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The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate

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1 The interaction of two dissociative anaesthetics, ketamine and phencyclidine, with the responses of spinal neurones to the electrophoretic administration of amino acids and acetylcholine was studied in decerebrate or pentobarbitone-anaesthetized cats and rats.

2 Both ketamine and phencyclidine selectively blocked excitation by N-methyl-aspartate (NMA) with little effect on excitation by quisqualate and kainate.

3 Ketamine reduced responses to L-aspartate somewhat more than those of L-glutamate; the sensitivity of responses to these two putative transmitters was between that to NMA on one hand and that to quisqualate or kainate on the other.

4 On Renshaw cells, ketamine and phencyclidine reduced responses to acetylcholine less than those to NMA but more than those to quisqualate or kainate. Dorsal root-evoked synaptic excitation of Renshaw cells was reduced to a greater extent than that following ventral root excitation.

5 Intravenous ketamine, 2.5–20 mg/kg, and phencyclidine, 0.2–0.5 mg/kg, also selectively blocked excitation of neurones by NMA.

6 Ketamine showed no consistent or selective effect on inhibition of spinal neurones by electrophoretically administered glycine or γ-aminobutyricacid (GABA).

7 The results suggest that reduction of synaptic excitation mediated via NMA receptors contributes to the anaesthetic/analgesic properties of these two dissociative anaesthetics.

Introduction

Ketamine, a cyclohexylamine, is used clinically as a general anaesthetic agent in man and some animal species. Its anaesthetic properties are characterized by good analgesia, maintenance of many protective reflexes and poor muscle relaxation (McCarthy, Chen, Kaump & Ensor, 1965; Weisbroth & Fudens, 1972; Glen, 1973). These properties of ketamine differ from other general anaesthetics (McCarthy *et al.*, 1965) and have led to ketamine along with phencyclidine and other related agents being classified as 'dissociative anaesthetics' (Corssen & Domino, 1966).

The neurophysiological basis of dissociative anaesthesia has been the subjecy of attention for some time (see Domino, 1964). Several reports suggest that polysynaptic reflexes are reduced to a greater extent than monosynaptic reflexes with little effect on synaptic inhibitions (Tang & Schroeder, 1973; Lodge & Anis 1982). These results differ from those seen with many other anaesthetic agents, which enhance central inhibitions (see Richards, 1980).

Ketamine depresses the activity of single neurones, whether spontaneous (Conseiller, Benoist, Hammann, MacMaillard & Besson, 1972), evoked by afferent nerve or natural stimulation (Conseiller *et al.*, 1972; Raja & Guyenet, 1982) or induced by glutamate (Sinclair & Tien, 1979; Raja & Guyenet, 1982). This neuronal depression is not however universal; the excitability of some neurones and some pathways appears to be relatively unaffected (Conseiller *et al*, 1972; Kitahata, Taub & Kosaka, 1973) or even increased (Winters, Ferrar-Allado, Guzman-Flores & Alcaraz, 1972). Such effects of ketamine on single neurones and reflex or evoked potentials may be explained by a selective action on particular types of excitatory synapse.

Relevant to this idea is the possibility that L-aspartate and L-glutamate are the neurotransmitters respectively of some spinal interneurones and of some primary afferents (see Curtis & Johnston, 1974; Watkins & Evans, 1981). Since ketamine appears not to affect the release or uptake of amino

acids from nervous tissue (Minchin, 1981) it seemed possible that ketamine exerted some of its central depressant effects by a postsynaptic antagonist action at excitatory synapses. Receptors for the two naturally occuring excitatory amino acids have been classified into 3 subgroups characterized by N-methyl-aspartate, quisqualate and kainate (McLennan & Lodge, 1979; Davies & Watkins, 1979; Watkins & Evans, 1981). N-methyl-aspartate (NMA) antagonists have been found to reduce selectively polysynaptic reflexes in isolated spinal cord preparations (Evans, Francis & Watkins, 1978; Padjen & Smith, 1980) and polysynaptic excitation of Renshaw cells (Biscoe, Davies, Dray, Evans, Martin & Watkins, 1978; Lodge, Headley & Curtis, 1978; Davies & Watkins, 1979). We therefore decided to test the action of ketamine and phencyclidine on excitation of spinal neurones by a series of acidic amino acids. Our results show that these dissociative anaesthetics depress excitation by NMA receptor agonists.

Methods

Experiments were performed on a total of 23 cats, weighing 2–3 kg, and 34 rats, weighing 180–560 g, of either sex. Anaesthesia was induced by the intraperitoneal injection of pentobarbitone 35 mg/kg for 20 cats and 45–50 mg/kg for all rats and was maintained by the intermittent infusion of pentobarbitone at a level at which conjunctival reflexes were weak or absent. Three of the cats were induced by the short acting steroid alphaxalone/alphadolone injected intramuscularly at an initial dose of 12 mg/kg, and then decerebrated by mid-collicular section of the brain stem and subsequent removal of the nervous tissue rostral to this section. The decerebrate cats were subsequently paralysed with gallamine triethiodide and artificially ventilated to give an end-tidal CO_2 of about 4%.

After cannulation of the trachea, a radial or jugular vein and a carotid artery, the spinal cord was exposed by removing the dorsal laminae of the lumbar vertebrae. The cord was sectioned in cats at the thoraco-lumbar junction but was left intact in rats. After incising the exposed dura mater, dorsal roots and/or ventral roots were sectioned and prepared for stimulation and/or recording as appropriate.

