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Blocking Mechanisms of Ketamine and Its Enantiomers in Enzymatically Demyelinated Peripheral Nerve as Revealed by Single-channel Experiments

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Background: **Ketamine shows, besides its general anesthetic effect, a local anesthetic-like action that is due to blocking of peripheral nerve sodium currents. In this study, the stereoselectivity of the blocking effects of the ketamine enantiomers S(+) and R(−) was investigated in sodium and potassium channels in peripheral nerve membranes.**

Methods: **Ion channel blockade of ketamine was investigated in enzymatically dissociated *Xenopus* sciatic nerves in multiple-channel and in single-channel outside-out patches.**

Results: **Concentration-effect curves for the Na^+ peak current revealed half-maximal inhibiting concentrations (IC_{50}) of 347 μM and 291 μM for S(+) and R(−) ketamine, respectively. The potential-dependent K^+ current was less sensitive than the Na^+ current with IC_{50} values of 982 μM and 942 μM. The most sensitive ion channel was the flickering background K^+ channel, with IC_{50} values of 168 μM and 146 μM for S(+) and R(−) ketamine. Competition experiments suggest one binding site at the flicker K^+ channel, with specific binding affinities for each of the enantiomers. For the Na^+ channel, the block was weaker in acidic (*p*H = 6.6) than in neutral (*p*H = 7.4) and basic (*p*H = 8.2) solutions; for the flicker K^+ channel, the block was weaker in acidic and stronger in basic solutions.**

Conclusions: **Ketamine blockade of sodium and potassium channels in peripheral nerve membranes shows no stereoselectivity except for the flicker K^+ channel, which showed a very weak stereoselectivity in favor of the R(−) form. This potential-insensitive flicker K^+ channel may contribute to the resting potential. Block of this channel and subsequent depolarization of the resting membrane potential leads, besides to direct Na^+ channel block, to inexcitability *via* Na^+ channel inactivation. (Key words: Ion channel. Ketamine. Membrane potential. Patch clamp. *p*H dependence. Binding site. Stereoselectivity.)**

AS a general anesthetic, ketamine induces dissociative anesthesia after intravenous administration and has wide clinical applications (for an overview, see White *et al.*[1]).

Two enantiomers of the ketamine molecule exist due to a chiral center at the C_2 atom of the cyclohexanone ring. In laboratory investigations with rats, the S(+) form had a higher therapeutic index than did the racemate and R(−) ketamine.[2] In mice, S(+) ketamine had an analgesic effect that was three times more potent and caused less excitation.[3] In clinical studies with humans, S(+) ketamine produced more effective anesthesia. On the other hand, unwanted agitation was pronounced in patients who received the R(−) form.[4]

Laboratory investigations have revealed different target sites for the ketamine molecule. Blockade of synaptic transmission has been observed in guinea pig brain slices and rat olfactory cortex.[5,6] Furthermore, opioid receptors may be targets of ketamine, suggesting an opioid-like analgesic effect *via* these receptors,[7,8] although opposing views exist.[9] Frenkel and Urban[10] showed that batrachotoxin-activated human brain Na^+ channels in lipid bilayer membranes were blocked only by very high concentrations (half-maximal inhibiting concentrations [IC_{50}], 1.1 mM) of ketamine. Ketamine affects cholinergic[11,12] and N-methyl-D-aspartate receptors,[13,14] and it is speculated that the latter effect is the main mechanism for ketamine's general anesthetic action. The N-methyl-D-aspartate receptor also has shown a stereoselectivity for ketamine in favor of S(+).[15,16] Zeilhofer *et al.*[16] found an IC_{50} of 0.8 μM for S(+) and 1.5 μM for R(−) ketamine. In the end, however, the overall mechanism of ketamine action on the nervous system remains unclear.

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Besides the general anesthetic effect, ketamine also shows local anesthetic properties when applied at the peripheral nerve. In clinical studies, ketamine has been used for intravenous regional,[17,18] spinal,[19] and epidural[20] anesthesia and for regional pain treatment.[21] The local anesthetic effect has been related to a depression of the potential-sensitive Na^+ and K^+ currents in the peripheral nerve, as shown in voltage-clamp investigations.[22-24] The concentrations necessary, however, were much greater than those in clinical systemic administration of general anesthesia and could only be reached by local application.

Recently it has become possible to use the patch-clamp technique[25] to observe single ion channels at the membrane of the amphibian peripheral nerve[26] and even on human myelinated axons.[27,28] Drug effects can now be studied directly at the nerve membrane, where diffusion barriers such as the myelin sheath or connective tissue surrounding the fiber (peri- and epineurium) have been removed. Furthermore, the pharmacologic effect of a drug on each channel type of the axonal membrane can be observed independently, and the relative sensitivity of channels can be compared. Finally, investigation of potential-insensitive K^+ channels has become feasible. They are directly connected with the generation of the resting potential[29] and thus have another and as-yet underestimated effect on excitability.

We used all of these approaches to study the local anesthetic effect of ketamine and its enantiomers on different types of ionic channels and to investigate the mechanism of ketamine block at the peripheral nerve membrane.

Materials and Methods

Preparation

The patch-clamp method[25] was applied to sciatic nerves[26] of the clawed toad *Xenopus laevis*. Animals were killed by decapitation. These procedures were reported to the local veterinarian authority and are in accordance with German guidelines.

Nerves were dissected and desheathed mechanically and incubated with 3 mg/ml collagenase (Worthington type CLS II; Biochrom, Berlin, Germany) in Ringer's solution for 135 min and subsequently with 1 mg/ml protease (type XXIV; Sigma Chemical Co., St. Louis, MO) in Ca^{2+}-free Ringer's solution for 35 min at a temperature of 24°C. Afterward the preparation was washed in Ca^{2+}-free Ringer's solution, cut into 3-mm-long segments, and put into a culture dish coated with laboratory grease (Glisseal blue; Borer Chemie, Solothurn, Switzerland). Axons were used after a 30-min period of rest for as long as 10 h.

