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ENHANCEMENT OF DESENSITIZATION OF QUISQUALATE-TYPE GLUTAMATE RECEPTOR BY THE DISSOCIATIVE ANAESTHETIC KETAMINE

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Summary

Application of ketamine (10^{-4}–10^{-3} mol l^{-1}) to locust retractor unguis muscle produced a reversible, dose-dependent reduction in neurally evoked twitches, and blocked agonist-induced contractions. With increasing ketamine concentration (5×10^{-5}–10^{-3} mol l^{-1}), the amplitude of glutamate potentials was reduced and dose–response curves for ionophoresis of L-glutamate were shifted to the right, particularly after concanavalin A treatment. Ketamine (10^{-4} mol l^{-1}) enhanced the rate of desensitization to consecutive pulses of L-glutamate and this action was eliminated by concanavalin A. The amplitude of the excitatory postsynaptic current (EPSC) was reduced by ketamine (10^{-5}–5×10^{-4} mol l^{-1}) in a dose-dependent manner but without a concomitant reduction in EPSC rise time. The decay phase of the EPSC was usually biphasic in the presence of ketamine ($>5\times10^{-5}$ mol l^{-1}) but did not exhibit any voltage dependence. It is concluded that ketamine enhances desensitization and blocks the channel, particularly the closed form.

Introduction

Ketamine is a dissociative anaesthetic which also produces intense analgesia, often persisting after the return of consciousness. Total amnesia and a high incidence of hallucinations following ketamine anaesthesia are attributable to the drug being a congener of the hallucinogen phencyclidine (PCP), a readily synthesized cyclohexamine which has become a major drug of abuse. Ketamine blocks the response to acetylcholine in cut sciatic nerve preparations (Cronnelly *et al.* 1973), blocks noradrenaline uptake into nerve terminals of rat cerebral

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cortex (Smith *et al.* 1975) and increases dopamine levels in the rat thalamus and hypothalamus (Glisson *et al.* 1972). However, Jamieson & Lander (1984) demonstrated a positive inotropic effect of ketamine on bivalve mollusc heart muscle which was not mediated *via* cholinergic or aminergic receptors. Anis *et al.* (1983) showed that ketamine selectively inhibits excitation of cat Renshaw cells by *N*-methyl D-aspartate but is much less effective at reducing excitation induced by acetylcholine or glutamate.

Ketamine inhibition of the neurally evoked response of frog peripheral motor nerve endplate (Maleque *et al.* 1981; Volle *et al.* 1982) is *via* the open or activated ionic channel of the acetylcholine receptor channel complex. The reduction in amplitude and duration of miniature endplate current at the isolated mouse diaphragm during ketamine application (Torda & Gage, 1977) suggests that this drug shortens acetylcholine-induced channel lifetime, although some closed (i.e. non-activated) channel block of acetylcholine receptor channels may also occur (Volle *et al.* 1982). The lack of effect of ketamine on [^{125}I]bungarotoxin binding to microsacs of cultured chick myotubes (Volle *et al.* 1982) seemingly precludes an action on acetylcholine binding sites *per se*.

In contrast to our knowledge of the effects of ketamine upon peripheral vertebrate synapses where acetylcholine is the transmitter, there is, as yet, a lack of data on the site and mode of action of ketamine (and other anaesthetics) on the responses induced by excitatory amino acids in the mammalian central nervous system (e.g. see review by Watkins, 1978). There have also been few studies of the effects of anaesthetics upon the amino acid receptors which are relatively ubiquitous in the peripheral and central nervous systems of invertebrates (Usherwood, 1978; Nistri & Constanti, 1979). The present study investigates the effects of ketamine on the quisqualate-sensitive locust excitatory nerve–muscle junction which is a pharmacologically well-defined invertebrate excitatory amino acid receptor where glutamate is the receptor ligand. Transmission at this site is affected by many drugs of clinical usage, including trimetaphan (Ashford *et al.* 1987) and chlorisondamine (Ashford *et al.* 1988), and as such it may be a useful model for the actions of drugs on quisqualate-type glutamate receptors.

