexes was notably poorer than that reported with cats.

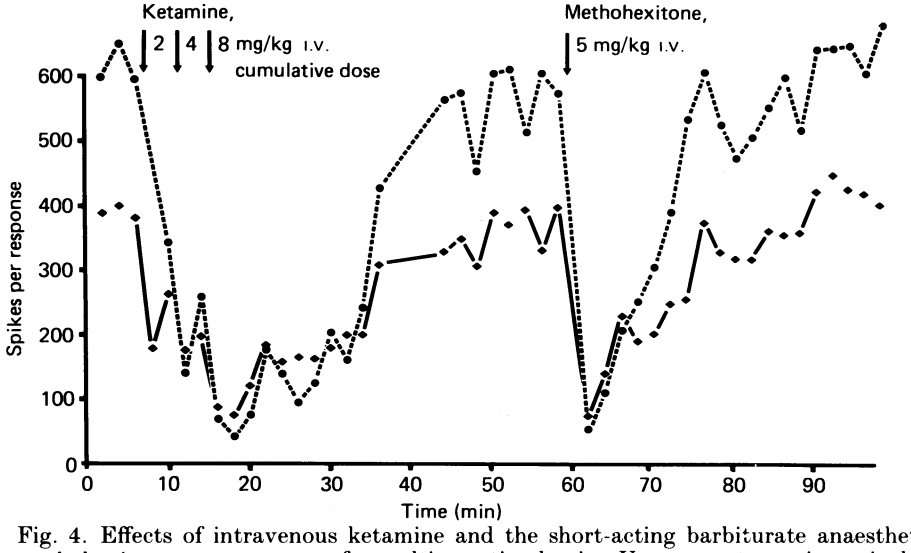

Fig. 4. Effects of intravenous ketamine and the short-acting barbiturate anaesthetic, methohexitone, on responses of a multireceptive lamina V neurone to noxious pinch of toe 2 (duration 15 s, repeated every 2 min). The ordinate indicates the number of spikes elicited during the first 5 s (◆) and the subsequent 10 s (●) of each pinch response. Both compounds reduced both phases of the response. The cell was not spontaneously active. α-Chloralose-anaesthetized spinalized rat.

As an alternative protocol, in two α-chloralose-anaesthetized rats we examined the ability of a noxious stimulus (a contact thermode alternating between 35 and 48–50 °C and placed over most of the foot) to condition the reflex evoked by electrical stimuli (3–5 × threshold, 0·1 ms, 0·5–1 Hz) to the posterior biceps–semitendinosus (p.b.s.t.) nerve. Although the noxious stimuli did, as predicted, enhance the otherwise weak or absent monosynaptic reflexes, the degree of conditioning was too small and variable for testing of intravenous drugs to be viable.

A similar protocol was followed in two decerebrate spinalized cats, in which near-threshold monosynaptic reflexes were evoked in L7 ventral root by electrical stimulation of the p.b.s.t. nerve. The resultant reflex was then conditioned alternately by a low- and a high-intensity peripheral stimulus; the low-intensity conditioning stimuli were electrical, either to the p.b.s.t. nerve or percutaneously to the central footpad, in either case at near-threshold intensity; the high-intensity conditioning was either a noxious natural stimulus to the foot (pinch or radiant heat) or high-intensity percutaneous electrical stimulation of the central footpad. The resultant potentiation of the monosynaptic reflex was tested for sensitivity to intravenous injections of ketamine at NMA-blocking doses. In a total of eleven tests with

doses of ketamine, 2–10 mg/kg, reflex potentiation was reversibly reduced (by a maximum of 60 %) on seven occasions. There was, however, no clear or consistent distinction between any of the various types of conditioning examined. No further tests of this kind were therefore carried out.

For these reasons all subsequent tests in the ventral horn were performed on single-cell responses to natural peripheral stimuli.

