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Blockade by local anaesthetics of the single Ca^{2+}-activated K^+ channel in rat hippocampal neurones

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1 Effects of local anaesthetics on single Ca^{2+}-activated K^+ channels were investigated using the inside-out configuration of the patch-clamp technique in single pyramidal neurones, which were freshly dissociated from rat hippocampus by use of proteolytic enzymes.

2 No significant effect was observed when 2 mM benzocaine was applied on either side of the membrane patch, or when 2 mM lignocaine or QX-314 was applied to the external surface of the membrane.

3 Lignocaine 1 mM, applied to the internal surface, slightly reduced the amplitude of the single K^+ channel current. When applied to the internal surface, QX-314 reduced the amplitude of the K^+ channel current, accompanied by an increase in noise in the open channel current, suggesting a fast flickering block. The blocking effect of QX-314 on the outward current increased with depolarization, suggesting a binding site for the drug at an electrical distance of about 0.5 across the membrane field.

4 The open time histogram showed one exponential component and the closed time histogram showed at least two components. The mean open time of the outward current was increased when the amplitude was reduced by the drugs.

5 The ionized form of the local anaesthetics had a similar action on the Ca^{2+}-activated K^+ channels to that on Na^+ channels, that is, they enter into the channel from the cytoplasmic side to induce open channel block. The blocking kinetics, however, might be so fast that they were beyond the frequency response of our recording apparatus, thus the recorded current amplitude was decreased. In contrast the K^+ channel was not accessible via hydrophobic pathways for the neutral form, which is also known to block the sodium channel.

Keywords: Lignocaine; benzocaine; QX-314; Ca^{2+}-activated K^+ channel; patch clamp; hippocampal neurone

Introduction

Mechanisms of ion channel blockade by local anaesthetics have been extensively studied in sodium (Na^+) channels in various tissues (Strichartz, 1973; Courtney, 1975; Khodorov *et al.*, 1976; Hille, 1977a,b; Hondeghem & Katzung, 1977; Schwarz *et al.*, 1977; Cahalan, 1978; Starmer, 1987; Strichartz & Ritchie, 1987). It is thought that an electrically neutral form of anaesthetic molecules applied in the external solution partition into the membrane. A proportion of these molecules directly affects the Na^+ channel, and others diffuse into the cytoplasm, where their cationic form blocks the channel from the internal mouth of the channel (Frazier *et al.*, 1970; Strichartz, 1976; Hille, 1977a,b). However, detailed studies on the effect of local anaesthetics on potassium (K^+) channels are still lacking at the single channel level, with the exception of work on the epithelial K^+ channels (Richards & Dawson, 1986). In the case of general anaesthetics, it has been demonstrated that they hyperpolarize the hippocampal pyramidal cells by increasing potassium conductance (Nicoll & Madison, 1982; see also Franks & Lieb, 1988). In this study, we describe the actions of local anaesthetics on single calcium (Ca^{2+})-activated K^+ channels.

The Ca^{2+}-activated K^+ channels, having a large conductance of around 250 pS at symmetrical 150 mM K^+ concentrations ($[K^+]$), are widely distributed at high densities in a variety of cells (Marty, 1981; Latorre & Miller, 1983). In neurones, the influx of Ca^{2+} through Ca^{2+} channels and/or release of Ca^{2+} from the endoplasmic reticulum increase the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) during the action potential, which in turn may activate the Ca^{2+}-activated K^+ channels. Therefore, the Ca^{2+}-activated K^+ channel may provide an outward current for repolarization and after-hyperpolarization (Kuba, 1980; Adams *et al.*, 1982; McDermott & Weight, 1982; Kuba *et al.*, 1983; Petersen & Maruyama, 1984; Storm, 1987; Tanaka & Kuba, 1987; for review see, Latorre *et al.*, 1989). Yoshida *et al.* (1991) recently demonstrated openings of the 235 pS K^+ channel immediately after the spontaneous action potential in the hippocampal cell, prepared in the same way as in the present study. It might be speculated that seizure following an overdose of a local anaesthetic is related to a possible blocking action of the drug on K^+ channels in the central nervous system (Usubiaga *et al.*, 1966; de Jong *et al.*, 1969; Warnick *et al.*, 1971). The present study demonstrates that ionized forms of the local anaesthetics block the Ca^{2+}-activated K^+ channels from the cytoplasmic side, in essentially the same manner as for Na^+ channels. Benzocaine (a neutral anaesthetic at physiological pH) failed to block the K^+ channel.