In some cats, combinations of the following left hind limb nerves were prepared for stimulation: including posterior biceps semitendinosus, gastrocnemius-soleus, flexor digitorum longus, plantaris, deep and superficial peroneal, sural and tibial nerves. The temperature of the animal and of the liquid paraffin pools associated with the laminectomy and the limb nerves were maintained near 38°C. Blood pressure was monitored continuously and experiments were terminated if systolic blood pressure fell consistently below 100 mmHg.

Extracellular recordings of action potentials from single neurones were made via the centre barrel (3.6 M NaCl) of seven barrel glass micro-electrodes of overall tip diameters 4–10 μm. Five of the outer barrels were used for administering drugs by microelectrophoresis (Curtis, 1964), automatic current balancing being effected through the sixth barrel (200 mM NaCl). The solutions used for electrophoresis were ketamine HCl (50 mM in 150 mM NaCl, pH 4.8), phencyclidine HCl (10 mM in 200 mM NaCl, pH 4.8), N-methyl-D-aspartate Na (NMA, 50 mM in 150 mM NaCl, pH 8.2), N-methyl-DL-aspartate Na (NMA, 200 mM, pH 8.1), quisqualate Na (5 mM in 200 mM NaCl, pH 7.8) kainate Na (5 mM in 200 mM NaCl, pH 8.2), L-glutamate Na (200 mM, pH 7.8), L-aspartate Na (200 mM, pH 8.0), L-homocysteate Na (LH, 200 mM, pH 7.7), α-amino-3-hydroxy-5-methyl-4-isoxazole propionate Na (AMPA, 10 mM in 200 mM NaCl, pH 7.5), 4-methyl-homoibotenate Na (4-MHI, 50 mM in 100 mM NaCl, pH 8.0), DL-α-aminoadipate Na (αAA, 200 mM, pH 7.8), 2-aminophosphonovalerate Na (APV, 50 mM in 150 mM NaCl, pH 7.6), acetylcholine Cl (ACh, 200 mM).

After suitable amplification, action potentials from single neurones were displayed on an oscilloscope along with the output of the spike height discriminator. The firing rate of the neurone was recorded continuously together with the electrophoretic ejecting currents used to produce approximately equal and submaximal responses to two or more excitants. Once reproduceable excitatory responses were established, the effects of ketamine or other drugs were tested by injecting them electrophoretically, or by ejecting them intravenously, without interrupting the cycle of excitant administration. Results were expressed as the percentage reduction of the excitatory response during the administration of the test substance in relation to the controls before and after its administration. Results from single cells on which the same combination of drugs was tested have been grouped together to show the overall effect of drugs on excitant responses. The mean together with its standard deviation are presented.

Results

Records showing the effect of ketamine or phencyclidine on excitatory responses to two or more excitants were obtained from 129 cats and 102 rat spinal neurones. Both ketamine and phencyclidine were found to depress responses of these neurones to NMA more than those to other excitants tested.

Ketamine and excitatory amino acids

In cats, the effects of ketamine on excitatory responses to NMA were compared with those to quisqualate on 55 cells, with those to kainate on 16 cells and those to both quisqualate and kainate on a further 14 cells. With ejecting currents of ketamine from 3 to 150 nA, responses to NMA were reduced by 78 ± 18% (mean ± s.d.), responses to quisqualate by 10 ± 12%, and to kainate by 9 ± 9%. On 12 rat spinal neurones, on which ketamine (5–20 nA) was tested against excitation by these three amino acids, responses to NMA were reduced by 65 ± 16%, those to quisqualate by 12 ± 13% and those to kainate by 12 ± 14%. Similar results were obtained with ketamine on 51 other neurones comparing quisqualate and NMA and on a further 21 neurones comparing kainate and NMA. There were no apparent differences in the effects of ketamine between decerebrate and pentobarbitone anaesthetized animals or between depression of the responses to the D-isomer or the racemic mixture of NMA. The effects of ketamine were occasionally accompanied by small reductions in the spontaneous firing rate although most of the neurones studied were not spontaneously active. At doses which just blocked responses to NMA there was usually little or no effect of ketamine on action potential amplitude or configuration. An example of the selectivity of ketamine as an NMA-antagonist is shown in Figure 1.

To control for the possibility that a combination of non-specific reduction in excitability, uneven distribution of agonists and antagonists and effects on transport processes might contribute to the selective NMA-antagonism described above, we have performed two other series of experiments.

Firstly, the effects of glycine or GABA were tested on responses of neurones to the above three excitatory amino acids. In neither case was there any evidence for a selective effect on responses to NMA.

Secondly, the effects of ketamine were compared on the action of NMA, which is not subject to inactivation by transport processes (Cox, Headley & Watkins, 1977) with those on the action of L-homocysteate (LH) an NMA receptor agonist (McLennan & Lodge, 1979) which is actively transported into cells (Cox *et al.*, 1977) and on the action of AMPA and 4MHI, two quisqualate receptor agonists which, unlike quisqualate (Lodge, Curtis, Johnston & Bornstein, 1980), are probably not actively

