Br. J. Pharmacol. (1988), **94**, 443–448

Antagonism of N-methylaspartate and synaptic responses of neurones in the rat ventrobasal thalamus by ketamine and MK-801

[1]T.E. Salt, D.G. Wilson & S.K. Prasad

Department of Physiology, University College, P.O. Box 78, Cardiff CF1 1XL

1 Extracellular single neurone recordings were made in the ventrobasal thalamus of urethane-anaesthetized rats.

2 Iontophoretically and intravenously administered ketamine and MK-801 were found to be selective antagonists of responses of neurones to iontophoretically applied N-methylaspartate.

3 Both antagonists, administered in N-methylaspartate-selective quantities, reduced the synaptic responses of ventrobasal thalamus neurones to a two-second air jet directed at the peripheral receptive field.

Introduction

Excitatory amino acid neurotransmitters can interact with one or more receptors characterized by the agonists kainate, quisqualate, and N-methyl-D-aspartate (NMDA). Both NMDA receptors and non-NMDA receptors have been implicated in synaptic transmission in various regions of the central nervous system (Watkins & Evans, 1981). In particular, somatosensory afferent transmission to the ventrobasal thalamus appears to involve both NMDA and non-NMDA receptors (Salt 1986; 1987). Recently, the dissociative anaesthetics, such as ketamine, and the anticonvulsant, MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine maleate), have been described as non-competitive NMDA receptor antagonists (Anis *et al.*, 1983; Harrison & Simmonds, 1985; Wong *et al.*, 1986). We have investigated the ability of both iontophoretically and systemically administered ketamine and MK-801 to antagonize responses of ventrobasal thalamus neurones to N-methylaspartate (NMA) and stimulation of sensory afferents. Preliminary communications of this work have been made (Salt & Wilson, 1987; Prasad & Salt, 1987).

[1] Present address: Department of Visual Science, Institute of Ophthalmology, Judd Street, London WC1.

Methods

Preparation

Experiments were performed on albino rats of either sex, weighing between 300 g and 400 g. The animals were prepared for single neurone recording and iontophoresis, under urethane anaesthesia (1.2 g kg^{-1}, i.p.), as previously described (Salt, 1987). The rats' heads were held in a stereotaxic frame with the incisor bar in the same horizontal plane as the ear bars. Five-barrelled glass microelectrodes were introduced into the ventrobasal thalamus at a point 4.9 mm anterior to the skull landmark, *lambda*, and 2.9 mm lateral to the mid-line suture. Extracellular recordings were made through one barrel of the electrodes which contained pontamine sky blue dye in 0.5 M NaCl/0.5 M Na acetate. One of the barrels contained 1 M NaCl (for automatic current balancing), and each of the remaining three barrels contained a drug solution for iontophoresis. Retaining currents of 15–20 nA, opposite direction to ejection, were used to prevent diffusion of drugs from the microelectrode.

Recording

Action potentials were gated by a window discriminator and timed by a computer system which could generate histograms of neural activity. This system

Table 1 Effects of iontophoretically-applied MK-801 and ketamine on responses to iontophoretically-applied agonists and to air jet stimulation

		% *reduction of response* (mean ± s.d.)			
Antagonist	n	*NMA*	*Kainate*	*Quisqualate*	*Air jet*
(A) MK-801	14	77 ± 28	17 ± 16		58 ± 24
	13	66 ± 13		11 ± 23	40 ± 25
(B) Ketamine	12	76 ± 20	12 ± 11		48 ± 14
	9	79 ± 10		16 ± 8	51 ± 14

NMA = N-methylaspartate.

also controlled the timing of iontophoretic agonist ejections and sensory stimulation. These were applied in regular cycles of between 90 and 180 s duration. Sensory stimulation was achieved using an electronically gated air jet which could be directed at the hairs or vibrissa(e) which comprised the receptive field of the neurone under investigation. Such