Materials and methods

All experiments were performed on muscle preparations from the metathoracic leg of the locust (*Schistocerca gregaria*). The preparations were continuously perfused with standard locust saline containing (in mmol l^{-1}): NaCl, 180; KCl, 10; Hepes, 10; $CaCl_2$, 2; pH 6·8. For twitch-tension recording the isolated retractor unguis (Usherwood & Machili, 1968) was used and ionophoretic data were obtained from fibres of the extensor tibiae muscle (Clark *et al.* 1980). Ketamine HCl (Parke-Davis) was dissolved in saline (adjusted to pH 6·8) and applied directly to the preparation *via* the superperfusate. Twitch responses and desensitization onset/recovery data were recorded on a Grass polygraph 7 and a Medelec oscilloscope with print-out facility. Dose–response curves were constructed using

oscilloscope data obtained during microionophoresis of L-glutamate onto single junctional sites in standard saline and drug-containing salines. Short (10 ms) pulses of glutamate were applied to the junctional sites using high (>100 MΩ) resistance electrodes containing 1 mol l^{-1} sodium L-glutamate (Sigma). An interval of 10 s was allowed between successive ionophoretic pulses. Several responses were obtained at each ionophoretic glutamate dose to ensure a constant junctional potential amplitude without variations caused by, for example, fluctuations in ejection current. Muscle preparations were exposed to standard saline at the end of a drug experiment and a final set of dose–response data was obtained. In some experiments the preparations were exposed to concanavalin A (3 μmol l^{-1}) for 30 min to abolish desensitization of the glutamate receptors (Mathers & Usherwood, 1976, 1978).

The methods used to study and analyse the effects of ketamine on the postsynaptic current (EPSC) at excitatory junctions on extensor tibiae muscle fibres were identical to those described by Ashford *et al.* (1987, 1988).

Results

Effect of ketamine on contractions of isolated retractor unguis muscle

When 10^{-4} mol l^{-1} agonist (L-glutamate or L-quisqualate) was applied to a retractor unguis preparation a phasic contraction ensued (Fig. 1) (Usherwood & Machili, 1968; Clements & May, 1974). Ketamine (100 μmol l^{-1} to 1 mmol l^{-1}) reduced the neurally evoked twitch amplitude (the rate and magnitude of the reduction being dose-dependent) and abolished the agonist-induced contraction (Fig. 1). The magnitude of the reduction in twitch amplitude was the same in previously stimulated preparations as in unstimulated ones. On removal of the anaesthetic the twitch contractions returned to control amplitude as did the response to bath-applied agonist (not shown).

Postsynaptic inhibition by ketamine

Ketamine (up to 1 mmol l^{-1}) had no effect on the resting potential and input conductance (slope and chord) of the extensor tibiae muscle fibre.

Ionophoresis of L-glutamate onto single, discrete excitatory junctional sites of this muscle causes a transient membrane depolarization. Addition of ketamine (50 μmol l^{-1} to 1 mmol l^{-1}) to the bathing solution reduced the amplitude of these potentials and dose–response curves constructed from such ionophoretic data were shifted to the right by ketamine in a dose-dependent and reversible fashion (Fig. 2).

An investigation was made of the effects of ketamine upon trains of glutamate potentials (generated by identical pulses of L-glutamate) recorded from a single junctional site (Fig. 3A,B). The preparation was rested for 1 min between trains. In the absence of ketamine, repetitive ionophoresis of L-glutamate was accompanied by a gradual decline in response amplitude due to desensitization of the postjunctional glutamate receptors (Fig. 3A). With ketamine in the bathing

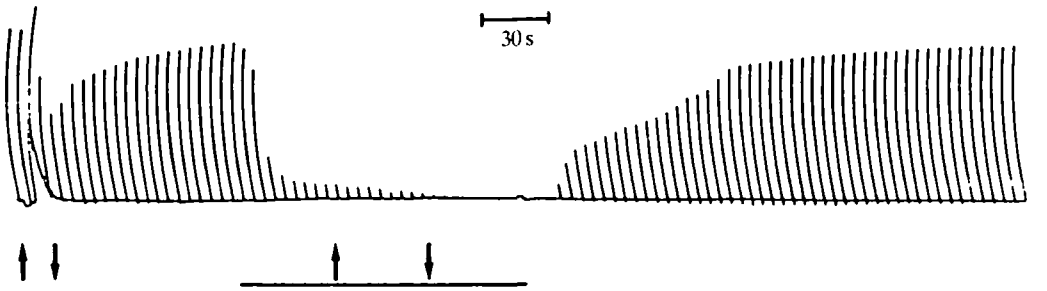

Fig. 1. Continuous record showing the effect of ketamine (1 mmol l^{-1}) on the neurally evoked twitch response and agonist (L-glutamate)-induced contraction of the isolated retractor unguis muscle of the locust *Schistocerca gregaria*. The arrows denote the duration of L-glutamate (100 μmol l^{-1}) application and the black bar indicates the presence of ketamine. L-Glutamate caused a phasic contraction of the muscle and a depression in amplitude of the twitch contraction evoked by stimulation of the retractor unguis nerve at a frequency of 0·25 Hz. After recovery of the preparation from the effects of the agonist, ketamine was added to the bathing medium and the amplitude of the neurally evoked twitch was rapidly reduced and eventually completely abolished. Application of 100 μmol l^{-1} L-glutamate in the presence of ketamine failed to excite the muscle.