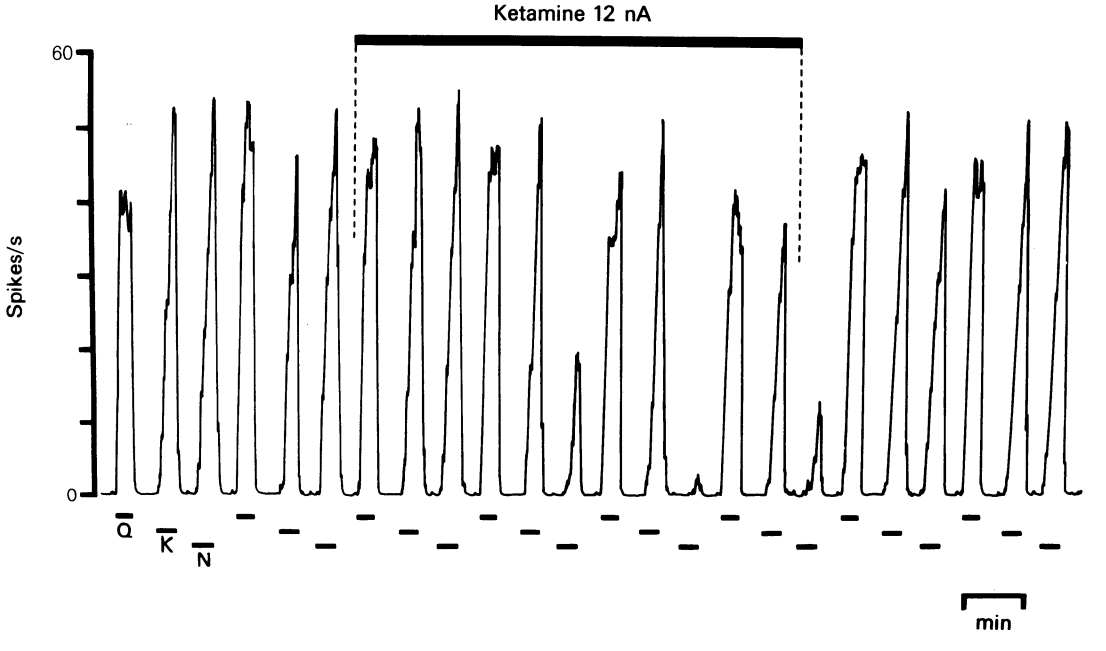

Figure 1 Effect of ketamine on excitation of a cat dorsal horn neurone by quisqualate, kainate and N-methyl-aspartate (NMA). The continuous record shows the firing rate of the neurone in response to the automatically-timed electrophoretic ejection of the three excitant amino acids, for the time indicated by the bar beneath the record. (Q, quisqualate 56 nA; K, kainate 35 nA and N, NMA 70 nA). Following control observations ketamine ejected with a current of 12 nA for 7.5 min, as indicated by the bar above the record, selectively reduced the excitant action of NMA. Full recovery of the response to NMA was observed 4.5 min after stopping the ejection of NMA. Ordinate scale: firing rate in spikes/s. Abscissa scale: time. Calibration bar = 1 min.

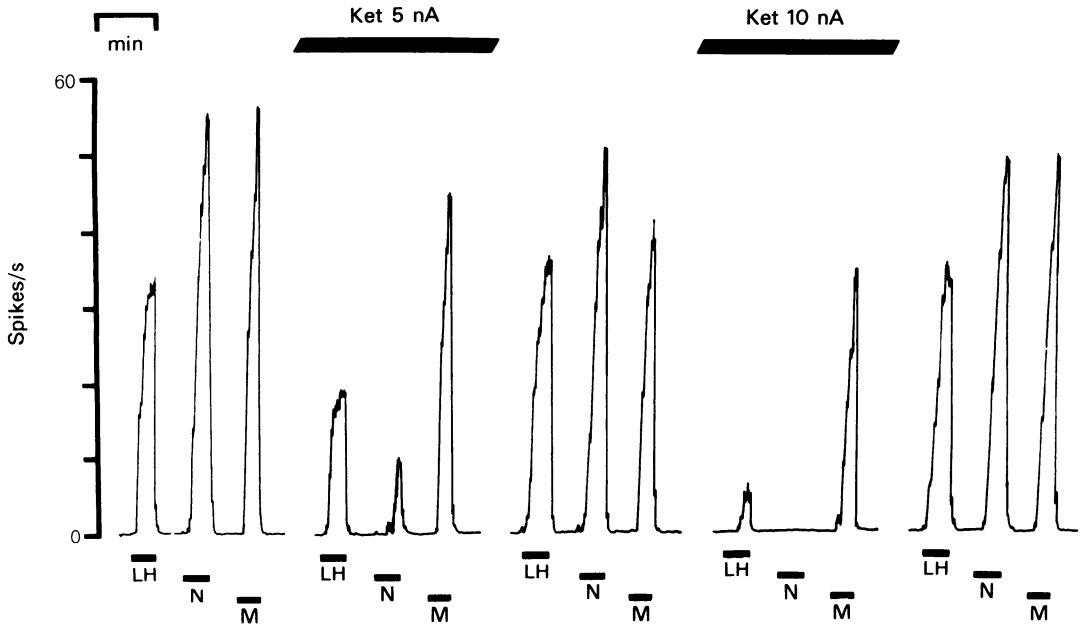

Figure 2 Effect of ketamine (Ket) on excitation of a cat dorsal horn neurone by L-homocysteate (LH), N-methyl-aspartate (NMA) and 4-methyl-homoibotenate (4-MHI). The record shows the firing rate of the neurone in response to the electrophoretic ejection of the three excitant amino acids as indicated by the bars beneath the record. Ketamine 5 nA ejected for 4–7 min and ketamine 10 nA ejected for 3–6 min, as indicated above the record reduced responses to NMA (N, 36nA) more than those to LH (48 nA), and those of LH more than those to 4-MHI (M, 28 nA). The centre panel shows responses 3–6 min after stopping the ejection of ketamine 5 nA and immediately before the ejection of ketamine 10 nA. Recovery, far right panel, to near control values occurred 5–8 min after ending the ejection of ketamine 10 nA. Ordinate scale: firing rate in spikes/s. Abscissa scale: time. Calibration bar = 1 min.

transported (Krogsgaard-Larsen, Honore, Hansen, Curtis & Lodge, 1980). Responses to LH (5 cells) were reduced to a somewhat lesser extent than those to NMA whereas responses to AMPA (5 cells) and 4MHI (11 cells) were only marginally reduced.

