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Mechanism of action of ketamine in the current and voltage clamped myelinated nerve fibre of the frog

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1 The effects of the general anaesthetic ketamine, on the frog isolated node of Ranvier, were studied under current and voltage clamp conditions.

2 Ketamine (0.5 and 1 mM) reversibly decreased the amplitude of the action potential and increased both the duration of the action potential and the threshold potential. When the K current was blocked, spontaneous action potentials appeared after washout of the drug.

3 Ketamine rapidly blocked the Na current and more slowly modified a fraction of Na channels (about 10%) to give rise to a non-inactivatable (late) Na current. After washout of the drug, the block reversed more rapidly than the ketamine-induced late Na current disappeared.

4 Steady-state outward, peak Na and ketamine-induced late Na currents were rapidly and reversibly blocked by ketamine with an apparent dissociation constant of 0.7 mM.

5 Both peak Na and ketamine-induced late Na currents were reversibly blocked by procaine.

Introduction

Ketamine, a less potent derivative of phencyclidine, clinically produces a rapid state of unconsciousness (in about 20 to 60 s), described as 'dissociative anaesthesia' (Domino *et al.*, 1965) and characterized by anaesthesia, analgesia and amnesia. Frequently, following intravenous administration of ketamine, excitatory phenomena occur, such as for instance: cardiovascular stimulation (Tweed *et al.*, 1972); increased skeletal muscle activity (Corssen & Domino, 1966); behavioural and EEG changes representing seizure activity (Domino *et al.*, 1965; Winters *et al.*, 1972). It has also been clinically observed that ketamine induces psychic feelings: during recovery from the drug anaesthesia (which occurs in about 10 to 15 min) emergence reactions are common (Zsigmond & Domino, 1980).

Both the depressant and excitatory effects of the general anaesthetic have been studied *in vivo* and *in vitro* on various preparations. In general, the results are fully consistent with the clinical observations described previously. In particular, the mechanism of ketamine action has been studied on frog sciatic nerve (Diamond *et al.*, 1975) and on squid giant axon (Shrivastav, 1977). Nevertheless, no clear explanations have been given to account for both types of ketamine effects.

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The aim of the present work was to study the action of ketamine on the current and voltage clamped node of Ranvier and to try to understand better the mechanisms of its effects.

Methods

The experiments were carried out on single myelinated nerve fibres from the sciatic nerve of the frog *Rana esculenta*. The membrane potential and membrane currents were recorded under current and voltage clamp conditions, using the method of Nonner (1969). The normal resting potential was assumed to be −70 mV, corresponding to 30% of Na inactivation. Membrane currents were calculated assuming an axoplasmic resistance of 10 MΩ. Linear leakage and capacity currents were subtracted electronically from the total current.

The Ringer solution had the following composition (mM): NaCl 111.5, KCl 2.5, $CaCl_2$ 1.8, $NaHCO_3$ 2.4, pH 7.4. When recording action potentials or K current, the fibre ends were cut in a solution containing 120 mM KCl, which was used in the end pools throughout the experiments. When monitoring Na current, K current was suppressed by replacing the end pool solution with 110 mM CsCl + 10 mM NaCl and adding tetraethylammonium (10 mM) to the external

solution. The temperature was maintained at 14–16°C.

Results

Effects on membrane potential

The effects of external ketamine on membrane potential are shown in Figure 1. Figure 1 (a–d) shows action potentials elicited by depolarizing stimuli, under control conditions (Figure 1a), 4 min after the addition of 0.5 mM ketamine to the Ringer solution (Figure 1b), 3 min after the addition of 1 mM ketamine to the Ringer solution (Figure 1c) and after 3 min wash with Ringer solution (Figure 1d). In the presence of the drug, the threshold potential and action potential duration increased whereas the spike amplitude decreased. These effects were reversed after washout of ketamine with normal Ringer solution. When the K current was blocked (by internal CsCl and external tetraethylammonium), spontaneous action potentials appeared for a prolonged period 5 to 10 min after washout of the drug (Figure 1e).