medium the amplitude of the first response in a train was reduced compared with controls. Ketamine also enhanced the rate and magnitude of reduction in response amplitude with ionophoretic pulse repetition (Fig. 3B). After treatment of the preparation with concanavalin A (3 μmol l^{-1}), which blocks desensitization (Mathers & Usherwood, 1976, 1978), the decline in amplitude (due to desensitization) of successive glutamate potentials with ionophoretic pulse repetition was observed to be completely blocked (Fig. 3C). When ketamine was now applied to the preparation the amplitude of the first response in a train was reduced compared with that in a concanavalin-A-treated control train (as occurred in the absence of concanavalin A) but there was no further reduction in glutamate potential amplitude with repetition of the ionophoretic glutamate pulse (Fig. 3D). Identical data were obtained during ketamine application to concanavalin-A-treated preparations with glutamate pulse trains of frequencies between 1 and 3 Hz (Fig. 3E). Thus ketamine not only causes a reduction in the amplitude of the glutamate potentials but also appears to produce an enhancement of desensitization similar to that observed for trimetaphan (Ashford *et al.* 1987).

In concanavalin-A-treated preparations, ketamine shifted the glutamate dose–response curve to the right in a dose-dependent fashion with apparent reductions in the maximum amplitude of the glutamate potential (Fig. 4).

Recovery from desensitization of junctional sites untreated with concanavalin A was studied using a double ionophoretic pulse technique similar to that described previously (Clark *et al.* 1979; Anis *et al.* 1981). Recovery rates in standard saline were similar to those obtained in previous studies and to those obtained in the presence of ketamine (Fig. 5).

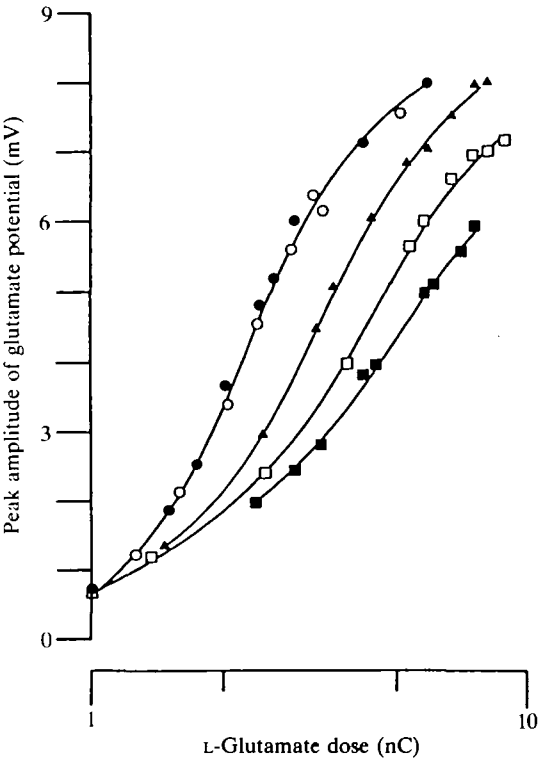

Fig. 2. Semilogarithmic dose–response curves drawn from data obtained at a single excitatory junctional site in a locust extensor tibiae muscle fibre during application of L-glutamate by ionophoresis. Data were obtained in standard saline at the start (●) and end (○) of the experiment and in the presence of (▲) 50 μmol l^{-1} ketamine; (□) 100 μmol l^{-1} ketamine; (■) 200 μmol l^{-1} ketamine. The preparation was equilibrated for 5 min in each solution before the dose–response data were obtained. An interval of 10 s was allowed between each application of L-glutamate to eliminate interactions between responses. Lines were fitted by eye. Resting potential of the muscle fibre was −65 mV throughout the experiment.