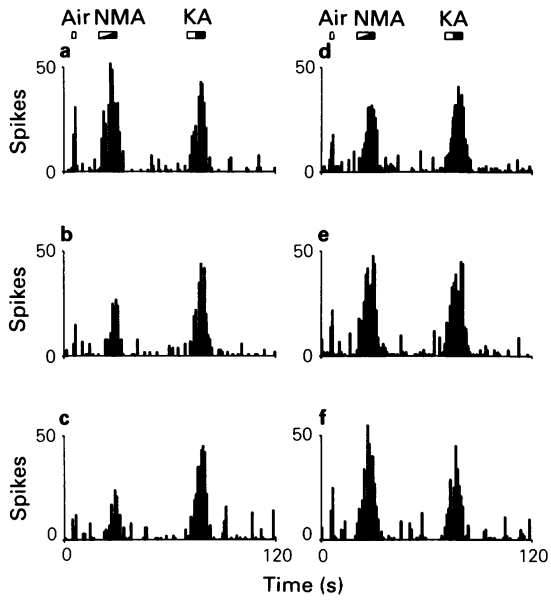

Figure 1 Peri-stimulus time histograms of action potentials counted into 1000 ms time epochs (bins). The marker bars above the upper two records indicate air jet stimulation (Air) and iontophoretic ejection of N-methylaspartate 5 nA (NMA) and kainate 35 nA (KA). These markers apply to all six records. (a) Control, (b) a record taken 5 min after the start of a continuous iontophoretic ejection of MK-801 (1 nA). Note that the antagonist reduced responses to air jet stimulation and NMA whilst having little effect on the response to kainate. (c–f) Records taken 10, 20, 35 and 50 min, respectively, after the end of MK-801 ejection. Note the progressive recovery of the responses to air jet stimulation and NMA.

stimuli, of two seconds duration, were used in all experiments and were followed by iontophoretic ejections of NMA and either kainate or quisqualate. Response magnitudes were calculated as the number of action potentials evoked by a given stimulus, and the effects of either ketamine or MK-801 were evaluated by making a concurrent iontophoretic ejection of the antagonist once stable responses to cycles of iontophoretically applied agonists and the air jet stimulus had been achieved. Wherever possible, multiple antagonist ejections (with different currents) were made in order to determine the current of ketamine or MK-801 which produced the most selective antagonism of NMA compared to either kainate or quisqualate. The effects of systemic MK-801 or ketamine administration were evaluated by intravenous injection of a bolus of drug in 0.9% saline via a cannula in the femoral vein.

Drugs

Solutions for iontophoresis were made up as follows: Na N-methyl-D,L-aspartate (0.1 M, pH 8.0), Na kainate (50 mM in 150 mM NaCl, pH 8.0), Na quisqualate (50 mM in 150 mM NaCl, pH 8.0), ketamine HCl (50 mM in 150 mM NaCl, pH 4.0), MK-801 (5 mM in 150 mM NaCl, pH 4.0). Excitatory amino acid agonists and urethane were purchased from Sigma Chemicals. Ketamine HCl and MK-801 were generous gifts from Parke-Davis Veterinary (Pontypool) and Merck, Sharp & Dohme (Harlow), respectively.

Results

MK-801

The effects of iontophoretically applied MK-801 were investigated on 27 neurones, using currents of 0 nA (retaining current switched off) to 20 nA. It was found that responses to iontophoretically applied NMA were reduced, on average, by 72% of control values, whilst responses to either kainate (14