Electrophysiologic Techniques

Patch pipettes were pulled from borosilicate glass tubes (GC 150; Clark Electromedical Instruments, Pangbourne, UK), coated with Sylgard 184 (Dow Corning, Seneffe, Belgium), and fire-polished before the experiment. The pipettes used had a resistance of 8-35 MΩ. Currents were measured using an EPC 7 patch-clamp amplifier (List, Darmstadt, Germany) and stored on video tape using a modified PCM-501ES pulse code modulation unit (Sony, Tokyo, Japan). For analysis, data were filtered with a four-pole low-pass Bessel filter and digitized with a Labmaster TM-40 AD/DA board (Scientific Solutions, Solon, OH). Recordings were made at 14 ± 2°C. Membrane potentials (E) are given for the inner side with respect to the outer side of the membrane.

Experiments were performed with external solutions containing either 105 mM KCl, 13 mM NaCl, 2 mM $CaCl_2$, 5 mM BES (N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid), and 100 nM tetrodotoxin (TTX) (105 mM K_o) or 110 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 5 mM BES and either 100 nM TTX (Ringer-TTX) or 10 mM tetraethylammonium (Ringer-TEA) all adjusted to *p*H 7.4 with tris(hydroxymethyl)aminomethane (TRIS) base. For *p*H experiments, external solutions were titrated to *p*H 6.6 and *p*H 8.2 with HCl and TRIS base. The internal solution contained 105 mM KCl, 13 mM NaCl, 5 mM BES, 3 mM ethylenebis(oxyethylenenitrilo)-tetraacetate adjusted to *p*H 7.2 with TRIS base (KCl_i). In the internal solution used for sodium current investigations, KCl was replaced by CsCl ($CsCl_i$). S(+) and R(−) ketamine and the racemic mixture (preservative free) were provided by Dr. Gebhardt, Parke Davis, Freiburg, Germany. The substances were dissolved in distilled water to yield 100 mM stock solutions. Drugs were applied to excised outside-out patches using a multiple-barrel perfusion system. In some experiments, the drug was also applied to the axoplasmic side of the membrane using excised inside-out patches.

Data Analysis

Current records were digitized and analyzed using pCLAMP 5.5.1 software (Axon Instruments, Burlingame, CA). Numeric values are given as means ± SEM or fitted values ± SE of the fit, and error bars are plotted as SEM. Significance of differences in means was tested using the Student's *t* test for equal variances, and equal-

ity of variances were tested using Fisher's F test. Concentration-effect curves and histograms were fitted with a computer by nonlinear least-squares fit with the corresponding function as noted in Materials and Methods. Statistical analysis, fittings, and preparation of the figures were done with Fig. P 6.0 software (Biosoft, Cambridge, UK).

Na^+ Currents

For the investigation of Na^+ currents, potassium currents were suppressed using Ri-TEA as the external and $CsCl_i$ as the internal solution. The membrane holding potential (E_h) was set to −90 mV in the voltage-clamp mode and Na^+ currents were elicited using a 50-ms step to −40 mV after a hyperpolarizing prepulse of 50 ms to −130 mV. When formed at the nodal region of the axon, outside-out patches contained many Na^+ channels, which resulted in a macroscopic Na^+ current up to 100 pA. Twenty traces were averaged to measure the peak Na^+ current for further analysis (fig. 1A). To investigate the potency of the ketamine isomers, the averaged peak Na^+ current was determined in control solution and in drugs. Fractional block was calculated as the peak current in ketamine divided by the peak current in control. For direct comparison of the blocking potency on the peak Na^+ current, 300 μM S(+), R(−), and racemate were applied randomly on each patch in separate experiments.

To investigate the effect of *p*H alterations on ketamine block, peak Na^+ currents were first measured at different *p*H values (6.6, 7.4, and 8.2) without ketamine and then after adding 300 μM ketamine racemate to each of the three solutions. To compare the results, values of the peak Na^+ current were normalized to the control at *p*H 7.4 in drug-free Ringer's solution. The peak Na^+ current in control was decreased at lower *p*H and increased in the basic solution (fig. 1C).