Influence of ketamine on the voltage-clamped EPSC

Amplitude

The EPSC amplitude (recorded intracellularly under voltage-clamp) was linearly related to membrane potential (over the range −40 mV to −140 mV) with an extrapolated reversal potential of +1·9 ± 3·7 mV (S.D.; $N = 5$), a value similar to that obtained in previous studies (see Anwyl & Usherwood, 1974; Ashford *et al.* 1987, 1988). A reduction in peak amplitude of the EPSC was seen with ketamine at a concentration as low as 10 μmol l^{-1} ($N = 1$), but usually at least 50 μmol l^{-1} was required to produce a marked (>20 %) change in this parameter. The reduction in peak EPSC amplitude in the presence of ketamine (10 μmol l^{-1} to 0·5 mmol l^{-1}) was independent of membrane potential in four out of five experiments. The

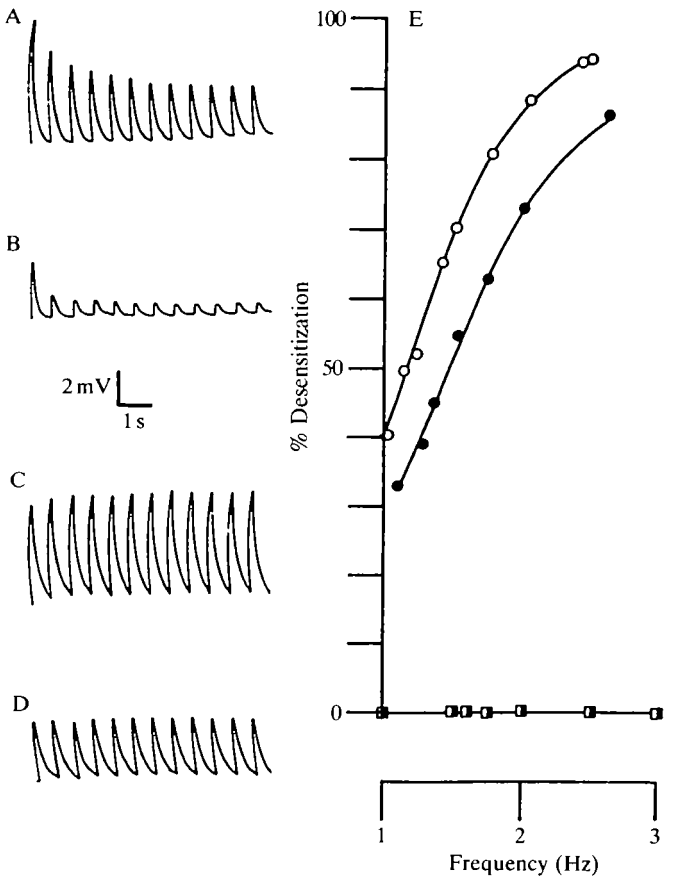

Fig. 3. Enhancement of desensitization by ketamine. (A) A train of ionophoretic glutamate potentials obtained in standard saline. Note the decline in amplitude of the potential with repetition, due to desensitization. (B) Data obtained from same site as in A but after equilibration for 15 min in saline containing 100 μmol l^{-1} ketamine. Note the reduction in amplitude of the initial response in the train compared with the initial response in A and further reduction of glutamate potential amplitude with repetition of ionophoretic pulse. A period of 10 min was required for full recovery of the control response pattern after removal of ketamine (data not shown). (C) Complete block of densitization after exposure of the muscle preparation for 30 min to 3 μmol l^{-1} concanavalin A. (D) After concanavalin A treatment ketamine still reduced the amplitude of the glutamate potential but no further reduction occurred with repetition of the ionophoretic pulse. (E) Data collected from a single junctional site using the above procedure but with pulse trains of different pulse repetition frequencies (1–3 Hz). (●) In standard saline; (○) in 100 μmol l^{-1} ketamine; (■) in standard saline after concanavalin A pretreatment; (□) in 100 μmol l^{-1} ketamine after concanavalin A treatment. Percentage depression of the glutamate potential amplitude was determined by comparing the amplitude of the tenth response in a train with that of the first response in the train.