A result showing the effect of ketamine on excitation of a dorsal horn neurone by NMA, 4MHI and LH is presented in Figure 2. Such selectivity is very similar to that reported previously for α-amino adipate (McLennan & Lodge, 1979; Krogsgaard-Larsen *et al.*, 1980). On both cat and rat spinal neurones the reduction by ketamine of the responses to the two putative excitatory neurotransmitters, L-glutamate and L-aspartate, was variable. In general though responses to L-aspartate and L-glutamate were reduced less than those to NMA and more than those to quisqualate. For example, on 47 rat spinal neurones, ketamine (5–80 nA) reduced responses to L-aspartate by 55 ± 13% and those to L-glutamate by 36 ± 16%. Responses to L-aspartate were reduced to a greater extent than those of L-glutamate on 35 cells, to the same extent on 2 cells and to a less extent on 10 cells. On 23 neurones on which responses to NMA, aspartate, glutamate and quisqualate were compared, the action of NMA was reduced by 79 ± 19%, that of aspartate by 46 ± 20%, that of glutamate by 43 ± 25% and that of quisqualate by 15 ± 17%. Similar results were also obtained from experiments in the cat.

Recovery from the NMA-blocking effect of ketamine usually occured within 5–10 min of terminating the ketamine ejection.

Selectivity of excitatory amino acid antagonism

It is possible to get an idea of the selectivity of NMA antagonists by observing the effect of increasing the dose above that required to block NMA actions on responses to quisqualate and kainate. On several cells tested in this way in both cats and rats, increasing the ketamine ejection current five to ten fold had relatively little effect on the quisqualate or kainate responses. In most cases large reductions of quisqualate or kainate responses were only seen concomitant with reductions in action potential amplitude.

When compared with αAA on 11 cat cells and 2-aminophosphonovalerate (2-APV) on 9 cat cells and after allowance had been made for various drug

dilutions, ketamine was found to be approximately 4–6 fold and 0.3–2 fold more potent than αAA and 2-APV respectively. With regard to their selectivity as NMA antagonists, ketamine was somewhat more selective than αAA and about the same as APV on most cells tested.

An example of these actions of ketamine and αAA are shown in Figure 3 from which it can be seen that ketamine was a more selective NMA-antagonist than αAA.

On 18 rat spinal neurones on which the actions of αAA and ketamine were compared, ketamine (11–50 nA) reduced responses to L-aspartate by 58 ± 29% and those to L-glutamate by 32 ± 23% and αAA (11–25 nA) reduced responses to L-aspartate by 57 ± 24% and those to L-glutamate by 30 ± 26%. Using Scheffe's method for *post-hoc* contrasts ketamine and αAA discriminated significantly between aspartate and glutamate actions but there was no significant difference in selectivity between the effects of ketamine and αAA.

Does ketamine act at the same receptor as other N-methyl aspartate antagonists?

It is generally assumed that established organic NMA antagonists e.g. αAA and 2-APV act at the NMA receptor (Watkins & Evans, 1981). The amplitude of the effect of the concurrent administration of two antagonists relative to their effects when given separately should show whether they act at the same or different sites (Barlow, 1980). Accordingly we tested the effects in cats of combining the ejection of ketamine and αAA on NMA excitation: in no case was there clear evidence of a multiplicative effect. Usually the effects were somewhat less than additive, suggesting that ketamine and αAA may act at the same site. On 3 cells where it was attempted, ketamine appeared to shift to the right the NMA dose-response curve which retained a similar slope and maximal value.