Responses of single motoneurones to natural peripheral stimuli. We have found it relatively easy to evoke responses in motoneurones to noxious peripheral stimuli. So many cells respond to noxious pinching of a restricted peripheral area (such as one toe) that making unambiguously unitary recordings from ventral root filaments can become a problem. By no means all of these nociceptive cells respond consistently to cutaneous heating to temperatures which are generally regarded as noxious ($\geqslant$ 45 °C) but which do not cause overt tissue damage within a few repetitions of the stimulus; with our stimulus protocol such damage occurs at above 50 °C in rats and above about 54 °C in cats. We have not quantified the relative sensitivity to these two stimuli of all motoneurones encountered, but our subjective impressions are that less than a quarter of pinch-sensitive cells respond consistently to noxious heat, with the proportion being lower in rats than in cats.

Many of these motoneurones, and some for which no adequate noxious stimulus can be found, respond to non-noxious peripheral cutaneous or deep somatic stimuli. Under our experimental conditions the great majority of such responses attenuate rapidly ($\leqslant$ 5 s) and often completely, so that only a small proportion of cells can be coerced to respond throughout a 10–15 s stimulus and even fewer to respond consistently to successive stimuli. As a result our sample of motoneurones with consistent non-nociceptive responses is small, three in rats and two in cats.

The analysis below is made on the basis of 234 technically satisfactory tests (see Methods) of intravenous ketamine on eighty motoneurones in forty-five rats and five cats. Of the 234 tests, thirty-three were repeat tests on the same cell of a particular dose; in these cases the technically superior result has been selected, and analyses made of the remaining 201 intravenous tests, of which 173 were on seventy motoneurones in rats and twenty-eight on ten cells in cats.

In rats the effects of ketamine have been examined both in twenty-eight spinally sectioned and in seventeen spinally intact animals; with seven of the latter the cord was reversibly 'cold-blocked'. In common with others we have found little evidence in anaesthetized rats of tonic descending inhibitions of synaptic responses. Thus the general nature of nociceptive responses was similar in intact and spinalized animals, but it was not within the scope of the present study to examine this point in detail.

The effects of ketamine in spinalized animals. At doses of I.V. ketamine which correspond to the NMA-antagonizing dose (i.e. 2–5 mg/kg), nociceptive responses of motoneurones were almost always reduced and sometimes entirely blocked. Fig. 5 shows the effect of ketamine on responses of a cat motoneurone to noxious pinch stimuli and illustrates how the late phase of the pinch response was often very much more sensitive to ketamine than was the early part (which we presume to have less nociceptive content; see Methods).

In this series we have tested ketamine on alternating pinch and heat stimuli on nineteen cells in fifteen rats. There was no consistent difference in sensitivity of the

responses evoked by these two stimuli. Ketamine was tested at 4 mg/kg on fourteen of these cells and reduced the late phase of pinch responses to a mean of 35 % of control and the heat responses to 34 % of control. Higher and lower doses of the NMA antagonist similarly caused equal reductions of the two types of response. An example of such an effect is shown in Fig. 6.