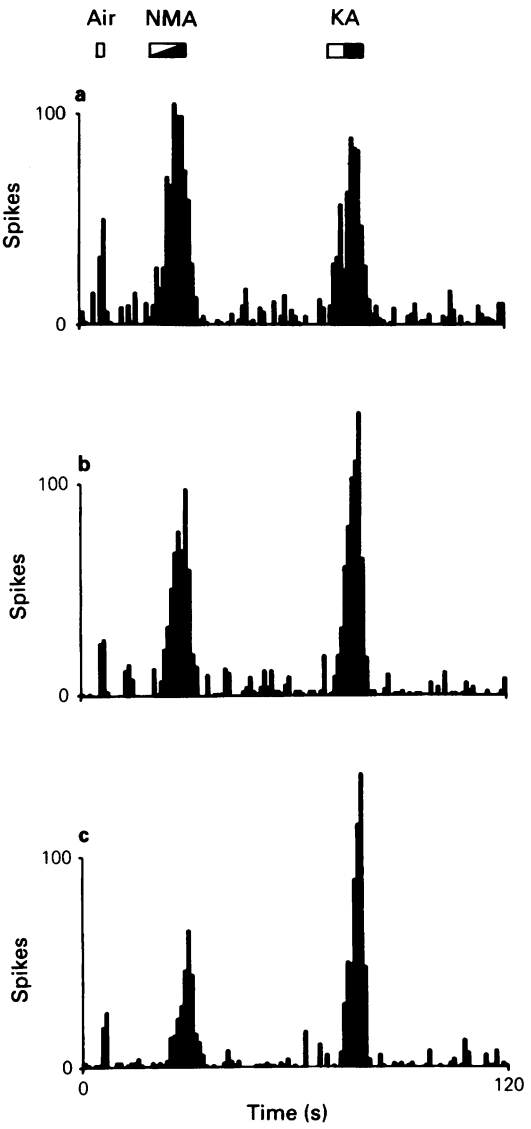

Figure 2 Data from the same neurone as in Figure 1. As in Figure 1 the marker bars indicate air jet stimulation (Air) and iontophoretic ejection of N-methyl-aspartate 5 nA (NMA) and kainate 35 nA (KA), but each histogram is a cumulative record of two successive cycles of stimuli. Three intravenous injections of MK-801 were made, separated by 10 min. (a) Control, taken after the study with iontophoretic MK-801 had been completed. (b) Six minutes following the second of two intravenous doses of 0.1 mg kg^{-1} MK-801 (cumulative dose of 0.2 mg kg^{-1}); responses to air jet stimulation and NMA were reduced. (c) Six minutes after a further injection of 0.2 mg kg^{-1} MK-801 (cumulative dose of 0.4 mg kg^{-1}); responses to the air jet and NMA were reduced further.

neurones) or quisqualate (13 neurones) were less affected. At the same time, responses to air jet stimulation were also reduced by an average of 49%. Recovery from these effects was seen in 19 of the neurones studied. These results are summarized in Table 1A, and an example is shown in Figure 1. It is noteworthy that, with MK-801, it was difficult to obtain large reductions in NMA responses without also producing some reduction of responses to kainate or quisqualate. Furthermore, recovery from the effects of the antagonist was prolonged, typically in the region of 20 to 50 min (Figure 1).

The effects of intravenously given MK-801 (0.1–0.4 mg kg^{-1}) were studied on seven neurones, five of which had already been studied with iontophoretically applied MK-801. Effects were generally seen after a period of five to ten minutes, and cumulative doses were spaced accordingly (Figure 2). As with iontophoretically administered antagonist, responses to NMA and air jet stimulation were reduced more than responses to either kainate or quisqualate (Table 2A). Recovery from the effects of intravenously administered MK-801 was not seen, even though recordings were maintained for up to three hours following antagonist injection, during which time responses to either kainate or quisqualate were still observed.

Ketamine

Ketamine, ejected with iontophoretic currents of between 0 nA and 15 nA, was found to antagonize responses to NMA and air jet stimulation by an average of 77% and 49% of control values, respectively, whilst responses to either kainate (12 neurones) or quisqualate (9 neurones) were relatively unaffected (Figure 3). These results are summarized in Table 1B. Recovery from the effects of iontophoretically applied ketamine appeared to be more rapid than with MK-801, typically in the region of 5 to 15 min.