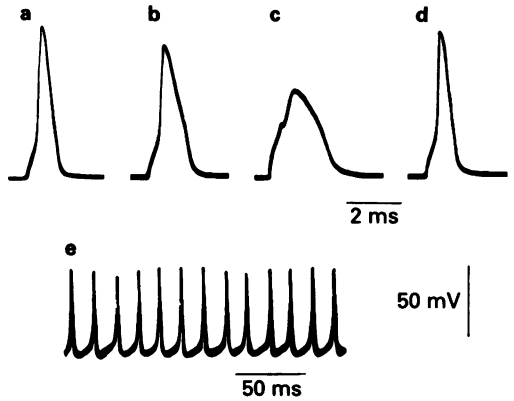

Figure 1 Effects of ketamine on the action potential. (a) Control action potential in Ringer solution. (b and c) Action potentials recorded 4 min (b) and 3 min (c) after the addition of 0.5 mM (b) and 1 mM (c) ketamine to the Ringer solution. (d) Action potential recorded 3 min after washout of the drug with Ringer solution. Fibre: 7-03-85 I. (e) Spontaneous action potentials recorded 7 min after washout of 0.5 mM ketamine with Ringer solution containing 10 mM tetraethylammonium. The internal solution was 110 mM CsCl + 10 mM NaCl. Fibre: 5-03-85.

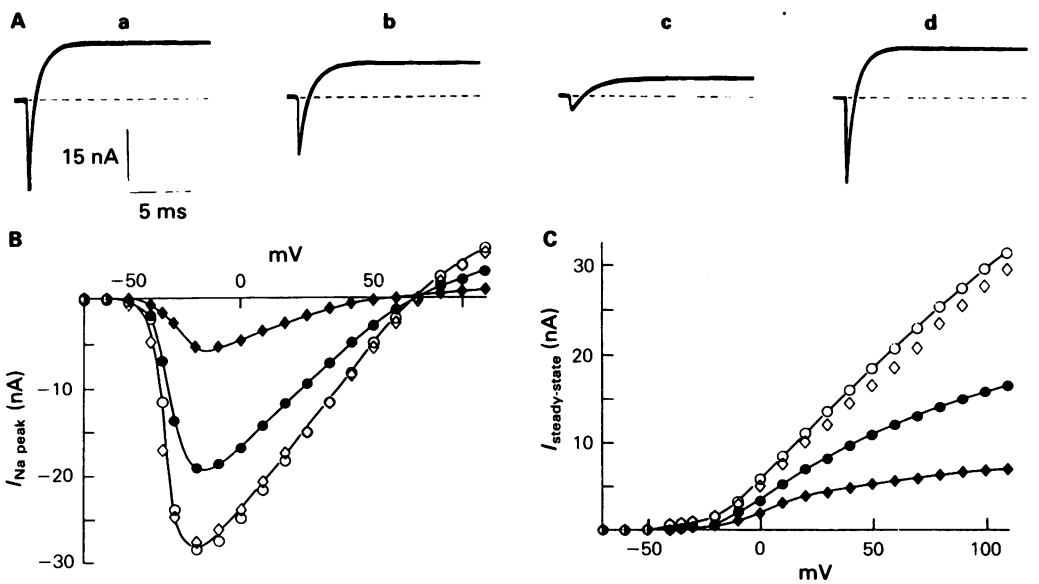

Figure 2 Effects of ketamine on membrane currents. (A) Traces of membrane currents recorded under control conditions (a), in the presence of 0.5 mM (b) and 1 mM (c) ketamine and after washout of the drug (d), during depolarizations to 0 mV preceded by 50 ms hyperpolarizations to − 120 mV. (B and C) Current-voltage relationships of peak Na (B) and steady-state outward (C) currents, under control conditionsa (○), in the presence of 0.5 mM (●) and 1 mM (◆) ketamine and after washout of the drug (◇), during depolarizations of various amplitudes preceded by 50 ms hyperpolarizations to − 120 mV. Fibre: 7-03-85 I.

Effects on membrane currents

Figure 2 shows the effects of external ketamine (0.5 and 1 mM) on membrane currents. Linear leakage and capacity currents were not modified by the drug. Ketamine blocked both steady-state outward and peak Na currents in about 3 to 5 min, without any noticeable changes either in current kinetics (Figure 2A) or in the shapes of the current-voltage relationships (Figure 2B and C). The effects were not dependent on the frequency of stimulation and were reversed by about a 3 to 6 min wash with Ringer solution. It is noteworthy that the effects of ketamine on the steady-state outward current were not fully reversible, in contrast to those on the peak Na current (see below).