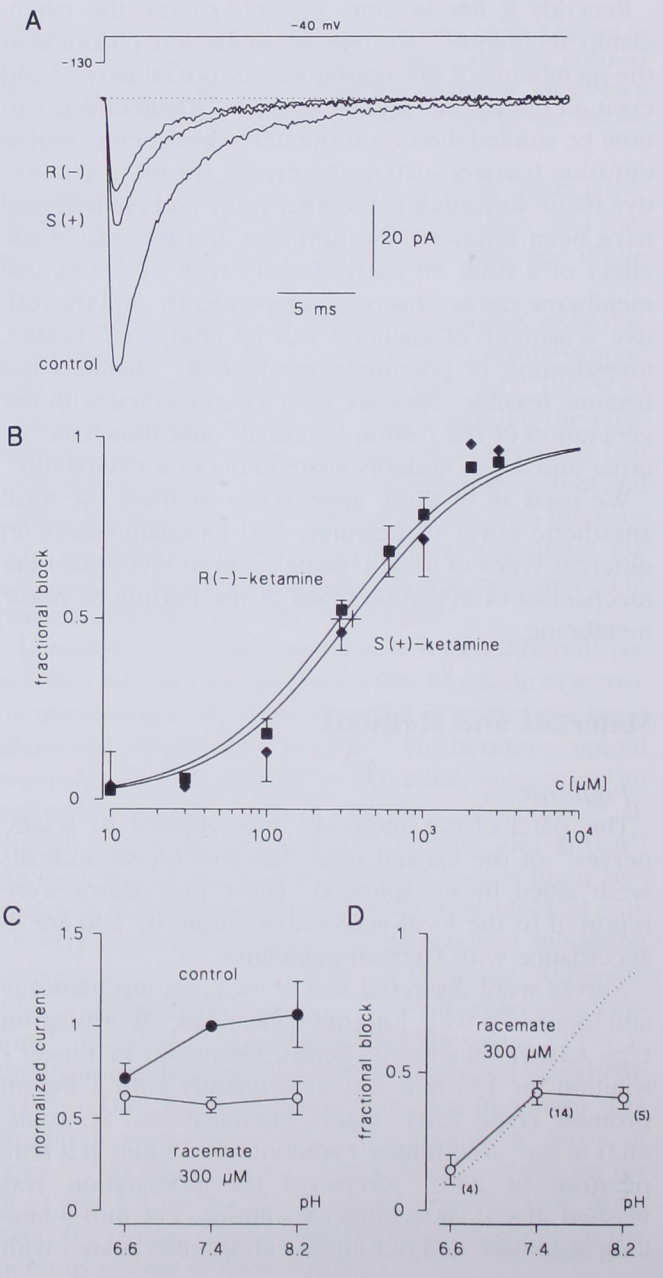

Fig. 1. Blockade of Na^+ currents by ketamine. (*A*) Recordings of a multiple Na^+ channel outside-out patch in Ri-TEA solution (control) and after addition of 300 μM S(+) and R(−) ketamine to the external solution. Each trace represents averaged peak Na^+ current of 20 recordings; the pulse protocol is given above the traces: 50 ms prepulse to −130 mV, 50 ms test pulse to −40 mV, holding potential E_h = −90 mV. The solution in the pipette contained $CsCl_i$, the filter frequency was 2 kHz, and the temperature was 12.5 ± 1 °C. The dotted line gives zero current level. Leakage and transient currents were subtracted as described in Materials and Methods. (*B*) Concentration (c) dependence of fractional block of peak Na^+ current. Curves represent nonlinear least-squares fits of the function $f(c) = 1/[1 + (IC_{50}/c)^h]$ to the data points (mean ± SEM) assuming a one-to-one reaction (Hill coefficient of h = 1), giving IC_{50} values of 347 μM and 291 μM (marked as crosses) for S(+) (diamonds) and R(−) ketamine (squares), respectively. Data are from 23 patches, including that of figure 1*A*. (*C*) Peak Na^+ currents measured at *p*H 6.6, 7.4, and 8.2 in Ringer-TEA solution (control, filled circles) and after addition of 300 μM ketamine racemate (open circles) normalized to the control value at *p*H 7.4. E_h = −90 mV, pulse protocol as in figure 1*A*. The number of patches is noted in figure 1*D*. (*D*) *p*H dependence of fractional racemate block at the corresponding *p*H values. The number of outside-out patches is indicated next to each symbol. Data were calculated from figure 1*C*. The dotted line gives the theoretical fraction of deprotonated (neutral) ketamine independence of *p*H (pK_a = 7.5).

Delayed Rectifier K^+ Currents

When K^+ channels were investigated in Ri-TTX to suppress Na^+ currents, the internal solution was KCl_i. The holding potential was set to −90 mV and then stepped for 50 ms to +60 mV to elicit potential-activated K^+ outward currents in control solution and in different ketamine concentrations. To reduce noise, 20 recordings were averaged, and the fractional block was calculated as the ratio of the current amplitudes measured during the last 20 ms of the 50 ms pulse in ketamine divided by the amplitude in control.

Potential-insensitive Flicker K^+ Currents

Investigations were conducted on outside-out patches of mainly thin nerve fibers (approximately 5 μm in diameter). The bath consisted of 105 mM K_o, and KCl_i served as the pipette solution, giving a potassium reversal potential of 0 mV. Holding potential was continuously set to −90 mV, and no test pulses were applied. To quantify the ketamine block, the mean current was measured over at least 30 s each in control and in the test solutions. In some experiments, inside-out patches were used to administer ketamine from the axoplasmic side. In these experiments, the pipette contained 105 mM K_o and the bath solution was KCl_i.

To determine if S(+) and R(−) ketamine act at the same binding site on the channel, competition experiments were done with these two agents. In these experiments, flicker channels were blocked to a certain extent with a fixed concentration of R(−) ketamine. Concentration-effect relations with these partly blocked channels reveal half-maximal blocking concentrations ($IC_{50,app}$) for S(+) ketamine, depending on the amount of previously blocked channels and on the number of binding sites.

Assuming competition for one binding site, higher (*i.e.*, shifted) IC_{50} values should result, depending on the simultaneously applied R(−) concentration. If the two isomers act at different binding sites, the IC_{50} data for the S(+) isomer should be independent of the R(−) concentration. The mathematical relation for competition of two ligands for one binding site is $IC_{50,S(+),app} = IC_{50,S(+)} \cdot (1 + [R(-)]/IC_{50,R(-)})$, where $IC_{50,S(+),app}$ is the apparent (shifted) half-maximal blocking concentration of S(+) ketamine, [R(−)] is the concentration of R(−) ketamine, and $IC_{50,S(+)}$ and $IC_{50,R(-)}$ are half-maximal inhibiting concentrations of the corresponding ketamines.

In three sets of experiments, patches were preincubated with 200, 500, or 1,000 μM R(−) ketamine, blocking the mean current to a certain degree, and then different concentrations of S(+) ketamine were added.

Table 1. Blocking Potency of Ketamine Enantiomers (IC_{50})

	S(+) (μM)	R(−) (μM)	Racemate (μM)
I_{Na^+} peak	347 ± 55 (6)	291 ± 23 (9)	325 ± 35 (5)
I_{K^+} peak	982 ± 152 (6)	942 ± 110 (6)	
I_{K^+} flicker	168 ± 26 (7)	146 ± 19 (8)	152 ± 23 (7)
I_{K^+} flicker (internal)			216 ± 22 (4)

Values obtained from nonlinear least-squares fit ± standard error of the fit (number of experiments in parentheses).