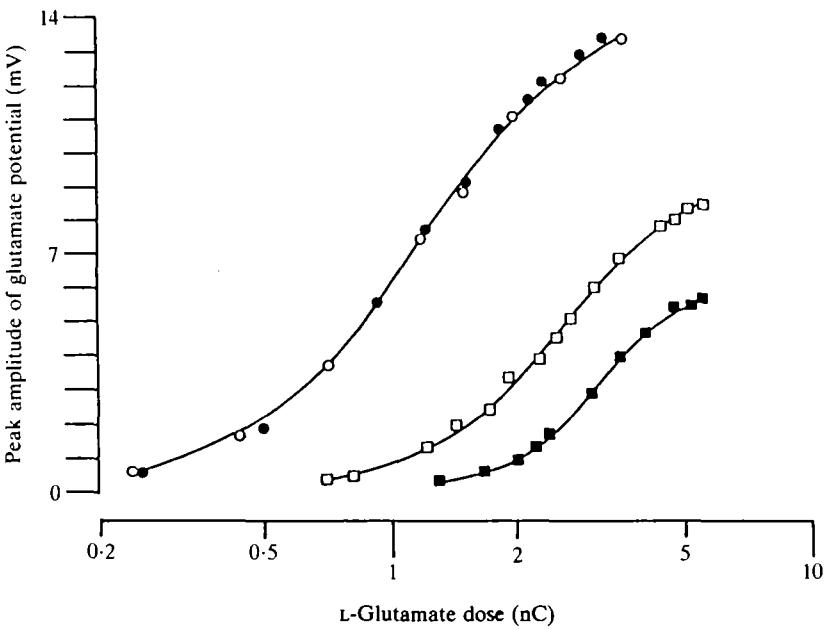

Fig. 4. Dose–response data from a preparation pretreated with saline containing concanavalin A (3 μmol l^{-1}) for 30 min in standard saline at the start (●) and end (○) of experiment, (□) in 400 μmol l^{-1} ketamine, (■) in 1 mmol l^{-1} ketamine. Lines were fitted by eye.

relationship between peak EPSC amplitude and membrane potential was approximately linear with similar extrapolated reversal values to those measured in standard saline (data not shown). However, in one experiment there was a slight voltage dependence of EPSC amplitude in the presence of ketamine (Fig. 6A).

Rise time of EPSC

In the absence of ketamine the EPSC rise time (20% to 80% peak EPSP amplitude) was between 0·6 and 1·0 ms ($N = 5$) (see also Ashford *et al.* 1987, 1988). For concentrations of ketamine <100 μmol l^{-1} there were no overall significant ($P > 0{\cdot}5$; Student's t-test) changes in the EPSC rise time (Fig. 7) but with 1 mmol l^{-1} and on some occasions 0·5 mmol l^{-1} ketamine a 20% reduction in rise time was recorded which was significant at the 0·01% level (data not shown). In the absence of drug, and for all concentrations of ketamine tested (10 μmol l^{-1} to 1 mmol l^{-1}), the EPSC rise time exhibited no obvious voltage dependence. These data are in contrast to the action of trimetaphan on the EPSC rise time (Ashford *et al.* 1987) where reduction in rise time is strongly dependent on concentration and voltage. An example of this voltage dependence for the effect of 50 μmol l^{-1} trimetaphan on rise time is shown in Fig. 7 for comparison.

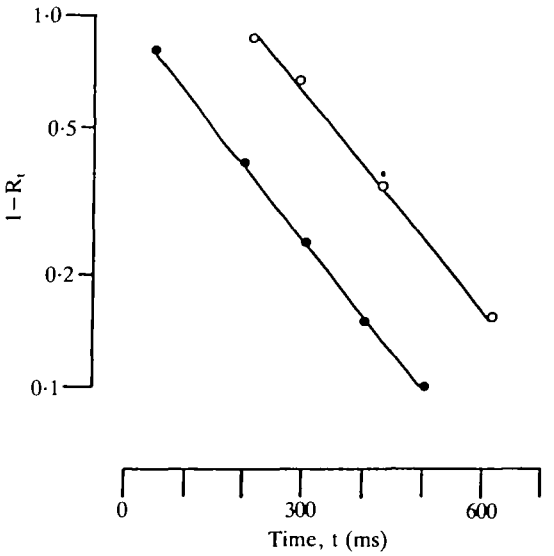

Fig. 5. Recovery from desensitization in the presence and absence of ketamine. Semilogarithmic plot of $1-R_t$ (where R_t is the ratio of the amplitude of a 'test' junctional response to an ionophoretic pulse of L-glutamate to that of a 'control' response to an identical pulse and t is the interval between the two pulses in ms) against interpulse intervals (in ms) showing the rate of recovery from desensitization. (●) In standard saline; (○) in 100 μmol l^{-1} ketamine. Note that although the plot is shifted to the right in the presence of ketamine, the rate of recovery of the test response with increasing t is similar in standard and ketamine-containing saline. Identical pulses of L-glutamate were applied in the presence and absence of drug. Rate constant for recovery in each case was 5 s^{-1} (cf. Anis *et al.* 1981). The resting potential of the muscle fibre was −60 mV. Lines were fitted by linear regression. Qualitatively similar data were obtained with 10 μmol l^{-1} and 1 mmol l^{-1} ketamine.