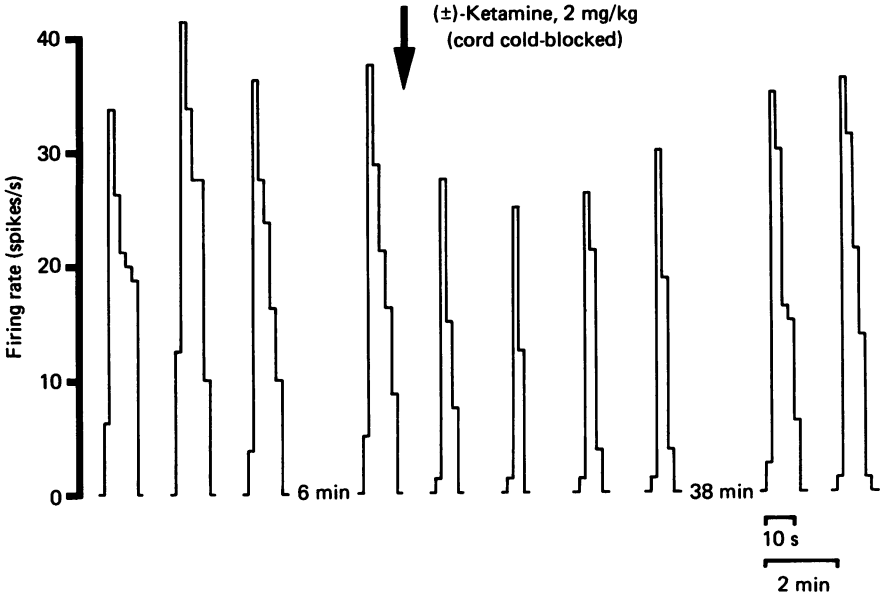

Fig. 5. Effect of low, NMA-blocking dose of intravenous racemic ketamine on responses of a motoneurone to noxious pinch of toe 3 (10 s duration) repeated at 2 min intervals. The record is of spikes counted in 2 s epochs, but the ordinate is calibrated as spikes per second; interstimulus periods are omitted. On this cell the initial phase of the response was relatively resistant to ketamine whereas the late phase was abolished. Substantial recovery occurred 45 min after the ketamine injection. Unit recorded in S1 ventral root of a decerebrate cat reversibly spinalized with a cold-block device.

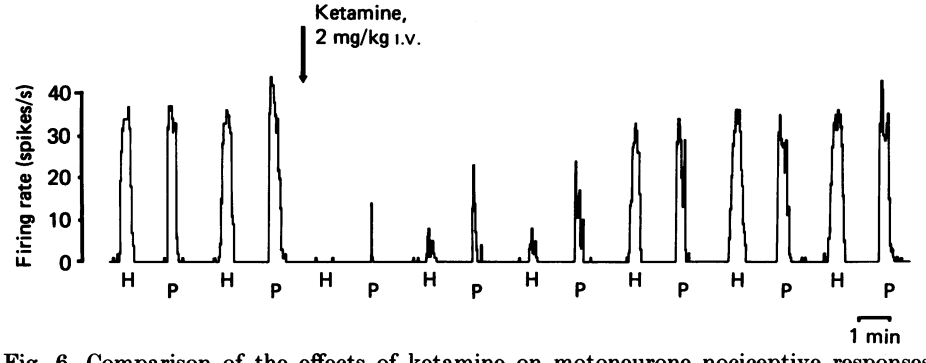

Fig. 6. Comparison of the effects of ketamine on motoneurone nociceptive responses evoked by alternating heat (H, ramp from 36 to 49·5 °C for 30 s to plantar foot) and pinch stimuli (P, 15 s to toe 5). Unit in L5 ventral root of an α-chloralose-anaesthetized spinalized rat.

Such low-threshold responses as have been tested independently have proved variably susceptible to ketamine, although as illustrated in Fig. 5, the early phase of pinch responses was often more resistant than the late phase. With one of the two cells on which the direct comparison was made, non-nociceptive responses were reduced appreciably less than nociceptive responses; this result is illustrated in Fig. 7. Although the non-nociceptive responses (to 50 Hz vibration) were reduced somewhat, the parallel reduction and recovery of the spontaneous firing of the cell suggests that the reduction of the vibration response is best explained by a general reduction of cell excitability. The response to noxious pinch was clearly reduced to a considerably greater degree.

Comparison of the effects of ketamine in spinalized and intact states. Little difference in effectiveness on motoneuronal responses to pinch was revealed by comparing eighty-nine tests of I.V. ketamine in fourteen rats with spinal cords functionally intact, with 116 comparable tests in twenty-eight spinally sectioned and seven cold-blocked rats. Thus in tests at the 2 mg/kg dose, ketamine reduced total pinch