The fractional block induced by these S(+) ketamine concentrations was measured with respect to the partially R(−)-blocked current.

Current-clamp Experiments

For current-clamp experiments, outside-out patches were formed in 105 mM K_o bath solution and checked for the presence of flicker channels in the voltage-clamp mode, as described previously. After switching to current-clamp mode, a spontaneous membrane potential of approximately 0 mV was measured. Changing the bath solution to Ringer's immediately hyperpolarized the membrane to −70 mV. Ketamine was then applied externally to determine its effect on the spontaneous resting potential of an outside-out patch.

Results

During and after enzymatic treatment, the myelin sheath of some axons gradually retracted from the node of Ranvier, leaving parts of the excitable membrane clean and well accessible to the patch pipette. Either nodal or paranodal regions could be chosen for forming outside-out patches. This is important because only the narrow nodal region of the excitable axon membrane contains Na^+ channels at high densities, whereas most of the K^+ channel types also can be found in paranodal regions.[26] The blocking effects of racemate and stereoisomers of ketamine were investigated on Na^+ and different K^+ conductances.

Na^+ Currents

S(+) and R(−) ketamine blocked the peak axonal Na^+ current in a concentration-dependent manner (fig. 1A). The block was fully reversible in a few seconds after washout (data not shown).

Concentration-effect curves were constructed from the fractional block of the peak inward Na^+ current induced by different concentrations of S(+) and R(−) ketamine (fig. 1B). Table 1 lists the half-maximal in-

Table 2. Blocking Potency of Ketamine Enantiomers (Fractional Block)

	S(+)	R(−)	Racemate
I_{Na^+} peak (300 μM)	0.40 ± 0.05 (9) (NS)	0.50 ± 0.03 (12) (NS)	0.47 ± 0.03 (6)
I_{K^+} peak (1000 μM)	0.51 ± 0.03 (6)	0.51 ± 0.03 (6)	
I_{K^+} flicker (200 μM)	0.47 ± 0.02 (7)*	0.58 ± 0.03 (8)*	0.51 ± 0.05 (8)
I_{K^+} flicker (200 μM/internal)	0.37 ± 0.07 (5) (NS)	0.50 ± 0.04 (5) (NS)	0.47 ± 0.07 (5)

Values are mean ± SEM (number of experiments in parentheses).

* Significant difference ($P < 0.01$) of mean values.

hibiting concentrations (IC_{50}), as revealed by nonlinear least-squares fit. For comparison, table 2 lists fractional block values induced by 300 μM of the different ketamines. These data were also included in the concentration-effect relations mentioned previously. In figure 1D, the fractional block of 300 μM ketamine is given for each *p*H value as related to the current in drug-free solution at the corresponding *p*H. Table 3 lists the fractional block values. Obviously the ketamine block is stronger at basic *p*H than in the more acidic solution.

Delayed-rectifier K^+ Currents

The number of K^+ channels in the outside-out patches was high enough to obtain macroscopic delayed-rectifier currents. The ketamine enantiomers also suppressed this current (fig. 2A), but higher concentrations were needed to obtain the same effect as for the sodium channel. The block was fully reversible. Concentration-effect curves were also constructed for this potential-dependent K^+ current for S(+) and R(−) ketamine (fig. 2B, table 1). For direct comparisons of the fractional block induced by 1 mM of the two enantiomers, see table 2. Because of the relative insensitivity of these channels, the blocking effects of ketamine and its enantiomers on the known subtypes I, F, and S of the delayed-rectifier K^+ channels[26] have not been studied in detail.

Potential-insensitive Flicker K^+ Currents

Ketamine also blocked a TEA-insensitive, voltage-independent flickering background K^+ channel.[29] Figure 3A presents a recording from a macropatch containing many flicker K^+ channels, which can be identified easily by their flickering appearance (see also figure 4A). Because of the symmetrical K^+ concentration at either side of the membrane used in these experiments, K^+ currents were inwardly directed at the holding potential (E_h) of −90 mV, which is close to the normal resting potential of the peripheral nerve. Thus voltage-dependent K^+ channels are not activated at this potential and thus cannot disturb the measurements.

As shown in figure 3A, increasing ketamine concentrations decrease the mean current through flicker K^+ channels. The block was fully reversible after washout of ketamine. The concentration relation for S(+) and R(−) ketamine for this channel is given in figure 3B, the corresponding IC_{50} values are listed in table 1, and direct comparison of the fractional block induced by 200 μM is given in table 2.

Dependence of flicker K^+ channel block on *p*H was measured in the same manner as for the Na^+ currents, but with mean currents of multiple-flicker K^+ channel patches (figs. 3C and D). Again the block was lower in acidic and stronger in basic solutions. In contrast to the Na^+ channel, the ketamine block at this channel was much more dependent on *p*H (table 2).

Internal application of ketamine using inside-out patches revealed a slightly lower blocking potency than was the case when it was applied externally (tables 1 and 2).

Mode of Single-channel Block

Using pipettes with higher resistance (see Materials and Methods), we could record only one flicker K^+

Table 3. *p*H Dependence of Ketamine Racemate Block (Fractional Block)

	*p*H		
	6.6	7.4	8.2
I_{Na^+} peak (300 μM)	0.15 ± 0.05 (4)	0.43 ± 0.04 (14)	0.41 ± 0.04 (5)
I_{K^+} flicker (200 μM)	0.24 ± 0.06 (3)	0.59 ± 0.05 (11)	0.86 ± 0.02 (3)

Values are mean ± SEM (number of experiments in parentheses).