Decay of EPSC

The decay of the EPSC consists of two or three components (Ashford *et al.* 1987) but is usually monophasic and exponential when measured between 80 % and 20 % peak EPSC amplitude (Anwyl & Usherwood, 1974, 1975; Ashford *et al.* 1987). In two experiments ketamine (10–100 μmol l^{-1}) had no significant effect on the EPSC decay time (80 % to 20 % of peak EPSC amplitude). However, in three other experiments the time course of the EPSC was lengthened by ketamine (50 μmol l^{-1} to 0·5 mmol l^{-1}) with the decay becoming biphasic, having an early fast phase (faster than the control decay) and a late slow phase (slower than the control decay). A drug-induced biphasic decay of the EPSC time course is normally considered to be a good indicator that open-channel block is occurring. However, the EPSC decay, both in the absence and in the presence of ketamine (10 μmol l^{-1} to 0·5 mmol l^{-1}), did not exhibit any consistent voltage sensitivity between −40 and −140 mV (e.g. Fig. 8). Thus if open-channel block is the

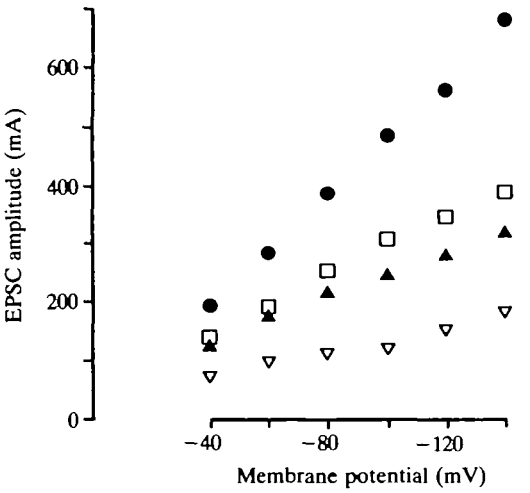

Fig. 6. Relationship between EPSC amplitude and voltage-clamped membrane potential in the absence (●) and presence of ketamine (□, 50 μmol l^{-1}; ▲, 100 μmol l^{-1}; ▽, 500 μmol l^{-1}). In this experiment the reduction in EPSC amplitude in the presence of ketamine (500 μmol l^{-1}) was possibly slightly voltage-dependent as indicated by the deviation of the relationship from linearity and an extrapolated reversal potential different from the control. It was not possible routinely to study events at membrane potentials of less than −40 mV but where this was possible there was no evidence that ketamine affected the reversal potential for the EPSC. Each datum point is the mean ± S.E.M. ($N > 20$). In all cases the standard error bars were smaller than the symbols.

mechanism by which the currents become biphasic, then it is likely that the uncharged form of ketamine (pKa of 7·5; Cohen & Trevor, 1974) is active.

Discussion

The results from previous studies on acetylcholine receptor channel complexes have suggested that ketamine may produce two forms of inhibition, open- and closed-channel block (Maleque *et al.* 1981; Volle *et al.* 1982). It has been proposed that ketamine blocks the open conformation of the acetylcholine receptor channel in a voltage-dependent and concentration-dependent manner by binding to a site on the receptor 17 % of the way within the membrane electric field (Maleque *et al.* 1981) and that the closed-channel block induced by ketamine is not voltage-dependent (Volle *et al.* 1982). Data presented in this study suggest that this anaesthetic also exerts at least two modes of action on the locust muscle glutamate receptor; it enhances receptor desensitization and it blocks the receptor channel complex in its closed and/or open state.