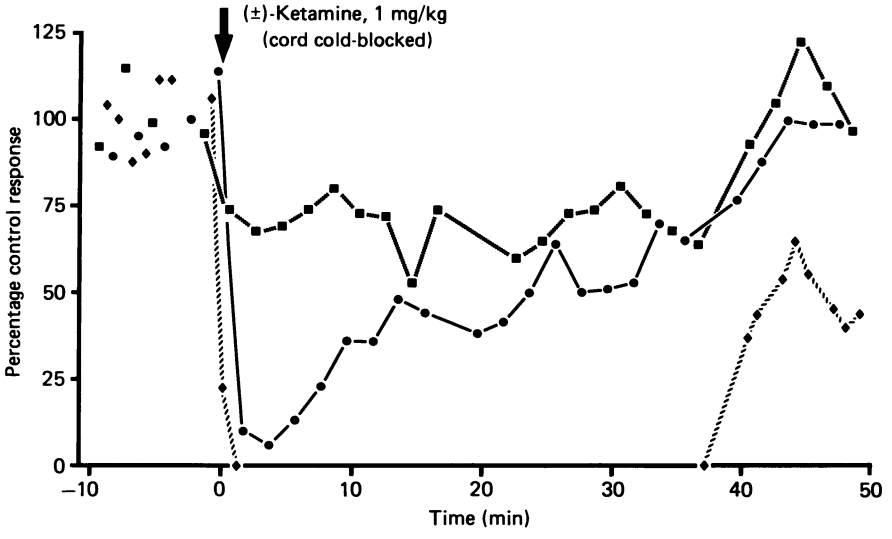

Fig. 7. Comparison of effects of ketamine on motoneurone responses to alternating noxious pinch and non-noxious vibration stimuli. Counts of spikes evoked per response, and of spontaneous discharge during interstimulus intervals, were averaged over four cycles to provide mean pre-ketamine control values which were normalized to 100%. All values of spikes per response are expressed as a percentage of the appropriate control mean. Unit in S1 ventral root of a decerebrate cat which was in a state of reversible spinalization by cold-block. ●, pinch to main footpad; ■, vibrating rod over ischium; ◆, spontaneous activity.

responses in the functionally intact state to a mean of 58% control (seventeen cells) whereas in spinalized animals the reduction was to 56% (fourteen cells). Equivalent tests have not been made in sufficient numbers for adequate comparison of other synaptic responses in rats; nor have any tests been performed in spinally intact cats.

Fig. 8 provides an example of the effect of ketamine on nociceptive pinch responses of a rat motoneurone before and during a period of reversible spinalization by

cold-block, and indicates both that the over-all response amplitude was little altered by the cold-block and that ketamine was equally effective in the two states. The blood pressure trace shows how, with the cord in the intact state, each pinch stimulus evoked a small but fairly consistent increase in blood pressure. During cold-block these cardiovascular responses were absent, indicating the effectiveness of the conduction block of ascending pathways. The trace also indicates that in the intact state ketamine did not affect the cardiovascular responses, implying that it was not blocking ascending nociceptive activity at a dose which did abolish the spinal reflex.