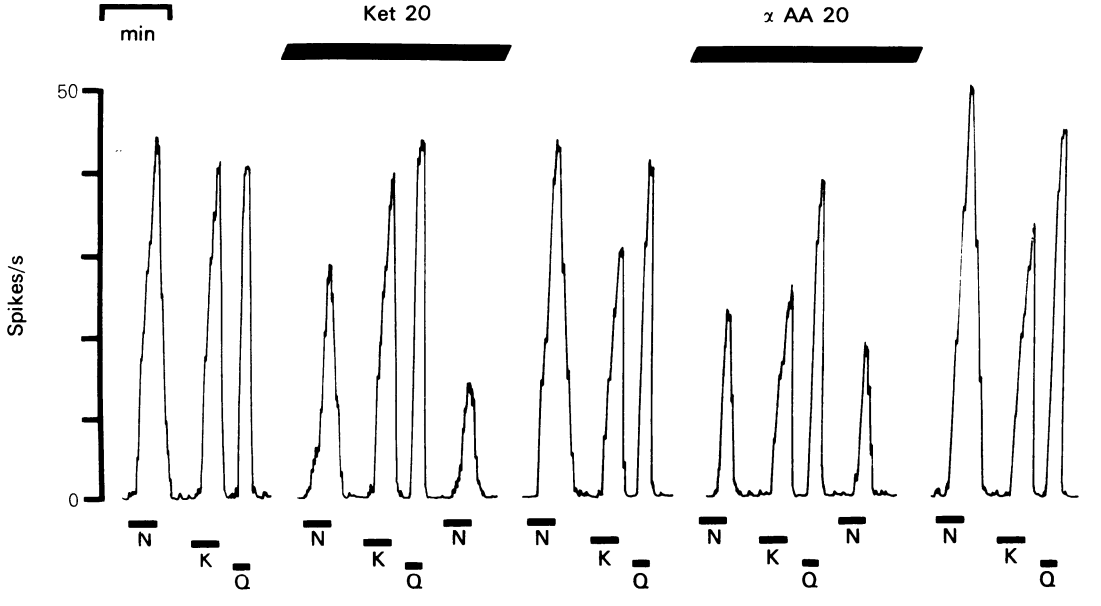

Figure 3 Comparison of selectivity and potency of ketamine (Ket) and DL-α-aminoadipate (αAA) as N-methyl-aspartate (NMA) antagonists. The record shows the firing rate of a dorsal horn neurone in response to the electrophoretic ejection of NMA (N, 63nA), kainate (K, 31 nA) and quisqualate (Q, 62 nA) for the times indicated below the record. The ejection of the two antagonists using the same current, 20 nA, and for the same period of time, 2–5 min, reduced the action of NMA by 60–70%. Such records when taking into account the drug dilutions in the electrodes (50 mM in 150 mM NaCl for ketamine and 200 mM for αAA) indicate that ketamine is approximately 4 times more potent than αAA. It should also be noted that the response to kainate, and to a less extent that of quisqualate, were also reduced during αAA ejection. Ordinate scale: firing rate in spikes/s. Abscissa scale: time. Calibration bar = 1 min.

Effect of ketamine on acetylcholine excitation of Renshaw cells

On 25 cat Renshaw cells, (±)-ketamine (2–30 nA) reduced responses to ACh by 37 ± 22% and those to NMA by 77 ± 18%. On no cat cells was the action of ACh reduced to a greater extent than that of NMA. On 14 of these neurones responses to quisqualate (11 cells) and kainate (3 cells) were reduced by 14 ± 9%.

Similar results were obtained on 21 rat Renshaw cells although the differences between ACh- and NMA-antagonism were less pronounced. Thus responses to NMA were reduced by 67 ± 20% and those to ACh by 53 ± 16%. On 6 other rat Renshaw cells responses to L-aspartate were reduced by 57 ± 13%, those to L-glutamate by 34 ± 16%, and those to ACh by 28 ± 11% by ketamine (12–25 nA) and on these same cells αAA (12–50 nA) reduced responses to L-aspartate by 35 ± 10%, those to L-glutamate by 22 ± 18%, and those to ACh by 2 ± 6%.

Phencyclidine: effects on cat spinal neurones

On 21 cat neurones including 6 Renshaw cells, phencyclidine (2–40 nA) reduced responses to NMA (21 cells) by 83 ± 19% whereas responses to quisqualate (14 cells) were reduced by 8 ± 11%, to kainate (9 cells) 11 ± 12% and to ACh (6 cells) by 33 ± 20%.

With 11 cells on which both ketamine and phencyclidine were tested, phencyclidine was approximately 10 times more potent than ketamine and showed very similar selectivity. Increasing the phencyclidine ejecting currents above those required to abolish responses to NMA often led to a reduction in action potential amplitude which was accompanied by reduction in the observed effects of the other excitants. This effect on action potential configuration, which was more evident than with ketamine, reversed rapidly whereas the NMA-blocking effect often required an hour or more to fully reverse.

An example of the selectivity of phencyclidine is presented in Figure 4. Ejected with a current of 8 nA, phencyclidine reduced only the NMA effect but at 12 nA, when the response to NMA was completely blocked, the action of ACh was reduced by almost 50%. Gradual recovery from the anti-cholinergic and anti-NMA actions was observed after stopping the phencyclidine ejection.

Effect of intravenous ketamine and phencyclidine

Ketamine (2.5–20 mg/kg) and phencyclidine (0.2–0.5 mg/kg) have been administered intravenously (i.v.) whilst recording responses of 12 cat spinal neurones to the electrophoretic administration of excitatory amino acids and ACh. NMA actions were