Biochemical methods using receptor-enriched membranes from electric organs of *Torpedo* indicate that a number of substances which have been described electrophysiologically as channel blockers may also enhance desensitization

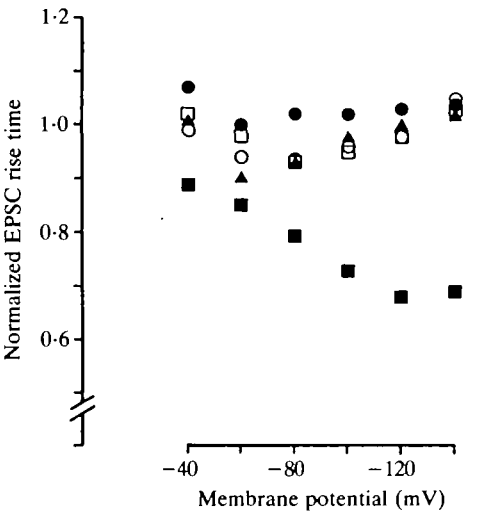

Fig. 7. Effect of ketamine on EPSC rise time (20–80 % of peak EPSC amplitude) at various membrane potentials. (●) Drug-free saline; (□) 50 μmol l^{-1} ketamine; (○) 100 μmol l^{-1} ketamine; (▲) 500 μmol l^{-1} ketamine. There is little evidence for either a change of the EPSC rise time in ketamine or any voltage sensitivity of this parameter. The voltage-dependent effect of trimetaphan (50 μmol l^{-1}) on the rise time of the EPSC in this preparation is included for comparison (■) (see also Ashford *et al.* 1987). These data have been normalized as the ratio of the observed EPSC rise time to that observed in drug-free saline at −60 mV. Each datum point is the mean from at least 20 EPSCs (S.E. bars, when larger than the symbols, have been omitted for the sake of clarity).

(Eldefrawi & Eldefrawi, 1980). For example, the antidepressant imipramine, PCP and histrionicotoxin supposedly act on the activated, but nonconducting form of the acetylcholine receptor channel (Burgermeister *et al.* 1977) without influencing the rate of recovery of the receptor from its desensitized state. The lack of a means of differentiating between enhancement of desensitization and open-channel block of the nicotinic acetylcholine receptor channel complex has prevented unequivocal testing of these conclusions using electrophysiological techniques.

However, the argument supporting a bimodal action for ketamine at the locust glutamate receptor is based upon comparisons between preparations treated with concanavalin A and those not exposed to this lectin. Concanavalin A has two effects on locust glutamate receptors: it abolishes desensitization (Mathers & Usherwood, 1976, 1978) and it eliminates the voltage sensitivity of the receptor-gated channel lifetime (Mathers, 1981). The rate of onset of desensitization of the locust glutamate receptor is dependent upon agonist concentration whereas the rate of recovery from desensitization is independent of the level of desensitization (Anis *et al.* 1981). In ionophoretic studies with trains of glutamate pulses, the rate of onset of desensitization can be notionally controlled by varying the glutamate pulse frequency. The data from such experiments clearly show that ketamine reduced the steady-state amplitude of the first response obtained in a train and

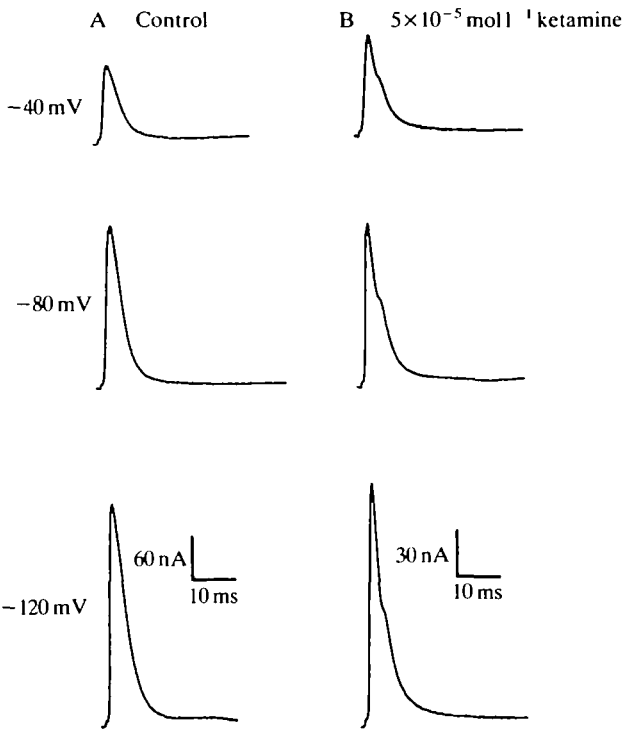

Fig. 8. Averaged EPSCs recorded under voltage-clamp from a locust extensor tibiae muscle fibre at different membrane potentials in drug-free saline (A) and in a saline containing 50 μmol l^{-1} ketamine (B). The preparation was equilibrated in the ketamine-containing solution for 15 min before the data in B were recorded. Note the biphasic decay of the EPSC in ketamine saline. Each trace is the average of at least 20 consecutive individual EPSCs.