A careful analysis of the effects of ketamine on the Na current, was undertaken after block of the K current. In addition to the reversible reduction of the peak Na current, ketamine (0.5 mM) induced, during long lasting depolarizations, a maintained (late) Na current which increased and persisted for a prolonged period after washout of the drug (Figure 3). Both peak and late Na currents were suppressed by 300 nM tetrodotoxin. The presence of the late inward Na current, after washout of the ketamine, may explain the non-fully reversible effects of the drug on the steady-state outward current recorded when K channels were not blocked (see Figure 2C).

The kinetics of ketamine action on peak and late Na currents are presented in Figure 4. When ketamine (0.1 mM) was added to the external solution, the peak current rapidly decreased and reached a steady level

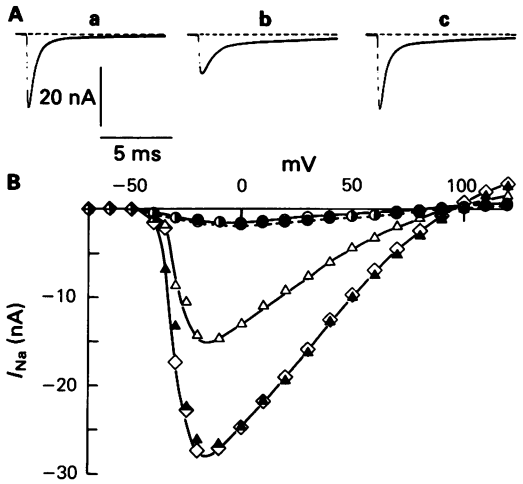

Figure 3 Effects of ketamine on Na current. (A) Traces of Na current recorded under control conditions (a), in the presence of 0.5 mM ketamine (b) and 4 min after washout of the drug (c), during depolarizations to 0 mV preceded by 50 ms hyperpolarizations to −120 mV. (B) Current-voltage relationships of peak and late Na currents. The peak Na current was recorded under control conditions (◇), in the presence of 0.5 mM ketamine (△) and 4 min after washout of the drug (▲), during depolarizations of various amplitudes preceded by 50 ms hyperpolarizations to −120 mV. The late Na current was recorded in the presence of 0.5 mM ketamine (○) and 4 min after washout of the drug, at the end of 19 ms depolarizations of various amplitudes preceded by 50 ms hyperpolarizations to −120 mV. Fibre: 5-03-85.

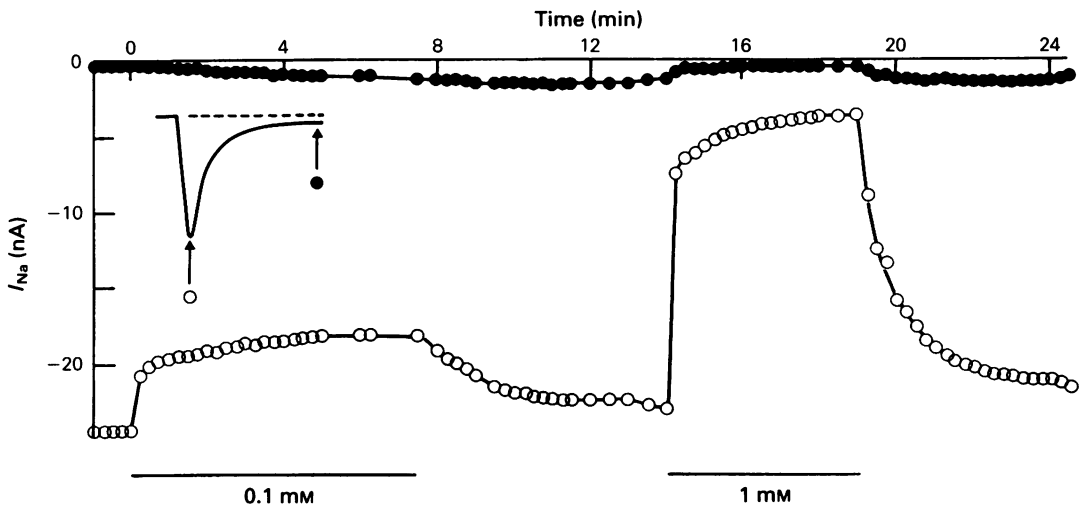

Figure 4 Kinetics of the action of ketamine on peak and late Na currents. The peak (○) and late (●) Na currents were recorded respectively during and at the end of 19 ms depolarizations to 0 mV preceded by 50 ms hyperpolarizations to −120 mV. The following solutions were applied successively: Ringer solution, 0.1 mM ketamine (7 min), Ringer solution (6 min), 1 mM ketamine (5 min) and finally Ringer solution. Fibre: 7-03-85 II.