Methods

Single hippocampal pyramidal cells were isolated from the brain of Wistar rats (7–10 days old) of either sex by a technique similar to that developed by Kaneda *et al.* (1988). Briefly, the brain was rapidly dissected out and sliced at thicknesses of 400–600 μm, which were then treated for 20 min at 37°C with collagenase (0.04%, Sigma, type I) and actinase (0.056%, Kaken Chemical Co., Japan) dissolved in the control external solution. The enzyme was washed out with external solution containing 20% foetal calf serum. The treated brain pieces of hippocampal tissue were stored in the external solution. The neurones were mechanically isolated just before use.

A drop of the cell suspension was added to the external solution in the recording chamber. After the cells settled down on the glass bottom, perfusion of the recording chamber was started. Single channel currents were recorded in the inside-out configuration (Hamill *et al.*, 1981) by use of a patch clamp amplifier (EPC-7, List, Darmstadt, F.R.G.). The glass electrode was usually filled with a 150 mM K^+ pipette solution,

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and had a tip resistance of 5–10 MΩ. The local anaesthetics were applied in the internal solution by use of the rapid application technique (Kakei & Ashcroft, 1987; Akaike *et al.*, 1986). All experiments were performed at room temperature (20–25°C). Statistical values are given in mean ± s.d. (*n*).

The single channel current was replayed and fed to the computer via a Bessel-type low pass filter (28 dB/octave) with a cut-off frequency of 2.5 kHz. The sampling frequency of the a.d. converter was 5 kHz. In some preliminary experiments, a current noise induced by applying local anaesthetics was examined on the oscilloscope using a higher cut-off frequency (10 kHz), but we failed to see clear improvement of the frequency resolution.

The composition of the control external solution was (in mM): NaCl 150.0, KCl 5.0, $MgCl_2$ 1.0, $CaCl_2$ 1.0, glucose 10.0, N-2-hydroxyethylpiperazine-N'-ethanesulphonic acid (HEPES) 10.0 and titrated to pH 7.4 with Tris base (Tris(hydroxymethyl)aminomethane). The composition of the standard internal solution used for the inside-out patch recordings was as follows (in mM): KCl 150.0, HEPES 5.0, ethyleneglycol-bis(β-aminoethylether)N,N'-tetraacetic acid (EGTA) 1.0 (pH 7.2 with KOH). The concentration of free Ca^{2+} ($[Ca^{2+}]$) was adjusted by adding an appropriate amount of $CaCl_2$ according to Fabiato & Fabiato (1979) and Tsien & Rink (1980). The pipette solution contained (in mM): KCl 150.0, $CaCl_2$ 1.0, and HEPES 5.0 (pH = 7.4 with KOH).

Drugs

The drugs used were purchased from the following companies: collagenase (Sigma type 1, U.S.A.), actinase (Kaken Chemical Co., Japan), foetal calf serum (Dainippon Chemical Co., Japan). The local anaesthetics used were lignocaine (lidocaine; pK_a = 7.9, Sigma, U.S.A.), QX-314 (N-ethyl derivative of lignocaine) predominantly in the cationic form (Ishizu Chemical Co., Japan), and uncharged benzocaine (pK_a = 2.6, Tokyokasei, Japan).

Results

Effects of lignocaine and benzocaine applied to the internal surface

After a gigaohm seal had been made on a hippocampal cell, the patch electrode was drawn back to excise the patch membrane in the control external solution. Under this inside-out patch condition, the activity of Ca^{2+}-activated K^+ channels was observed in more than 90% of the membrane patches. Representative records of the K^+ current activated by 12.6 μM $[Ca^{2+}]_i$ in symmetrical 150 mM K^+ solutions are illustrated in Figure 1. The channel activity increased with increasing depolarization, and at +20 and at +40 mV the channel showed sustained bursting activity. The channel closing events of relatively long durations observed at negative potentials became less frequent with depolarization, leaving the repetitive brief closing events in the burst. This K^+ channel had an ohmic conductance of about 235 pS. In 18 experiments, the average conductance was 234 ± 10 pS (n = 18). Our study on the control characteristics (Yoshida *et al.*, 1991) also indicated that blocking of the channel by tetraethylammonium (TEA) and Ba^{2+}, as well as the channel gating kinetics produced by divalent cations are consistent with those of the Ca^{2+}-activated K^+ channel (BK channel) described in other tissues (Latorre *et al.*, 1989).