increased the rate at which successive glutamate potentials declined in amplitude in a dose-dependent fashion. This could be interpreted either as an enhancement of desensitization or as open-channel block with a sufficiently low recovery rate to ensure interaction between glutamate potentials occurring at frequencies of 1–3 Hz. However, in the double-pulse experiments the rate of recovery of the glutamate potential was identical in the presence and absence of ketamine with both recoveries being single exponential processes (see also Anis *et al.* 1981). Although it is possible that recovery from open-channel block by ketamine occurs at exactly the same rate as recovery from desensitization this appears unlikely. The obvious conclusion is that ketamine enhances desensitization of the locust muscle glutamate receptors. (A similar explanation has been invoked to account for one of the effects of trimetaphan on locust muscle, Ashford *et al.* 1987.) The observation that pretreatment with concanavalin A to remove desensitization blocks this effect of ketamine adds further weight to the argument that desensitization is the causative factor. In addition, it is unlikely that concanavalin A treatment prevents open-channel block by drugs as it has been shown previously

that this treatment has no effect upon the block of the open channel of the locust muscle glutamate receptor by trimetaphan (Ashford *et al.* 1987) and by chlorisondamine (Ashford *et al.* 1988).

Because the amplitude of the glutamate potential is reduced by ketamine even after pretreatment of locust muscle with concanavalin A, it is necessary to invoke a second mode of action for this drug on the glutamate receptor. Although ionophoretic dose–response data are difficult to interpret (even when desensitization is not a complicating factor) the production by ketamine of a rightward shift and depression of the maximum in the glutamate dose–response curves for the concanavalin-A-treated locust muscle preparation are supportive of closed- rather than open-channel block, although this is not an unequivocal conclusion. The lack of voltage dependence of the changes in EPSC amplitude caused by ketamine and the lack of effect of this drug on the rise time of the EPSC support the contention that open-channel block is perhaps not a factor in the mode of action of this anaesthetic on the locust glutamate receptor. However, its variable effects on EPSC decay are more difficult to interpret if this is true, since biphasic EPSC decays which were seen in some experiments are usually interpreted as due to open-channel block. If ketamine does block the open glutamate channel then this block is at best only weakly dependent on membrane potential, despite the fact that ketamine is predominantly positively charged at pH 6·8. However, if the glutamate receptor channel is blocked by unionized ketamine then one would not expect voltage-dependent actions on the EPSC amplitudes, rise time and decay times, although one might expect greater concentration-dependent actions (on rise times and decay times) than those recorded in this study.

Also, even assuming some open-channel block does occur, the concentration-dependent reduction in the EPSC amplitude induced by ketamine is unlikely to be explained by this mechanism. Maleque *et al.* (1981) and Torda & Gage (1977) have suggested that the reduction in peak amplitude of acetylcholine-activated currents by ketamine was due to the shortened open-channel lifetime (i.e. the channel 'closes' faster so reducing overall current amplitude). However, this is not a convincing explanation here because ketamine (particularly at concentrations $<100\,\mu\text{mol}\,l^{-1}$) reduces the peak EPSC amplitude without a parallel effect on rise time (which was observed for trimetaphan and chlorisondamine, Ashford *et al.* 1987, 1988), and in two out of five experiments this concentration range did not affect the EPSC decay. Thus a simpler explanation for the reduction, by ketamine, in the amplitude of glutamate potentials in concanavalin-A-treated muscles and in the voltage-clamped EPSCs is through a non-voltage-dependent closed-channel block, agreeing with the conclusions of Volle *et al.* (1982) for ketamine inhibition of acetylcholine-induced current amplitude.

An alternative explanation (to open-channel block) for the biphasic EPSC decays in ketamine is the possibility that they could result from a slowing of diffusion of transmitter from the synaptic cleft owing to the appearance of high-affinity binding sites for glutamate, since desensitization of the nicotinic acetylcholine receptor is thought to involve the appearance of a high-affinity binding

site. The observation that the fast component of the biphasic decay seen in ketamine is faster than the decay of the control EPSC suggests that desensitization in the presence of the drug may be fast enough to influence the time course of the EPSC. It would have been interesting to have tested the effects of ketamine on the EPSC after concanavalin A treatment, but unfortunately the lectin does not reach all the junctions on a muscle fibre which contribute to the generation of the synaptic current. Single-channel studies, which provide the best opportunity to test some of these ideas, are already in progress.

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