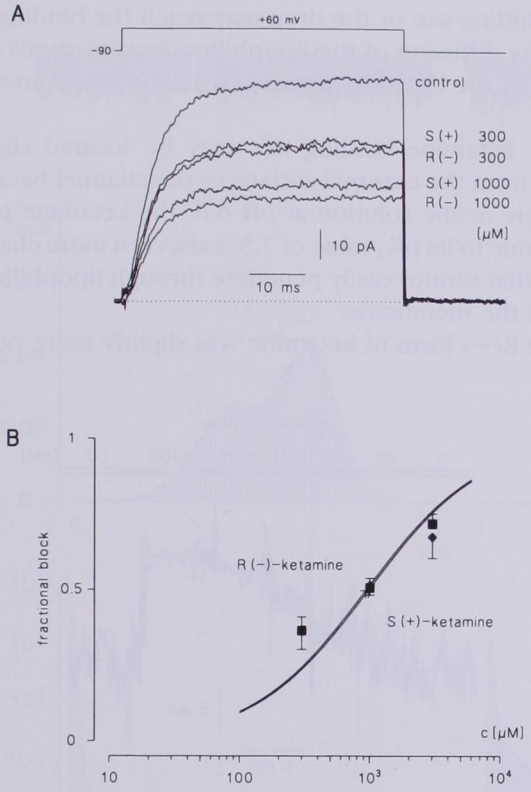

Fig. 2. Blockade of potential-dependent K^+ currents by ketamine. (*A*) Steady-state outward K^+ currents recorded from a macroscopic outside-out patch in Ringer-TTX solution (control) and after addition of 300 μM and 1 mM S(+) and R(−) ketamine, respectively. Each trace represents the averaged current of 20 recordings; pulse protocol is shown above the traces: 50 ms test pulse to +60 mV, E_h = −90 mV. Solution in pipette, KCl_i; filter frequency, 1 kHz; temperature, 14°C. Dotted line shows zero current level. (*B*) Concentration dependence of fractional steady-state outward K^+ current blockade. Curves fitted as in figure 1*B* using h = 1, giving IC_{50} values of 982 μM and 942 μM for S(+) and R(−) ketamine, respectively. Symbols for isomers at 300 μM and 1 mM are superimposed; corresponding error bars for R(−) are given upward and for S(+) ketamine are shown downward. Data are from four patches, including that of figure 2*A*.

channel from outside-out patches. Figure 4A shows the kinetic behavior of this channel under control conditions and when perfused with test solution at the external side. The blocking effect of the ketamine enantiomers is induced by short closures of the channel, rather than by reducing single-channel conductance, which becomes obvious from the single-channel recordings. To quantify this observation, amplitude histograms were constructed from recordings lasting one minute each in control and in 200 μM S(+) and R(−) ketamine (fig. 4B). The single-channel current calculated from the amplitude histograms was 3.1 ± 0.2 pA in control, 3.3 ± 0.2 pA in S(+) ketamine, and 3.3 ± 0.3 pA in R(−) ketamine (n = 3 each) at E_h = −90 mV. Thus the amplitudes were not different, indicating that ketamine does not affect the single-channel amplitude.

Closed-time histograms (fig. 4C) constructed from the same recordings typically showed many short closures in control solution, which correspond to the flickering of the channel. The closed-time distribution in control solution was fitted with a single exponential time constant of 0.19 ± 0.01 ms (n = 6), despite the presence of a few longer closures that contribute to a second and third closed-time constant.[29] But because these closures are comparatively few (a fraction of 10^{-3}), they were omitted from the analysis. This fast time constant (τ_{cf}) remained nearly unchanged in the presence of S(+) and R(−) ketamine, where it was measured as 0.20 ± 0.03 ms (n = 5) and 0.18 ± 0.02 ms (n = 3), respectively. However, the isomers induced many closures that overlapped the brief flickering events. These longer closures clearly induced a second exponential component (τ_{cs}) in the closed-time histogram, with values of 2.70 ± 0.10 ms (n = 5) for S(+) ketamine and 3.84 ± 0.20 ms (n = 3) for R(−) ketamine, revealing a highly significant difference in closed time. These time constants represent the mean duration of the ketamine molecule bound to the channel structure. Thus we conclude that the affinity of the R(−) ketamine to the flicker K^+ channel is slightly higher than that of the S(+) form.

Competition between the Two Enantiomers

To determine whether ketamine block was induced by different channel binding sites for S(+) and R(−) isomers, we did competition experiments on multiple-channel patches. Different R(−) ketamine concentrations were used to induce block of flicker K^+ channels to different extents. Dose-response relations done with S(+) ketamine with partially blocked channels revealed increasing apparent IC_{50} values with increasing R(−) concentrations (fig. 5), indicating that both enantiomers compete for the same binding site.

Membrane Potential

Because the open probability of the flicker K^+ channel has been described to be fairly potential insensitive,[29] its activity may well contribute to the normal resting membrane potential. Thus we studied the effects of the two ketamine isomers when applied to an outside-out macropatch under current-clamp conditions. In this experiment, both ketamine isomers caused immediate and completely reversible depolarization, which is en-

hanced by adding TEA (fig. 6), which had only had a weak depolarizing effect when used alone.

Discussion

Increasing sensitivity of ionic channels to ketamine at the peripheral nerve membrane has been found as delayed-rectifier K^+ channels < Na^+ channels < flicker K^+ channels. In rat dorsal root ganglion cells, the IC_{50} values for N- and L-type Ca^{2+} channels are 6,000 and 3,000 μM and 800 and 400 μM for S(+) and R(−) ketamine, respectively, and 2 μM for the racemate at the T-type Ca^{2+} channel.[30] Half-maximal blocking concentrations (IC_{50}) for the peak Na^+ current varied from 700 μM in the frog[23] and 2 mM in the clawed toad sciatic nerve.[24] The lower IC_{50} values of ketamine that we found in this study might be due to the difference in species and in experimental conditions. Specifically, the drug molecule was allowed direct access to the ion channel and diffusion barriers such as the endoneurium were removed during the previous enzymatic treatment.