After the control current had been recorded, 1 mM lignocaine or benzocaine was applied to the internal surface of the membrane. The gating kinetics of the channel were not markedly changed by these local anaesthetics. However, the amplitude of outward channel current was clearly decreased by 1 mM lignocaine. Benzocaine failed to affect the channel current.

The amplitude histograms were constructed from records obtained at +20 mV as shown in Figure 2. The open channel current showed a symmetrical distribution, and the very brief closing events caused a small continuous distribution between the full open level and the closed level in every experimental

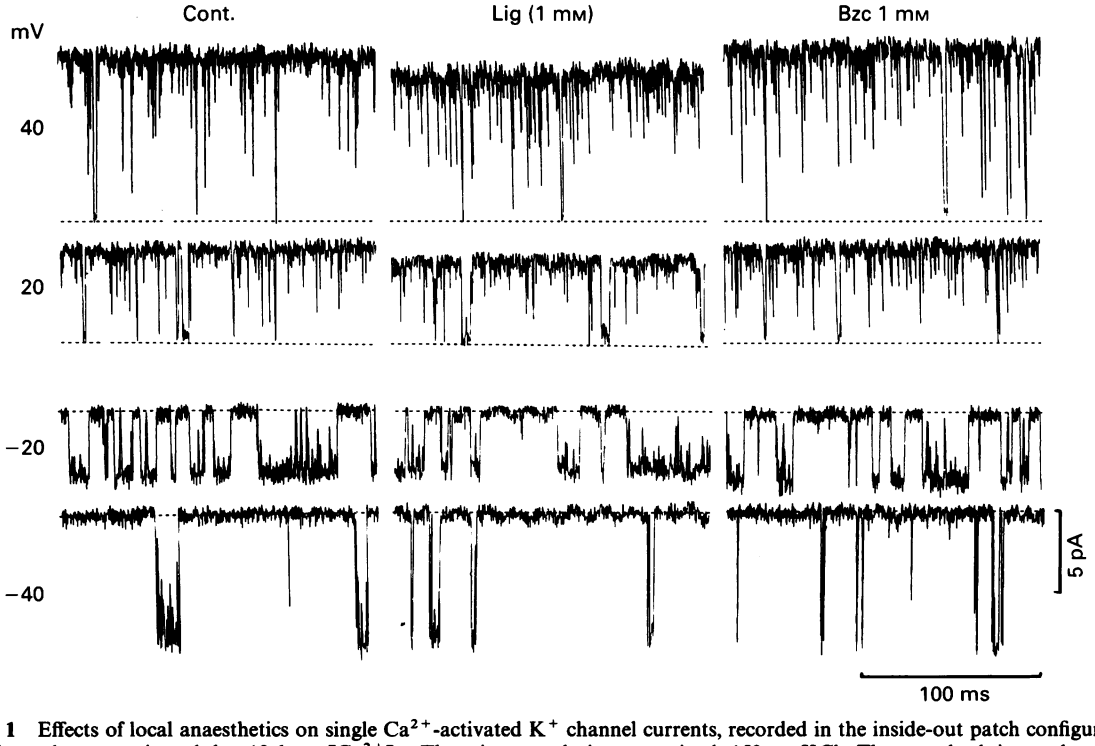

Figure 1 Effects of local anaesthetics on single Ca^{2+}-activated K^+ channel currents, recorded in the inside-out patch configuration. The channel was activated by 12.6 μM $[Ca^{2+}]_i$. The pipette solution contained 150 mM KCl. The standard internal solution (150 mM K^+) containing 1 mM lignocaine (Lig) or benzocaine (Bzc) was perfused after recording the control current (Cont). The membrane potentials are given at the left in mV. The upward deflections represent outward current, and the dashed lines indicate closed channel current level. All records were sampled at 5 kHz through a low pass filter of 2.5 kHz cut-off frequency. All data were taken from the same patch.