within about 4 min. After washout of the drug, the peak current recovered within about 5 min. A second application of ketamine (1 mM) almost completely blocked the peak current which recovered after washing with ketamine-free solution. The late current, which was almost nil before the first application of ketamine, increased in the presence of 0.1 mM ketamine and during washout. When ketamine was reapplied at a concentration of 1 mM, the late current was almost completely blocked. It recovered after washout of the drug and reached a steady level almost equal to that before the application of 1 mM ketamine.

The properties of the ketamine-induced late Na current, compared to those of the peak Na current, are summarized in Table 1 (see also Figure 3B). Peak and late Na currents were recorded in different fibres, after 4 to 6 min washout of ketamine (0.1 to 1 mM), respectively, during and at the end of 10 ms depolarizations of various amplitudes preceded by 50 ms hyperpolarizations to −120 mV. Under these conditions, the maximum late conductance represented about 10% of the maximum peak conductance and was not significantly dependent on the concentration of ketamine applied before washout. The voltage corresponding to the maximum late current was shifted by about 15 mV towards positive values, compared to that corresponding to the maximum peak current. The voltage corresponding to half maximum late conductance was about 10 mV more positive than that corresponding to half maximum peak conductance. Finally, the late current reversed at a voltage about 25 mV more negative than the peak current. No significant difference was observed between voltages corresponding to activation threshold of peak and late currents.

Figure 5 shows the steady-state Na current inactivation curves, under control conditions, in the presence of 0.5 mM ketamine and 6 min after washout of the drug. Ketamine did not significantly modify either the shape or the position along the voltage axis of the curve corresponding to the inactivatable current. However, for voltages more positive than −40 mV, ketamine induced a non-inactivatable Na current which increased after washout of the drug. This fraction of current corresponds to the late Na current previously described (see Figures 3 and 4).

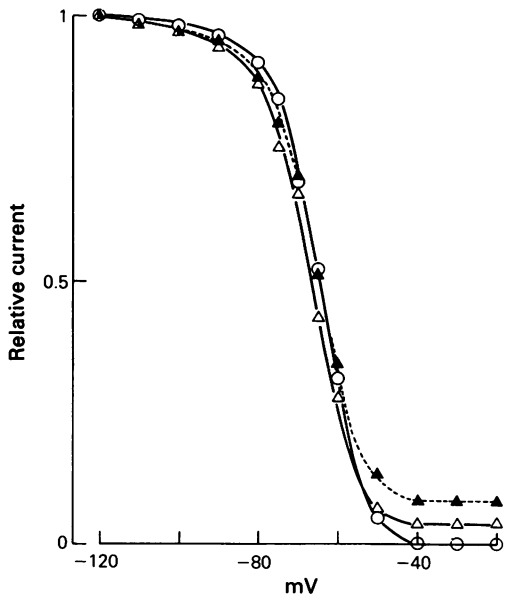

Figure 5 Effects of ketamine on the steady-state Na inactivation curve. Peak Na current was recorded under control conditions (○), in the presence of 0.5 mM ketamine (△) and 6 min after washout of the drug (▲), during depolarizations to 0 mV preceded by 50 ms pulses of various amplitudes. Fibre: 6-03-85.

Table 1 Effects of ketamine on voltage characteristics of Na currents and conductances

Fibre	[*Ket*] (mM)	g_L/g_P	V_{Ipmax} (mV)	V_{ILmax} (mV)	$V_{gP/2}$ (mV)	$V_{gL/2}$ (mV)	$V_{rev}I_P$ (mV)	$V_{rev}I_L$ (mV)
5-03-85	0.1	0.18	−15	0	−30	−28	+ 95	+80
6-03-85	0.1	0.11	−20	−5	−32	−18	+ 82	+70
7-03-85 II	1	0.05	−15	0	−34	−27	+107	+66
8-03-85	0.5	0.16	−15	0	−32	−10	+ 87	+60
Mean		0.12	−16	−1	−32	−21	+ 93	+69
± s.e.mean		±0.03	± 1	±1	± 1	± 4	± 5	± 4

[Ket] is the concentration of ketamine applied before washout.
g_L and g_P are the maximum late and peak conductances respectively.
V_{Ipmax} and V_{ILmax} are voltages corresponding to maximum peak and late currents respectively.
$V_{gP/2}$ and $V_{gL/2}$ are voltages corresponding to half maximum peak and late conductances respectively.
$V_{rev}I_P$ and I_L are reversal potentials of peak and late currents respectively.