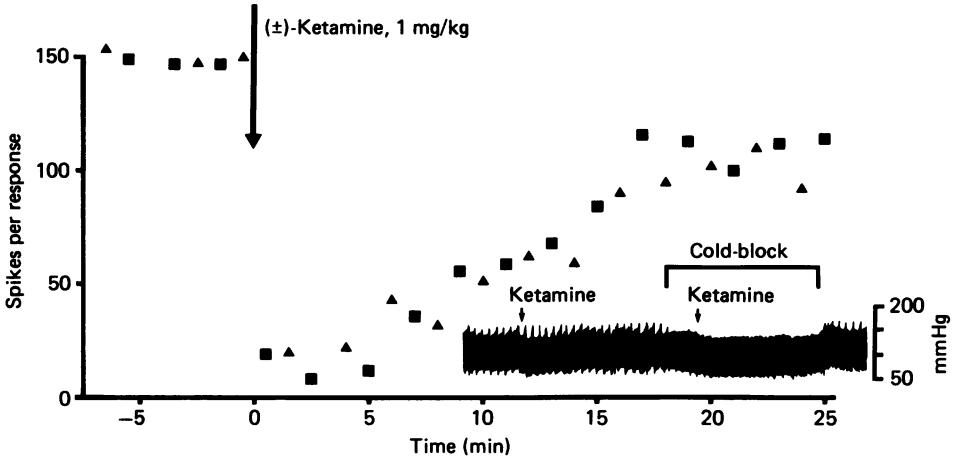

Fig. 8. Comparison of the effectiveness of ketamine on motoneuronal responses to noxious pinch of the tail in spinally intact (■) and subsequent reversibly spinalized states (▲). The inset shows the blood pressure record during the period represented in the graph. During reversible spinalization by cold-block the blood pressure deflexions previously elicited by each noxious stimulus were abolished, indicating effective block of ascending nociceptive activity. Ketamine in the spinally intact state did not have this effect. Unit in L6 ventral root of an α-chloralose-anaesthetized rat.

When considered over-all, the eighty motoneurones tested with ketamine showed a gradation of sensitivity to the NMA antagonist. Such a gradation complicates the analysis of the relation of ketamine effects to NMA receptor involvement. Because tests with high doses of ketamine have the disadvantage of long drug recovery times, it is impractical to test a full range of doses on all cells. Consequently the higher doses were tested only on the more resistant cells. This has the paradoxical consequence that if mean reductions per dose level are considered, the higher doses of the antagonist apparently reduce synaptic responses by little more than do lower doses.

In an attempt to compensate for this problem we have measured the lowest dose (in the logarithmic dose progression used) which reduced synaptic responses to below 50 % of control values. This analysis is presented in Table 1. It indicates that whilst many nociceptive responses were reduced to below 50 % of control (and often much lower) by doses of ketamine which block responses to NMA (taken here as $\leqslant 4$ mg/kg), the responses of other cells were considerably more resistant to the antagonist.

Responses of single ventral horn interneurones. The above results with motoneurones indicate that NMA-antagonizing doses of ketamine attenuate many motoneuronal responses, but those intravenous tests provide no evidence as to the site of this action and hence of the presumed NMA receptor involvement. In an attempt to answer this question we have recorded from ventral horn neurones in laminae VII and VIII and have tested both ketamine and the non-selective amino acid antagonist PDA on responses of such cells to peripheral stimuli and to electrophoretically administered amino acid analogues.

TABLE 1. Number of cells responding to noxious and non-noxious stimuli

Minimum dose of ketamine reducing responses to $\leqslant$ 50 % of control	Noxious pinch		Noxious heat		Non-noxious	
	Cord intact	Cord cut	Cord intact	Cord cut	Cord intact	Cord cut
$\leqslant$ 4 mg/kg	12	20	1	13	1	0
$\geqslant$ 8 mg/kg	8	12	0	5	1	2

Ketamine was administered intravenously in logarithmically increasing doses and the minimum dose which reduced the number of spikes evoked per response by at least 50 % was noted. The Table indicates, for each kind of peripheral stimulus, the number of rat spinal motoneurones tested on which this minimum effective dose of ketamine was at ($\leqslant$ 4 mg/kg) or above ($\geqslant$ 8 mg/kg) the NMA-blocking dose of this antagonist. The non-noxious stimuli were vibration, skin tapping, squeezing a muscle belly and extending a joint (one cell each).

Tests have been performed on twenty-six cells, twenty-two in eight spinalized cats, three in two spinally intact rats and one in one spinalized rat. Responses to noxious pinch were tested on twenty-one cells and responses to noxious heat on ten cells. With eighteen of these cells micro-electrophoretic NMA (with or without quisqualate and/or kainate) was included in the cycle.

Ketamine (10–80 nA) was tested micro-electrophoretically on twenty-six occasions on fourteen cells. On no occasion was there a parallel reduction of responses to the noxious stimuli and to NMA. Although nociceptive responses were reduced somewhat at the higher currents, quisqualate, when tested concurrently, tended also to be reduced, indicating non-selective reduction of cell excitability with these doses of ketamine.