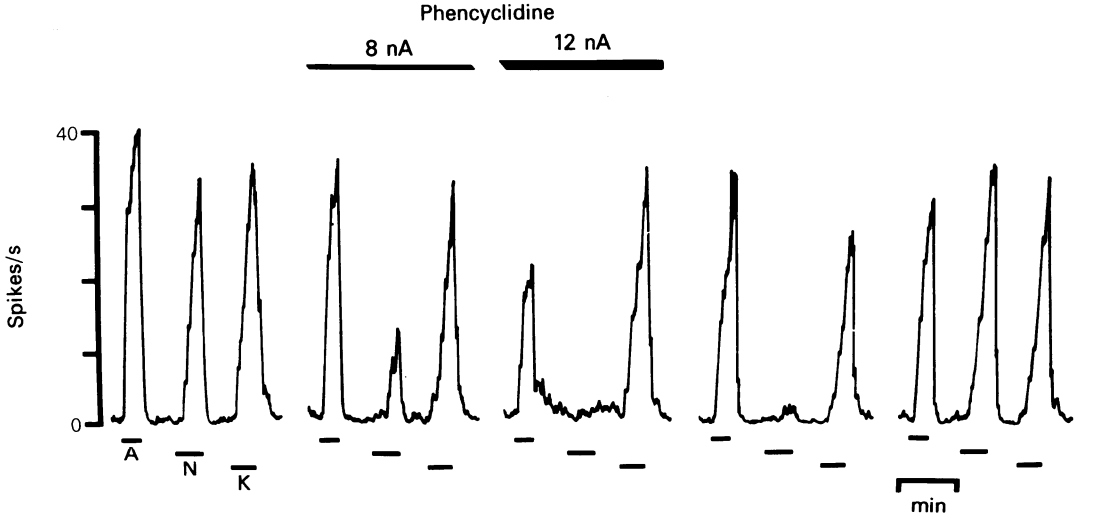

Figure 4 Action of phencyclidine on responses of a Renshaw cell to the electrophoretic ejection of acetylcholine (ACh), N-methyl-aspartate (NMA) and kainate (K). The records show the firing rate in response to the three excitants ejected at the times indicated. The ejection of phencyclidine 8 nA, 5–7.5 min selectively reduced the action of NMA (N, 25 nA) by 60% whereas at 12 nA, 6–8.5 min the response to NMA was abolished and that of ACh (A, 26 nA) reduced by 40–50%. Responses to kainate (K, 48 nA) were relatively unaffected by phencyclidine. Recovery from phencyclidine ejection was slow as can be seen in the panel second from the right, 8–10.5 min after the end of phencyclidine, full recovery being observed 30–32.5 min later, far right. Ordinate scale: firing rate in spikes/s. Abscissa scale: time. Calibration bar = 1 min.

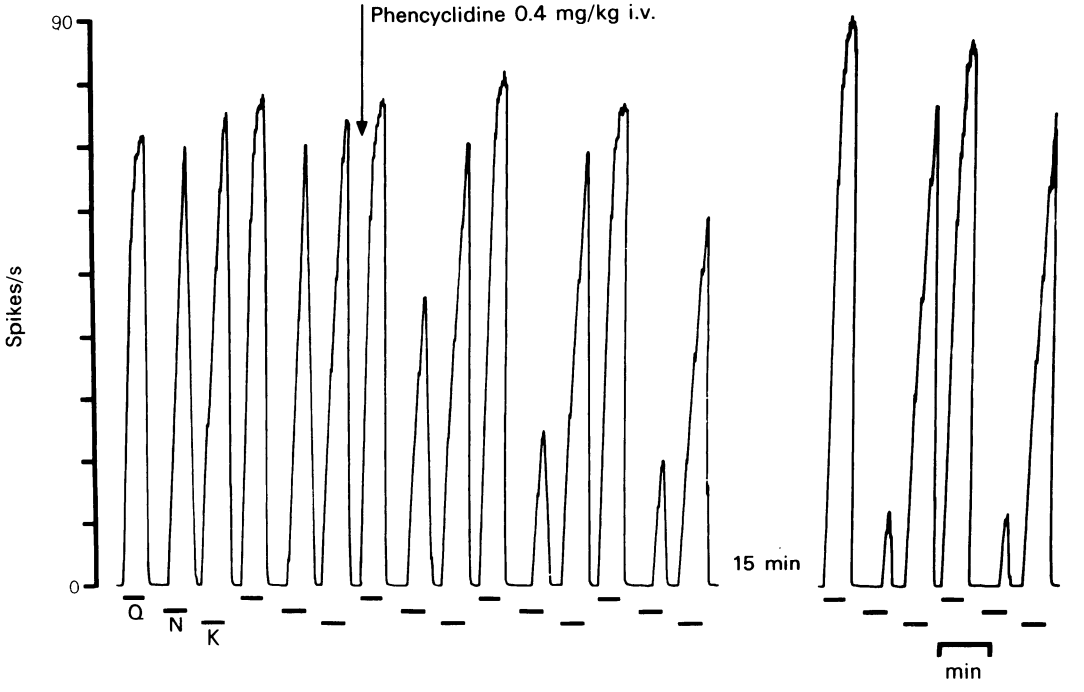

Figure 5 The effect of intravenous injection of phencyclidine, 0.4 mg/kg, on responses of a dorsal horn neurone to the electrophoretic ejection of quisqualate, N-methyl-aspartate (NMA) and kainate. The record shows the firing rate of the neurone in response to ejection of the excitants for the time indicated below. The injection of phencyclidine HCl (Sernylan; Bio-Ceutic Labs) was followed by the rapid and selective reduction of the response to NMA. Small changes in blood pressure and spike amplitude had disappeared 15 min later when responses to NMA (N, 50 nA) were still selectively reduced. Three hours later (not shown) the response to NMA was 70% of control, that of quisqualate (Q, 30 nA) 80% of control and that of kainate (K, 75 nA) 50% bigger than control. Ordinate scale: firing rate in spikes/s. Abscissa scale: time. Calibration bar = 1 min.

reduced by 87.5 ± 10% following i.v. ketamine and 72 ± 22% following i.v. phencyclidine. Responses to quisqualate and kainate were almost unaffected in all cases and excitation by ACh of 2 Renshaw cells following ketamine i.v. was reduced by 15 and 20%. Similar results were obtained in the rat following i.v. ketamine (1.0–7.5 mg/kg; 8 cells) and phencyclidine (0.4 mg/kg; 3 cells), responses to NMA being reduced by 87 ± 14%, those to quisqualate (7 cells) and kainate (3 cells) by 5 ± 16% and those to ACh by 54 and 31% (2 cells). Recovery toward control values was observed with ketamine during the following 10–120 min depending on dose. With phencyclidine recovery was much slower, taking several hours and rarely being complete. Changes in blood pressure were usually small and transient and on the basis of action potential amplitude and responses to kainate and quisqualate, movement of the microelectrode relative to the cell is unlikely to have contributed to the selective NMA-antagonism seen following i.v. administration. An example of the action of i.v. phencyclidine is presented in Figure 5. [For an equivalent result with ketamine see Figure 2 (Lodge, Anis & Burton, 1982)].