Dose-response curve of the effects of ketamine

Figure 6 shows the dose-response curve of the blocking effects of ketamine (0.01 to 1 mM) on steady-state outward and Na currents. Steady-state outward and peak Na currents were normalized to their respective values before the application of various concentrations of drug, whereas the late Na current block was expressed as the ratio of current amplitudes measured during and after the application of ketamine at a given concentration (see Figure 4). The effects of ketamine were almost identical on steady-state outward, peak Na and late Na currents and could be described assuming a one-to-one reaction between ionic channels and ketamine molecules, with an apparent dissociation constant of 0.7 mM.

Effects of procaine on the ketamine-induced late Na current

Procaine is often used clinically as a supplement during a variety of balanced anaesthetic techniques and specially during ketamine anaesthesia. It was observed that procaine greatly reduced the excitatory effects of ketamine (Wikinski *et al.*, 1980). In order to determine whether the ketamine-induced late Na current was sensitive to procaine, we performed the following experiment. The fibre was first exposed to 1 mM ketamine. After washing with Ringer solution a late Na current appeared (Figure 7a). The addition of 1 mM procaine to the external solution blocked both peak and late Na currents (Figure 7b). After washing with procaine-free solution, both peak and late Na currents reappeared (Figure 7c). However, probably due to a partial suppression of the long term effects of ketamine, the late Na current was smaller than before the application of procaine.

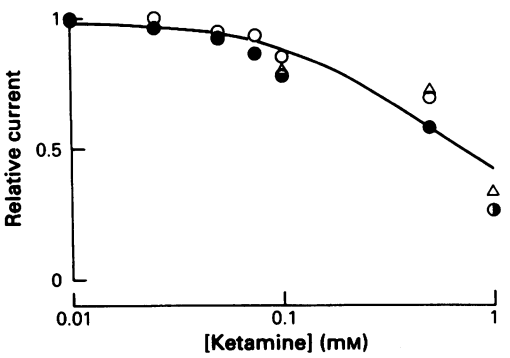

Figure 6 Ketamine dose-response curve for steady-state outward and Na currents. Peak Na current (○) and steady-state outward current (●) were normalized to their respective values in the absence of drug. Late Na current block (△) was expressed as the ratio of its amplitudes measured during and after application of a given concentration of ketamine. The currents were recorded during or at the end of 19 ms depolarizations to 0 mV preceded by 50 ms hyperpolarizations to −120 mV. The curve represents the block of currents assuming a one-to-one reaction between channels and ketamine molecules, with an apparent dissociation constant of 0.7 mM. The curve was drawn by a linear regression analysis ($r^2 = 0.90$).

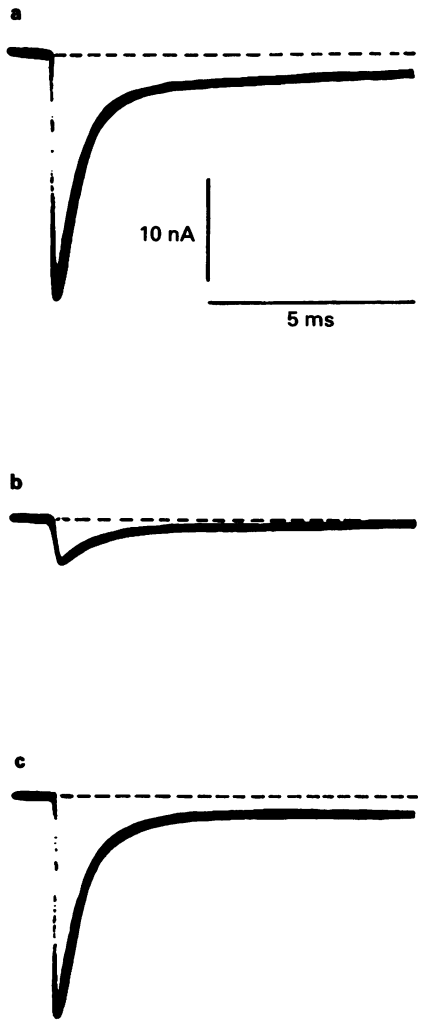

Figure 7 Effects of procaine on peak and late Na currents. Traces of Na current recorded after washout of 1 mM ketamine with Ringer solution (a), in the presence of 1 mM procaine (b) and after washout of procaine with Ringer solution (c), during depolarizations to 0 mV preceded by 50 ms hyperpolarizations to −120 mV. Fibre: 7-03-85 II.