In contrast, when ketamine was tested intravenously at NMA-antagonizing doses, it did reduce the nociceptive responses of some but not all of these lamina VII and VIII neurones, indicating that there is some involvement of NMA receptors at a pre-motoneuronal level. An example of such a result is given in Fig. 9. Of the twenty tests of ketamine $\leqslant$ 4 mg/kg performed on sixteen cells, nociceptive pinch or heat responses were reversibly reduced to below 50 % of control with twelve tests on eight cells.

PDA was tested micro-electrophoretically on six of the above cells. As expected, it reduced responses to amino acids. It was more effective than electrophoretic ketamine at reducing synaptic responses, as illustrated in Fig. 10, but the nociceptive responses were still only reduced with currents of PDA higher than those required to reduce or block responses to the amino acids; with the cell shown in Fig. 10, PDA at half the current still blocked responses to NMA whilst having only weak effects on the synaptic responses.

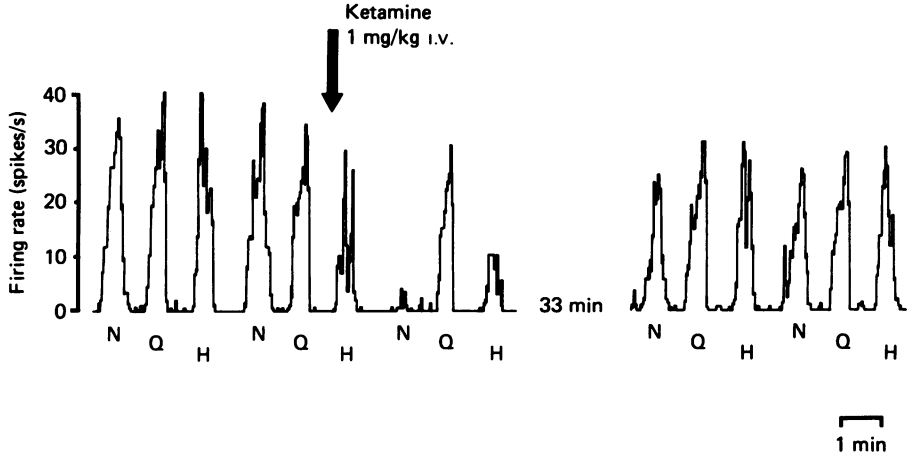

Fig. 9. Parallel reduction by ketamine of responses to electrophoretic NMA (N, 22 nA) and to a noxious heat ramp to the main footpad (H, 36–54·5 °C) whilst responses to quisqualate (Q, 10 nA) remained substantially unaffected. Firing rate of a neurone on the border between lamina VII and VIII in a decerebrate spinalized cat.

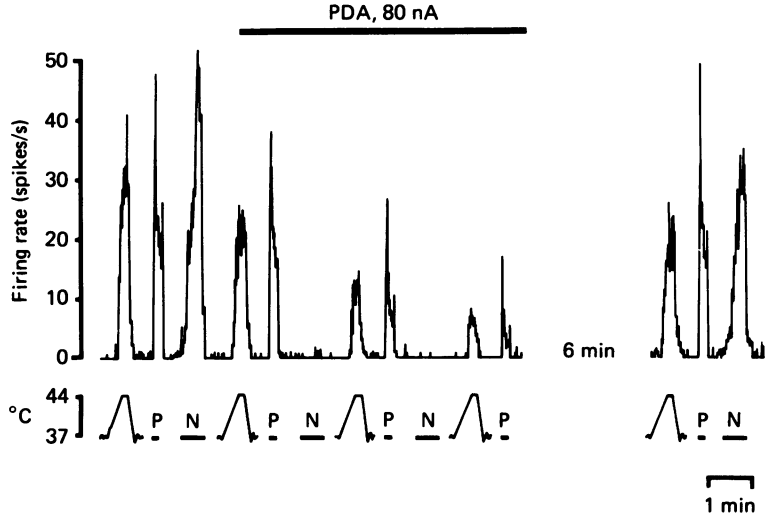

Fig. 10. Effects of the non-selective amino acid antagonist PDA on a lamina VII neurone responding to noxious heat of toe pad 4 (skin temperature ramped over 32 s from base line 37 to 44 °C where it was held for 8 s); to noxious pinch to the base of toe 5 (P, 10 s duration); and to electrophoretic NMA (N, 90 nA). Higher currents of PDA were required to affect synaptic responses than responses to electrophoretic amino acids. Decerebrate spinalized cat.