Effects of ketamine on synaptic excitations of spinal neurones

Renshaw cells receive excitatory inputs via afferents entering the dorsal root and from recurrent collaterals of ventral root motoneurone axons. Excitation following dorsal root (DR) stimulation has previously been shown to be sensitive to NMA antagonists (Biscoe *et al.*, 1978; Lodge *et al.*, 1978) whereas excitation from ventral root (VR) stimulation is reduced by ACh antagonists in both cat (Curtis & Ryall, 1966) and rat (Headley, Lodge & Biscoe, 1975). Ketamine administered electrophoretically or intravenously consistently reduced DR evoked responses of Renshaw cells to a greater extent than VR evoked responses. Such effects were consistent with reduction in responses to NMA and ACh respectively. Thus, in cats where the selectivity of ketamine between NMA and ACh actions is greater than in

rats, so the differential reduction of DR-, relative to VR-, evoked responses of 7 cat Renshaw cells was more pronounced than with 2 rat Renshaw cells.

On other interneurones, mostly in the dorsal horn, the effects of ketamine on excitations evoked by peripheral nerve stimulation were variable, on occasions synaptic excitations being clearly reduced although on other occasions very little effect was apparent even when ketamine was administered with currents in excess of those required to block NMA responses. Attempts to clarify this situation are now in progress and details will be published subsequently.

Ketamine and inhibitory amino acids

On 8 neurones in decerebrate and 3 in pentobarbitone-anaesthetized cats, ketamine was tested on inhibitory effects of glycine and GABA on spontaneous or kainate-induced activity. On none of the 11 cells was the inhibition by GABA selectivity enhanced as occurs with pentobarbitone (Lodge & Curtis, 1978): on 6 cells the actions of GABA and glycine were not clearly affected, on 4 they were both enhanced to a similar extent and on one cell both were reduced. On all 11 cells, selective NMA-antagonism was seen with ketamine at ejecting currents between 2 and 10 times less than those tested against the inhibitory amino acids.

Discussion

The major finding of the present study, that ketamine and phencyclidine selectively reduce responses of central neurones to NMA, has important implications both for the mode of action of general anaesthetics and the physiological role of NMA receptors.

Unlike anaesthetic barbiturates (e.g. Lodge & Curtis, 1978), ketamine did not selectively enhance the postsynaptic action of electrophoretically administered GABA. The result is consistent with the failure of dissociative anaesthetics to enhance long-lasting inhibitions (Tang & Schroeder, 1973; Lodge & Anis, 1982) which are enhanced by many other anaesthetic agents (see Richards, 1980).

The reduction by ketamine and phencyclidine of polysynaptic excitations in both spinal cord (Tang & Schroeder, 1973; Lodge & Anis, 1981) and higher brain centres (Dafny & Rigor, 1978; Ohtani, Kikuchi, Kitahata, Taub, Toyooka, Hanaoka & Dohi 1979; Angel & Gratton, 1982) may now be explicable in terms of the NMA antagonism reported here, since NMA receptors are thought to mediate synaptic excitations throughout the central nervous system (Curtis & Johnston, 1974; Watkins & Evans, 1981). The natural transmitter for these receptors is unknown although L-aspartate is a likely candidate (Curtis & Johnston, 1974; Biscoe *et al.*, 1978). The failure of ketamine and phencyclidine and also of other unrelated NMA-antagonists (e.g. Biscoe *et al.*, 1978; Lodge *et al.*, 1978), to block selectively L-aspartate-induced excitation can be explained in a variety of ways; L-aspartate may also act at non-NMA receptors, i.e. it has mixed agonist properties (see Curtis & Johnston, 1974; Watkins & Evans, 1981) or there may be uneven distribution of electrophoretically administered substances and of amino acid receptors (see Lodge *et al.*, 1978; Curtis, 1979). Our results are thus not inconsistent with those of Sinclair & Tien (1979) in which ketamine reduced both spontaneous and glutamate-evoked responses of cortical neurones but these authors made no comparison with other excitants. Reduction of spontaneous activity was not a consistent feature of our experiments.

The central effects of ketamine and related anaesthetics include dissociation of the somato-sensory cortex from other higher centres (Corssen & Domino, 1966; Weingarten, 1972). There is good evidence that excitatory amino acids act as transmitters in pathways to and from the cerebral cortex (see Watkins & Evans, 1981). In particular excitatory transmission in intracortical and cortico-caudate pathways is sensitive to D-α-amino adipate (Stone, 1976; 1979) and therefore presumably NMA-receptor block by ketamine could 'dissociate' the somato-sensory cortex.