Lower blocking potency in acidic solution corresponds with the general clinical observation in inflammatory tissue for local anesthetics. The lower blocking potency of ketamine at acidic *p*H values may be due to the pronounced protonation of the ketamine, which could impede its access to the drug-binding site. Thus a lipophilic interaction may exist between the drug and the binding site or the drug may reach the binding site only by diffusion of the lipophilic (deprotonated) drug through the lipid bilayer, as is true with local anesthetics.

The ketamine binding site may be located slightly away from the external surface of the channel because, in more acidic solution at *p*H 6.6, the ketamine molecule, due to its pK_a value of 7.5, exists in a more charged form that cannot easily penetrate through lipophilic layers of the membrane.

The R(−) form of ketamine was slightly more potent

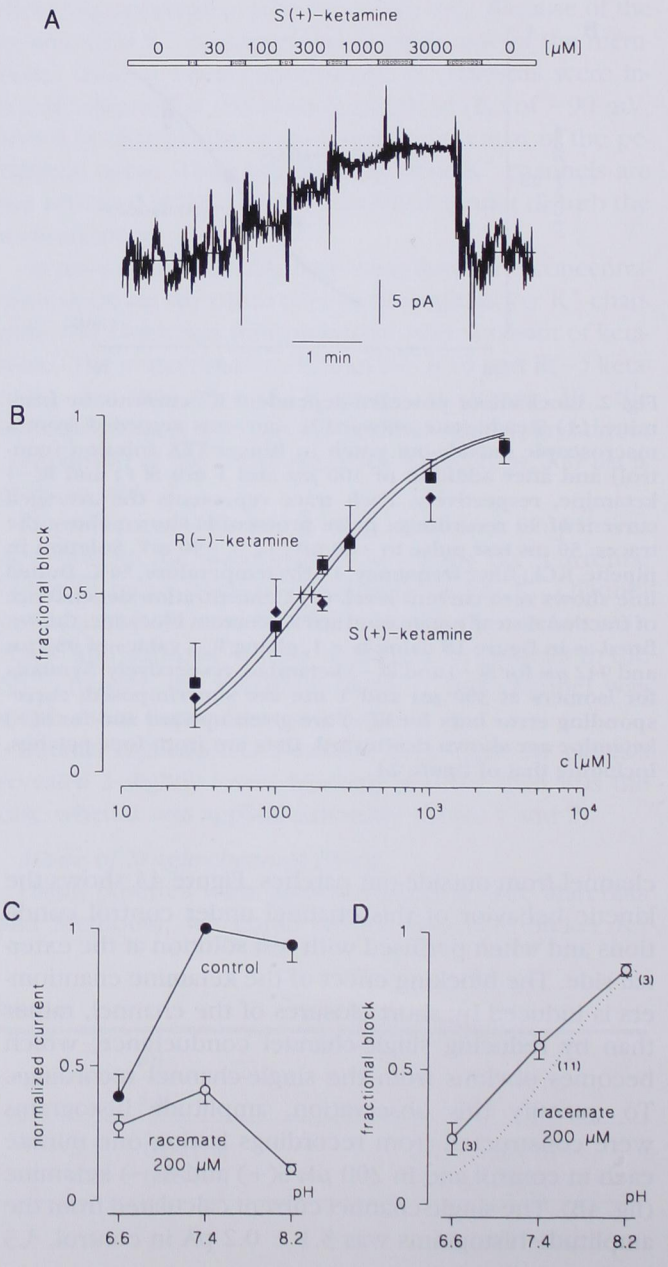

Fig. 3. Blockade of potential-insensitive flicker K^+ currents by ketamine. (*A*) decrease of flicker K^+ channel current due to increasing S(+) ketamine concentrations. Outside-out patch containing many flicker K^+ channels. Bath was composed of 105 mM K_o and the pipette was filled with KCl_i. Recording shows inward current at E_h = −90 mV, filter frequency of 60 Hz, and temperature of 14°C. Upper scale shows different concentrations of S(+) ketamine and duration of application; dotted interceptions represent periods of solution exchange. The dotted line shows the zero current level. Mean current amplitudes at corresponding concentrations are indicated as solid lines through the trace. (*B*) Concentration dependence of fractional flicker K^+ current block. Curves fitted as in figure 1*B* using h = 1, giving IC_{50} values of 168 μM and 146 μM for S(+) and R(−) ketamine, respectively. Data are from 23 patches, including that of figure 3*A*. (*C*) Mean flicker K^+ currents measured at *p*H 6.6, 7.4, and 8.2 in 105 mM K_o (control, filled circles) and after addition of 200 μM ketamine racemate (open circles) normalized to the control value at *p*H 7.4. E_h = −90 mV. The number of patches is noted in figure 3*D*. (*D*) *p*H dependence of fractional racemate block obtained as in figure 1*D*. The number of outside-out patches is indicated next to each symbol.