DISCUSSION

The above results indicate that the responses of many ventral horn neurones (including motoneurones) are reduced by low, subanaesthetic doses of ketamine but that responses of other ventral horn and of all dorsal horn neurones tested are relatively much more resistant to this dissociative anaesthetic.

The interpretation of these results in terms of amino acid receptor involvement in the mediation of the relevant responses depends on the assumption that ketamine is acting in this situation as a selective antagonist of actions mediated at NMA receptors. At the intravenous doses used, ketamine clearly selects between NMA-induced excitation and that induced by quisqualate or kainate (Anis *et al.* 1983; and see Fig. 9) and ketamine has been shown to be similarly selective in a variety of isolated tissue preparations (Harrison & Simmonds, 1985; Martin & Lodge, 1985; Thomson, West & Lodge, 1985). Ketamine does, however, have a variety of other effects on neurotransmitter systems (see review by White *et al.* 1982) and so the possibility arises that ketamine might be affecting synaptic responses via some of these other actions. It is generally accepted, however, that the parallel reduction of a neurotransmitter analogue and of a synaptic event is a strong argument in favour of a physiological role for the relevant receptor type in that synaptic event. We have performed such tests and have seen parallel reductions with some lamina VII and VIII neurones (see Fig. 9). The constancy of the intravenous dose required to reduce responses to NMA implies that it is not necessary to perform these technically difficult tests on all cells. Based on these arguments we interpret our results to mean that NMA receptors mediate some step in the synaptic pathway giving rise to ketamine-sensitive responses.

Dorsal horn

In the dorsal horn we found no evidence for NMA receptor mediation of nociceptive responses to peripheral pinch or heat. Ketamine is analgesic in man at subanaesthetic doses (see White *et al.* 1982) but this analgesia would seem not to be related to antagonism of NMA receptor-mediated events in the dorsal horn; moreover even full anaesthetic doses of ketamine have minimal effects on dorsal horn nociceptive responses (P. M. Headley & D. C. West, in preparation). This lack of effectiveness of ketamine on dorsal horn nociceptive responses is supported by its ineffectiveness on cardiovascular responses to noxious stimuli, as illustrated in Fig. 8. It is also consistent with the ineffectiveness of micro-electrophoretically administered D-α-aminoadipate (Davies & Dray, 1979).

Similarly we found no evidence for NMA receptor involvement in non-nociceptive responses of dorsal horn neurones. This result is consistent with results obtained in trigeminal dorsal horn with D-aminoadipate (Hill & Salt, 1982). There is, however, evidence that NMA antagonists can reduce the responses of spinal neurones to low-intensity mechanical (Davies & Dray, 1979) and electrical (Davies & Watkins, 1979, 1983; Lodge *et al.* 1983) activation of primary afferents. The reasons for the disparity are unclear. It may well be that in the electrical stimulation studies, the activation was of other types of afferent fibre than those examined in the present study; but Davies & Dray (1979) used similar stimulus parameters and preparations to ourselves, although their cats were anaesthetized with pentobarbitone. We have performed only one test on non-nociceptive responses in a pentobarbitone-anaesthetized cat; in this case ketamine, 2·5 mg/kg, clearly reduced responses to NMA but was without detectable effect on the responses to airjet deflexion of pedal hairs.