Ketamine and phencyclidine also have anti-cholinergic properties (Tsai, Albuquerque, Aronstam, Eldefrawi, Eldefrawi & Triggle, 1980) which may contribute to their anaesthetic effects (see Krnjević, 1974). Studies with the two optical isomers of ketamine (Lodge *et al.*, 1982) suggest that the (+)-isomer is approximately 3 times more potent than the (−)-isomer as an NMA-antagonist and only 1–1.5 times as potent as an ACh-antagonist. Since in anaesthetic/analgesic tests (Marietta, Way, Casta & Trevor, 1977; Ryder, Way & Trevor, 1978;) the (+)-isomer is 2–4 times more potent than the (−)-isomer, it appears that the NMA-antagonistic effects are likely to be related to these clinically useful properties of dissociative anaesthetics. Similar potency ratios, however, were obtained for the isomers as inhibitors of noradrenaline uptake *in vitro* (Smith, Azzaro, Zaldivar, Palmer & Lee, 1981) and this neurochemical effect has been used to explain the decrease in excitability of cerebellar neurones by phencyclidine and ketamine (Marwaha, Palmer, Woodruff, Hoffer & Freedman, 1980). In a preliminary study on 3 cats and 5 rat spinal neurones noradrenaline did not mimic the NMA-blocking action of ketamine. Thus it seems unlikely that this latter action of ketamine is effected via changes in extracel-

lular concentrations of noradrenaline. Further studies on the interaction between ketamine, monoamines, excitatory amino acids and ACh are in progress.

The intravenous dose of ketamine required to block NMA actions was considerably less than the 22 mg/kg needed for surgical anaesthesia in the cat (Weisbroth & Fudens, 1972; Glen, 1973). However, at subanaesthetic doses ketamine still has considerable analgesic effects (Corssen & Domino, 1966; Ryder *et al.*, 1978). It is possible that NMA-receptor block at synapses in the spinal cord may explain the reduction by ketamine of noxious reflexes, an effect which is apparently enhanced by chronic spinal section at the thoraco-lumbar level (Pekoe & Smith, 1982). Responses of nociceptive neurones both in the spinal cord (Conseiller *et al.*, 1972; Kitahata *et al.*, 1973) and in the brain stem (Ohtani *et al.*, 1979) to noxious inputs are reduced by ketamine and related anaesthetics. However, in our own studies on acutely spinalized cats (Anis, Headley, Lodge & West, 1982), responses of dorsal horn neurones to natural stimulation, whether noxious or non-noxious, were not altered by doses of ketamine sufficient to block completely NMA-induced excitation. It seems likely that reduction of synaptic excitatory responses mediated through NMA receptors in ascending pathways to higher centres such as the cerebral cortex, underlies ketamine analgesia. Indeed normal cortical function appears to be a prerequisite for the clinical efficacy of ketamine (Morgan, Loh, Singer & Moore, 1971; Janis & Wright, 1972).

A hypothesis suggesting a causal relationship between 'dissociative' anaesthesia and NMA-antagonism would lead to the prediction that if adequate brain levels of other NMA antagonists could be achieved a dissociative anaesthetic state would be induced. It is therefore relevant that although HA-966 appears from the results of Biscoe *et al.* (1978) to be a less potent and selective NMA-antagonist than reported here for ketamine, it does cross the blood barrier and induces behavioural, neurophysiological and EEG changes (Bonta, DeVos, Grijsen, Hilden, Noach & Sim, 1971) not dissimilar to those reported for the dissociative anaesthetics (Domino, 1964). Furthermore αAA given intrathecally to rats also produces a temporary loss of righting reflexes (Cahusac, Evans & Rodriguez, 1982). Such findings, together with the synaptic excitation and NMA-blocking actions of ketamine, add further support to an important role for NMA receptors in normal CNS function (Watkins & Evans, 1981).

Ketamine may prove to be a very useful tool for neuropharmacologists since it has a potency and selectivity as an amino acid antagonist similar to that of αAA and 2APV, established NMA antagonists (Watkins & Evans, 1981). Although phencyclidine is an order of magnitude more potent than ketamine as an NMA antagonist and as an anaesthetic agent, its effects on action potential configuration (see Results) make it less likely to be accepted as a neuropharmacological tool in electrophysiological experiments. There is no evidence for either cellular uptake or enzymic degradation of ketamine in the nervous system and thus relatively even distribution should follow electrophoretic administration minimizing problems of achieving high concentrations at distant sites, without non-specific effects near the electrode tip. Furthermore since ketamine crosses the blood brain barrier, systemic administration can be used to study the role of NMA receptors in synaptic transmission.

The complication of an anticholinergic action of ketamine in such neuropharmacological investigations may be overcome by the use of the isomers of ketamine (see above) and by comparison with the effects of more conventional centrally active cholinergic antagonists.

Ketamine, phencyclidine and related anaesthetics also offer the possibility of studying and isolating NMA receptors *in vitro*. From the rates of recovery following both electrophoretic and intravenous administration, ketamine and particularly phencyclidine appear to interact with the NMA receptor for longer than αAA, 2-APV or NMA itself. Binding of phencyclidine to rat brain has already been described (Vincent, Kartalovski, Geneste, Kamenka & Lazdunski, 1979; Zukin & Zukin, 1979; Quirion, Hammer, Herkenheim & Pert, 1981) and the binding site appears to be identical with the sigma opiate receptor. There are no published reports of the effect of classical NMA receptor agonists and antagonists on the binding of sigma opiates. It is however of interest that several sigma opiates like ketamine and phencyclidine have both analgesic and psychotomimetic properties (see Martin, 1981). Until the binding studies with the dissociative anaesthetics and NMA receptor agonists and antagonists are completed it remains impossible to know whether ketamine and phencyclidine act at the NMA recognition site or an associated part of the receptor-effector complex.

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