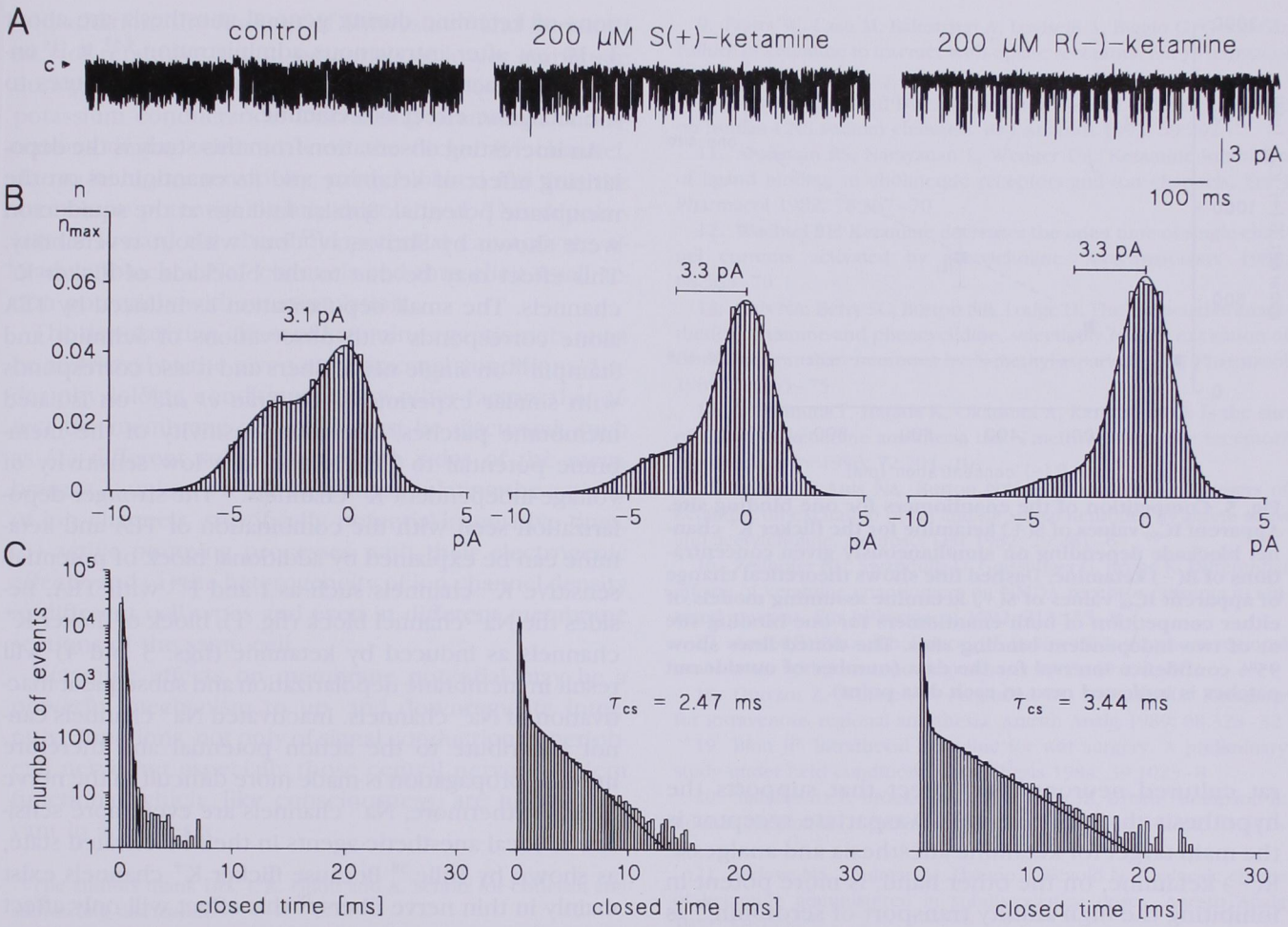

Fig. 4. Analysis of flicker K^+ channel blockade by ketamine. (*A*) Recordings of a single channel from an outside-out patch in 105 mM K_o (control) and after addition of 200 μM S(+) and R(−) ketamine to the external solution. Opening events are shown as downward deflections; *c* and arrowhead indicate the current level of the closed state. E_h = −90 mV; pipette contains KCl_1; filter frequency, 2 kHz; sample interval, 250 μs; temperature, 15 ± 1°C. (*B*) Estimation of single-channel amplitude using amplitude histograms from the same experiment as in figure 4*A*. Curves represent the sum of two Gaussian curves for the closed and open state of the channel. Filter frequency, 10 kHz; sample interval, 26 μs; bin width, 0.2 pA. Histograms are normalized as n/n_{max}, where n is the number of points in each bin and n_{max} is the total number of points. The single-channel current is measured as the difference between the two Gaussian peaks. (*C*) Kinetic properties of single flicker K^+ channel block. Frequency density is plotted *versus* time. Histograms were fitted with one exponential τ_{cf} = 0.17 ms in control solution and two exponentials in ketamine, revealing mean closed times τ_{cf} = 0.15 ms and τ_{cs} = 2.47 ms in S(+) and τ_{cf} = 0.14 ms and τ_{cs} = 3.44 ms in R(−) ketamine. Filter frequency was 5 kHz and sample interval was 20 μs.

than its optical antipode S(+) in blocking the channels we investigated, and except for the flicker K^+ channel there were no significant differences. These statistical differences, however, are too small to indicate clear stereoselectivity. According to Pfeiffer,[31] who formulated the general rule that stereoselectivity is high for substances with high potency and low for substances with low potency, this is not surprising because the ketamine concentrations needed in our experiments are comparatively high. At the flicker K^+ channel, however, closed-time histograms show a pronounced difference for the ketamine enantiomers.

In contrast to the higher affinity of the R(−) ketamine that we found, several studies in animals and in humans showed that S(+) ketamine is the more potent form. It has a better therapeutic index[2] and shows less agitation and emergence reactions after anesthesia than does the R(−) form.[4] On the molecular level, S(+) ketamine is about twice as potent in blocking N-methyl-D-aspartate receptor currents in