The non-selective amino acid antagonist *cis*-2,3-piperidine dicarboxylate (PDA)

was less effective in our experiments on spinal dorsal horn neurones than in previous studies on spinal (Davies & Watkins, 1983) and trigeminal neurones (Salt & Hill, 1981, 1983; Hill & Salt, 1982). In this series we have not tested PDA on heat-evoked responses which have previously been reported to be PDA resistant (Salt & Hill, 1983). Although we have recorded neurones on which responses to noxious and non-noxious mechanical stimuli were sensitive to PDA (see Fig. 2), with most cells we saw only partial and non-selective reductions of responses with currents as high as 200 nA. These latter results are difficult to interpret. Although PDA at high currents is still selective between amino acids and acetylcholine or substance P (Davies, Evans, Francis, Jones & Watkins, 1981; and for discussion see Salt & Hill, 1983) there are two possible interpretations when all the responses of a neurone are reduced by this antagonist: either amino acid receptors mediate the responses in question or they mediate other inputs to the cell, block of which could reduce cell excitability and hence all the synaptic responses tested. Despite these complications, our observation that on some cells PDA (but not ketamine) reduced responses, whilst having relatively little effect on spontaneous firing, is most readily interpreted as indicating non-NMA amino acid receptor mediation of both non-nociceptive and nociceptive responses to peripheral mechanical stimuli.

Ventral horn

The lack of ketamine's effectiveness on dorsal horn neurones is in sharp contrast to its ability to reduce responses of both spinal lamina VII and VIII neurones and motoneurones. This finding shows some similarities to the results of various behavioural investigations. Thus intrathecally administered NMA antagonists elicited only weak anti-nociception yet caused clear motor weakness in some (Tung & Yaksh, 1981; Cahusac, Evans & Rodriguez, 1983) though not all (Ahuja, 1983) reports. Systemically administered NMA antagonists have also been reported to have muscle relaxant properties (Turski, Schwarz, Turski, Klockgether, Sontag & Collins, 1985). Moreover there is neurochemical and electrophysiological evidence that aspartate is both more prevalent and more effective in the ventral horn than in the dorsal horn (for discussion see Biscoe, Headley, Lodge, Martin & Watkins, 1976).

In our experiments motoneuronal nociceptive responses to both mechanical and thermal stimuli were equally affected by NMA-antagonizing doses of ketamine whereas on the very small sample on which non-nociceptive responses were tested concurrently, the latter were relatively less affected. Such relative effectiveness is, however, superimposed on a considerable inter-cell variability in sensitivity to ketamine. We interpret this as implying a variable activation of pathways utilizing NMA receptors but we have no evidence as to the source of this variation. One obvious potential source of variation would be between motoneurone cell types, but for reasons outlined in 'Methods' we have not investigated this possibility.

Despite these reservations it is clear that with most (but not all) motoneurones, NMA receptors mediate some stage in the transmission of nociceptive and some other information. It is less clear precisely which neurones these NMA-receptor-mediated synapses may be, although at least some of them are pre-motoneuronal. Whenever tested, micro-electrophoretically administered ketamine failed to affect lamina VII and VIII neuronal responses even when intravenous ketamine fully blocked res-

ponses. Since the location of the relevant synapses on the cell is not known, such negative results with *locally* administered antagonists cannot be interpreted in terms of which cell has those NMA receptors which are affected by *systemic* ketamine; for although the NMA receptors are presumably not on the soma or proximal dendrites of the cell under study (in that this is where the recording pipette is most likely to be) they may still be on more distal dendrites of that neurone; or they may alternatively be on cells earlier in the pathway. For equivalent reasons it has not seemed worth attempting micro-electrophoretic administration of antagonists to motoneurones, particularly in view of the length of their dendrites.

As in the dorsal horn, PDA was more effective at reducing lamina VII and VIII neuronal sensory responses than was micro-electrophoretic ketamine; but similar reservations apply to the interpretation of this finding as were discussed above in relation to dorsal horn results.

Using 'natural' peripheral stimuli we have thus identified a role for NMA receptors in nociceptive and non-nociceptive pathways in the ventral but not the dorsal horn. This involvement applies to pathways activated by thermal and mechanical noxious and some mechanical somatic non-noxious stimuli. It remains to be established whether NMA receptors are selectively distributed in projections to particular cell groups; and whether these receptors mediate responses to other non-noxious as well as visceral stimuli.

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