Discussion

The present results show that, in the frog node of Ranvier, external application of 0.1 to 1 mM ketamine, has two major effects on membrane potential and membrane currents.

The first type of effect was to decrease reversibly the amplitude of the action potential and to increase both the duration of the action potential and the threshold potential. The same actions of ketamine on action potential have already been observed in the squid giant axon (Shrivastav, 1977), in the frog skeletal muscle (Marwaha, 1980), in the ventricular trabeculae carneae muscle preparation of the rat (Goldberg *et al.*, 1970) and in frog sciatic nerve (Diamond *et al.*, 1975). These effects can be explained by the reversible block of steady-state outward and peak Na currents by the drug (Figure 2 of the present paper; Marwaha, 1980). The block of membrane currents by ketamine is not significantly different in the frog node of Ranvier and in the squid giant axon. However, in the squid giant axon, the decrease of steady-state outward current was irreversible and the drug was more efficient after internal than after external application (Shrivastav, 1977). In the node of Ranvier, we observed no effects of the drug when it was applied internally at the two cut ends of the fibre. However, in our experimental conditions, ketamine could not diffuse well in the axoplasm and reach the nodal membrane. Nevertheless, as with other anaesthetics, even assuming that the membrane is permeable to ketamine, since the effects of external application of the drug were rapidly and fully reversible (see Figures 1 and 2), one can conclude that it acts preferentially from the outside of the nodal membrane.

The second type of effect was the induction of spontaneous action potentials after washout of the drug, when the K current was blocked. This could be as a consequence of the effects of the drug on membrane currents. Ketamine modified a fraction of Na channels to give rise to a non-inactivatable late Na current. Both normal and ketamine-induced late Na currents were blocked by ketamine (1 mM) but, after washing, the block of both currents reversed rapidly whereas the late Na current persisted. One can assume that, when the K current was blocked and during the first minutes of washout of the drug, the amplitude of the non-inactivatable late Na current was sufficient to depolarize the membrane to the action potential threshold, which could explain the spontaneous activity of the fibre. Depending on the amplitude of the late current, the membrane may or may not be depolarized and so spontaneous action potentials may or may not occur. In the squid giant axon, membrane depolarization and spontaneous firing have been observed after the application of ketamine (Shrivastav, 1977). This author did not mention the appearance of a late Na current either during or after ketamine application (but he had not blocked the K current). However, he observed that, in the presence of ketamine, the reversal potential of the peak current was shifted by about 25 mV towards negative values and the Na current inactivation was slowed. Since, in the node of Ranvier, the ketamine-induced late Na current did not, or only very slowly, inactivate and reverse at a potential more negative than the normal Na current, the observations of Shrivastav can be explained if one assumes that ketamine induces a larger late Na current in the squid giant axon than in the frog node of Ranvier. The last point which deserves attention is the observation that during washout of ketamine, spontaneous action potentials only appeared when the potassium hyperpolarizing current was blocked. In connexion with this observation, it must be noted that, in contrast to the frog node of Ranvier, the potassium current is normally lacking in the mammalian node of Ranvier (Chiu *et al.*, 1979; Brismar, 1980). Consequently, it can be surmised that ketamine should more readily induce spontaneous action potentials and excitatory effects in mammalian than in frog myelinated nerve fibres. Moreover, one can note that, if ketamine induces an important membrane depolarization, it exerts a depressant effect via the inactivation of the normal Na current (see discussion of Shrivastav, 1977).

We conclude that, in the peripheral nervous system, ketamine has both a depressant effect (due to blockade of membrane currents) and an excitatory effect (due to modification of a fraction of Na channels). These effects can explain some of the clinical observations: anaesthesia on the one hand, and excitatory phenomena on the other, following intravenous administration of ketamine.

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