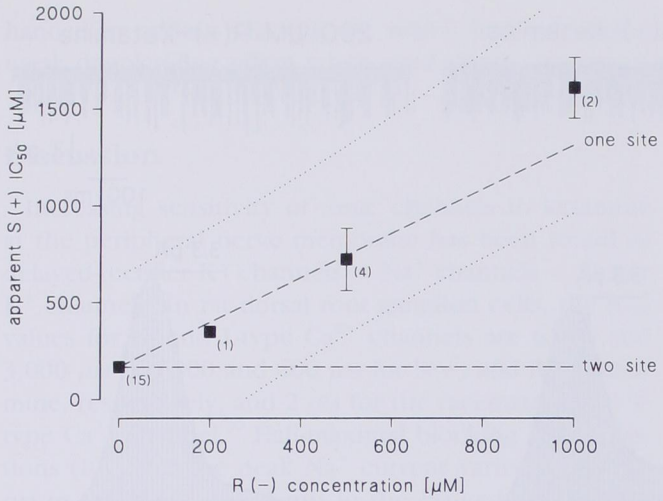

Fig. 5. Competition of the enantiomers for one binding site. Apparent IC_{50} values of S(+) ketamine for the flicker K^+ channel blockade depending on simultaneously given concentrations of R(−) ketamine. Dashed line shows theoretical change of apparent IC_{50} values of S(+) ketamine assuming models of either competition of both enantiomers for one binding site or of two independent binding sites. The dotted lines show 95% confidence interval for the data (number of outside-out patches is indicated next to each data point).

rat cultured neurons,[16] an effect that supports the hypothesis that the N-methyl-D-aspartate receptor is the main target for ketamine anesthesia and analgesia. R(−) ketamine, on the other hand, is more potent in inhibiting the high-affinity transport of serotonin[32] as well as inhibiting acetylcholinesterase.[33]

Axonal ion channels and other potential-dependent ion channels are relatively insensitive to general anesthetics, including ketamine. But the overall effects of anesthetics on axonal ion channels could be important for general anesthesia.[34] But because plasma concentrations of ketamine during general anesthesia are about 3–10 μM after intravenous administration,[4,35] it is unlikely that action on peripheral nerves contributes to the analgesic effect of ketamine.

An interesting observation from this study is the depolarizing effect of ketamine and its enantiomers on the membrane potential. Similar findings at the squid axon were shown by Shrivastav[22] but without reversibility. This effect may be due to the blockade of flicker K^+ channels. The small depolarization as induced by TEA alone corresponds with observations of Schmidt and Stämpfli[36] on single nerve fibers and it also corresponds with similar experiments by Bräu *et al.*[37] on isolated membrane patches. The low sensitivity of the membrane potential to TEA is due to a low sensitivity of voltage-independent K^+ channels.[28] The stronger depolarization seen with the combination of TEA and ketamine can be explained by additional block of potential-sensitive K^+ channels such as I and F[26] with TEA. Besides the Na^+ channel block (fig. 1), block of flicker K^+ channels as induced by ketamine (figs. 3 and 4) will result in membrane depolarization and subsequent inactivation of Na^+ channels. Inactivated Na^+ channels cannot contribute to the action potential and therefore impulse propagation is made more difficult in the nerve fiber. Furthermore, Na^+ channels are even more sensitive to local anesthetic agents in their inactivated state, as shown by Hille.[38] Because flicker K^+ channels exist mainly in thin nerve fibers,[29] this effect will only affect thin fibers with modalities such as pain, as already been observed by Weber *et al.*[39] Because the analgesic effect of ketamine is stronger with the S(+) isomer,[2] the former mechanism with a higher sensitivity to the R(−) form, however, may only play an additional role in ketamine analgesia.

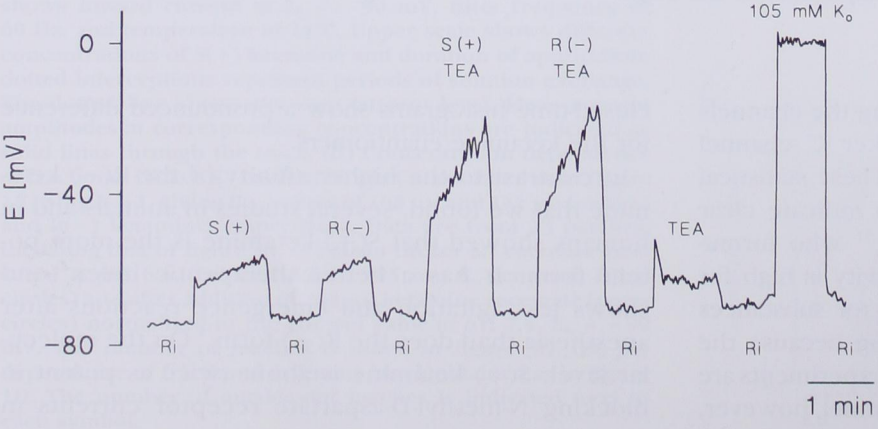

Fig. 6. Reversible depolarization of membrane potential. Outside-out macropatch in current-clamp mode. The bath solution contained Ringer's solution followed by Ringer's plus 1 mM S(+) ketamine, 1 mM R(−) ketamine, each enantiomer plus 10 mM TEA, 10 mM TEA, and 105 mM K_o. The pipette contained KCl_i, and the temperature was 14°C.

In addition, the findings of Shrivastav[22] and Benoit *et al.*[24] of repetitive action potentials of lower amplitude during ketamine washout can be explained by blocked potassium conductance. Decreasing ketamine concentrations during washout leave the flicker K^+ channel, due to its higher sensitivity, partly blocked and thus the membrane potential slightly depolarized. This depolarization toward the threshold potential can activate already unblocked Na^+ channels and thus elicit small action potentials without stimulation.

The depolarizing effect of ketamine enantiomers must be restricted to the given experimental conditions. Under physiologic conditions, many other factors that affect the membrane potential must be discussed, such as (1) different solutions on both sides of the membrane, (2) unknown mechanisms regulating the activity of ion channels, specifically potential-insensitive ones, (3) active pumping processes with their electrogenic effects, and (4) the heterogeneity of ion channel density in different cell types and even in different membrane sections of the same cell.

Ketamine effects on membrane potential may be a powerful mechanism to up- and downregulate integrative functions, not only of signal conduction in peripheral nerve but especially those central nervous system functions, which, like consciousness, are highly relevant in anesthesia.

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