Ketamine was administered intravenously (2.25–10.0 mg kg^{-1}) in the study of five neurones, four of which had already been investigated with iontophoretically applied ketamine. Similar effects to those seen with iontophoretic administration were obtained (Figure 4, Table 2B). The effects of ketamine were more rapid in onset than those of MK-801, and recovery was seen 15 to 45 min following the injection (Figure 4).

Discussion

The results presented here show that both ketamine and MK-801 are selective NMDA antagonists in the ventrobasal thalamus: this is consistent with pre-

Table 2 Effects of intravenous MK-801 and ketamine on responses to iontophoretically-applied agonists and to air jet stimulation

Antagonist	n	% *reduction of response* (mean ± s.d.) *NMA*	*Kainate/Quisqualate*	*Air jet*
(A) MK-801	7	70 ± 18	11 ± 22	39 ± 21
(B) Ketamine	5	85 ± 15	2 ± 4	56 ± 20

vious work in different brain areas by other workers (Anis *et al.*, 1983; Harrison & Simmonds, 1985; Aram *et al.*, 1986; Wong *et al.*, 1986). Furthermore, it is apparent that both antagonists, administered either iontophoretically or intravenously, can reduce synpatic responses to sensory stimulation during conditions of selective NMA antagonism. This is consistent with the previous finding from this laboratory, that sensory input to ventrobasal thalamus neurones can be blocked by the competitive NMDA antagonist D-2-amino-5-phosphono-valerate (APV) (Salt, 1986; 1987). However, it is noteworthy that, in the ventrobasal thalamus, both ketamine and MK-801 appear not to be as selective for NMA as APV

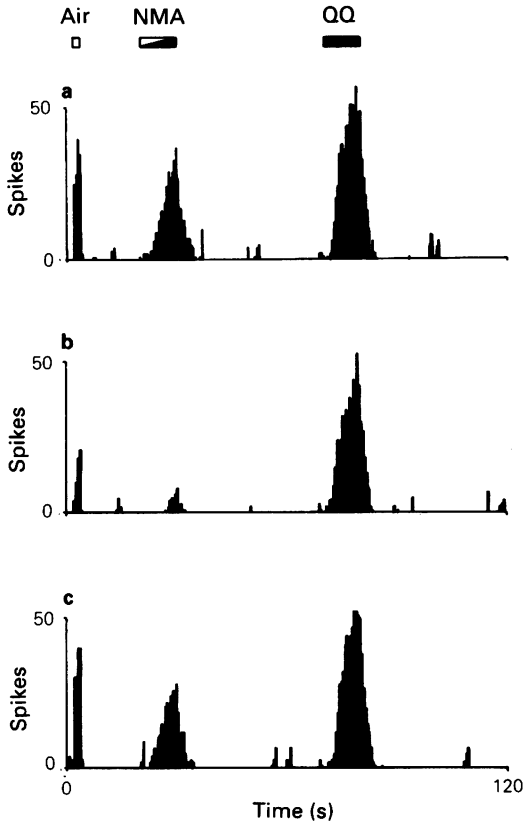

Figure 3 Responses of a neurone to air jet stimulation (Air) and iontophoretic application of N-methyl-aspartate 3 nA (NMA) and quisqualate 42 nA (QQ). Each histogram is cumulative over two trials. (a) Control; (b) responses during the continuous iontophoretic ejection of ketamine (10 nA for 160 s); responses to the air jet stimulus and NMA were reduced. (c) Recovery from the effects of ketamine, record taken 12 min after the end of the ejection of ketamine.

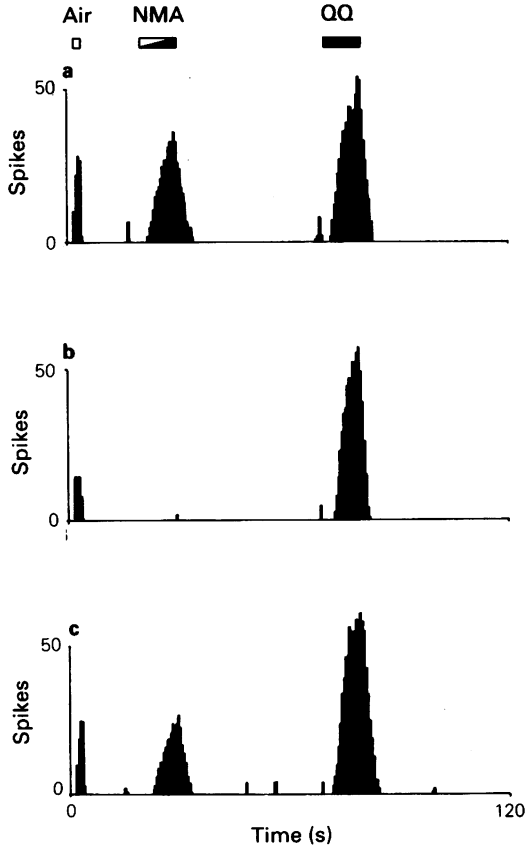

Figure 4 Similar records to those shown in Figure 3, taken from the same neurone. (a) Control; (b) the effects of a single intravenous injection of 5 mg kg^{-1} ketamine, 160 s following injection. (c) A substantial degree of recovery from the effects of ketamine 40 min after the injection.

was found to be under similar experimental conditions (Salt, 1987).

Both ketamine and MK-801, given intravenously at doses that are less than or similar to anaesthetic or anticonvulsant doses, respectively (Lawrence & Livingston, 1981; White *et al.*, 1982; Clineschmidt *et al.*, 1982), were found to reduce synaptic input to ventrobasal thalamic neurones, although this reduction was less pronounced than the reduction of NMA responses. It is of interest that the effects of both antagonists when given intravenously were similar to their effects on responses to NMA and responses to air jet stimulation when applied iontophoretically. This suggests that iontophoretically applied ketamine or MK-801 is able to penetrate synaptic receptors to a similar extent as are intravenously administered ketamine and MK-801. Furthermore, this similarity of effect suggests that ketamine and MK-801, when given intravenously, are not reducing sensory input to the thalamus by an action at a remote site, for example earlier in the sensory pathway. It is also noteworthy that, on some occasions, intravenous ketamine or MK-801 was virtually able to antagonize completely responses to iontophoretically applied NMA but did not antagonize the responses to the air jet stimulus to such a large extent (Figure 4). This suggests that NMDA receptors are not the only type of receptor involved in the responses to air jet stimulation, and indeed, it appears that there is involvement of non-NMDA excitatory amino acid receptors (Salt, 1987).

It is noteworthy that similar doses of ketamine to those used here were found to be effective against NMA responses but not sensory input in the dorsal horn of the spinal cord of rats and cats (Conseiller *et al.*, 1972; Headley *et al.*, 1987), and this would tend to argue against the dorsal horn as a major target site in ketamine anaesthesia. In contrast, the results presented here, showing that ketamine can antagonize sensory responses in the ventrobasal thalamus (a major supraspinal sensory relay nucleus) allows the possibility that some of the anaesthetic action of ketamine may be due to antagonism of thalamic synaptic NMDA receptors. It is, however, highly likely that the ventrobasal thalamic NMDA receptors are not the only substrate for the anaesthetic action of ketamine, and indeed, an interaction of this drug with cortical synaptic NMDA receptors has been demonstrated (Thomson, 1986).

MK-801 is not the only NMDA antagonist which has anticonvulsant actions (Meldrum, 1985), and indeed it has been reported that ketamine is an anticonvulsant (Reder *et al.*, 1980). Whether or not an interaction of these drugs with thalamic synaptic NMDA receptors is of major importance in determing anticonvulsant action remains to be elucidated, but it does seem possible that such an interaction could be a contributory factor.

We wish to thank Mrs V.E. Walker for her skilled technical assistance. This work was supported by the Medical Research Council.

References

ANIS, N.A., BERRY, S.C., BURTON, N.R. & LODGE, D. (1983). The dissociative anaesthetics ketamine and phencyclidine selectively reduce excitation of central mammalian neurones by N-methylaspartate. *Br. J. Pharmacol.*, **79**, 565–575.

ARAM, J., CHURCH, J., DAVIES, S.N., LODGE, D. & MARTIN, D. (1986). Comparison of phencyclidine, thienylcyclohexylpipridine and MK-801 as NMDA antagonists on rat spinal and cortical neurones. *Br. J. Pharmacol.*, **89**, 778P.

CLINESCHMIDT, B.V., MARTIN, G.E. & BUNTING, P.R. (1982). Anticonvulsant activity of (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine (MK-801), a substance with potent anticonvulsant, central sympathomimetic and apparent anxiolytic properties. *Drug Devel. Res.*, **2**, 123–134.

CONSEILLER, C., BENOIST, J.M., HAMANN, K.F., MAILLARD, M.C. & BESSON, J.M. (1972). Effects of ketamine (CI 581) on cell responses to cutaneous stimulations in laminae IV and V in the cat's dorsal horn. *Eur. J. Pharmacol.*, **18**, 346–352.

HARRISON, N.L. & SIMMONDS, M.A. (1985). Quantitative studies on some antagonists of N-methyl-D-aspartate in slices of rat cerebral cortex. *Br. J. Pharmacol.*, **84**, 381–391.

HEADLEY, P.M., PARSONS, C.G. & WEST, D.C. (1987). The role of N-methylaspartate receptors in mediating responses of rat and cat spinal neurones to defined sensory stimuli. *J. Physiol.*, **385**, 169–188.

LAWRENCE, D. & LIVINGSTON, A. (1981). Opiate-like analgesic activity in general anaesthetics. *Br. J. Pharmacol.*, **73**, 435–442.

MELDRUM, B. (1985). Possible therapeutic applications of antagonists of excitatory amino acid neurotransmitters. *Clin. Sci.*, **68**, 113–122.

PRASAD, S.K. & SALT, T.E. (1987). Interaction of MK-801 with NMDA receptors and synaptic transmission in the rat ventrobasal thalamus. *Br. J. Pharmacol. Proc. Suppl.*, **92**, 542P.

REDER, B.S., TRAPP, L.D. & TROUTMAN, K.C. (1980). Ketamine suppression of chemically induced convulsions in the two-day-old white leghorn cockerel. *Anesth. & Analgesia*, **59**, 406–409.

SALT, T.E. (1986). Mediation of thalamic sensory input by

both NMDA receptors and non-NMDA receptors. *Nature*, **322**, 263–265.

SALT, T.E. (1987). Excitatory amino acid receptors and synaptic transmission in the rat ventrobasal thalamus. *J. Physiol.*, **391**, 499–510.

SALT, T.E. & WILSON, D.G. (1987). Interactions of ketamine with N-methyl-D-aspartate receptors and synaptic transmission in the rat ventrobasal thalamus. *J. Physiol.*, **384**, 69P.

THOMSON, A.M. (1986). A magnesium-sensitive post-synaptic potential in the rat cerebral cortex resembles neuronal responses to N-methylaspartate. *J. Physiol.*, **370**, 531–549.

WATKINS, J.C. & EVANS, R.H. (1981). Excitatory amino acid transmitters. *Ann. Rev. Pharmacol. Toxicol.*, **21**, 165–204.

WHITE, P.F., WAY, W.L. & TREVOR, A.J. (1982). Ketamine – Its pharmacology and therapeutic uses. *Anesthesiology*, **56**, 119–136.

WONG, E.H.F., KEMP, J.A., PRIESTLY, T., KNIGHT, A.R., WOODRUFF, G. & IVERSEN, L.L. (1986). The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 7104–7108.

(Received September 29, 1987
Revised December 10, 1987
Accepted January 13, 1988)