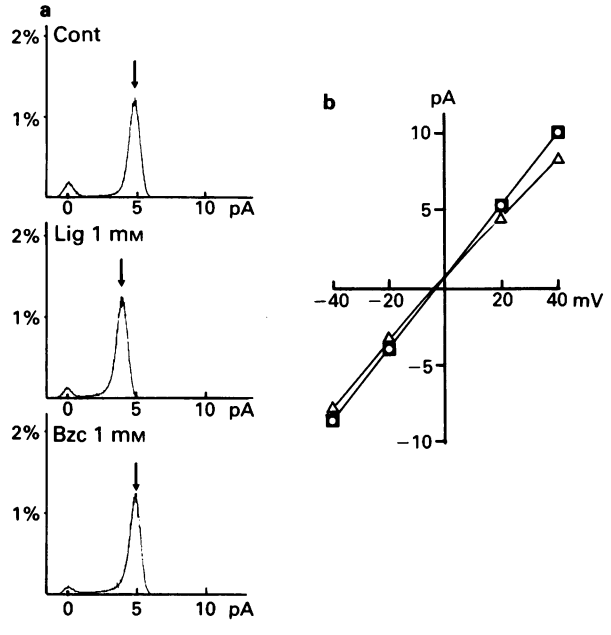

Figure 2 The amplitude histograms of the Ca^{2+}-activated K^+ channel currents recorded at +20 mV in the absence or the presence of internal lignocaine (Lig) or benzocaine (Bzc) (1 mM). Each histogram was determined from a 10–20 s segment of the current record. The arrows indicate the position of the distribution peaks, which were used to plot the I–V curve in (b). (b) (○) Control; (□) benzocaine and (△) lignocaine. The deviation of the reversal potential (−5 mV) from the expected K^+ equilibrium potential of 0 mV might be due to an experimental error in the voltage recording. Note that the density distribution at the closed current level was not much changed by the application of the local anaesthetics.

condition. The amplitude of the single channel current (i) was measured from the peak position, and were plotted against the membrane potential (V_m) in Figure 2a. Lignocaine reduced the channel conductance from 230 pS to 201 pS. The i–V_m relationship obtained in the presence of benzocaine was superimposable with the control.

Effects of QX-314 applied to the internal surface

The application of 1 or 2 mM QX-314 to the internal surface of the membrane reduced the current amplitude in a dose-dependent manner and increased noise in the open channel current (Figure 3). The voltage-dependent activation of the channel was not obviously modulated. The amplitude histograms of control and QX-314 records are compared at +20 mV in Figure 4a. The peak position of the open channel current was shifted to a lower level by the action of this drug, and the distribution of the open channel current was broader than in the control. The current amplitude at the distribution peak was plotted in Figure 4b. The channel inhibition was more marked for the outward current than for the inward current. The inhibition of the outward current increased with increasing depolarization. The current amplitude was decreased by 1 mM QX-314 to 80 ± 3, 69 ± 2, 65 ± 5, and 58 ± 5% control, at −40, −20, +20 and +40 mV, respectively. At 2 mM QX-314 the amplitude was 42 and 46% control at +40 mV and 68 and 68% control at −40 mV in two experiments. It should be noted that the conductance of single Na^+ channels is also apparently decreased by QX-314 for both the outward and inward currents, accompanied by an increase in noise (Wang, 1988).

The above findings with lignocaine and QX-314 might be explained in two alternative ways. Firstly, the channel conductance might be reduced by the drugs. In this case, the mean open probability (p_o) is given as follows:

$$p_o = I/(iN) \quad (1)$$

where I stands for mean patch current, i for the unit current amplitude, and N for channel number within the patch. In the present study N was 1 in every experiment analyzed. If i is measured from the distribution peak in the amplitude histogram, values of p_o are 0.90, 0.87 and 0.93 in the control, 1 mM, and 2 mM QX-314, respectively. However, the increase in the open channel noise induced by QX-314 makes an alternative mechanism more likely; that channel conductance remained constant in the open conformation, but the channel was blocked in a flickering manner by the drug, basically in the same manner as in the Na^+ channel, thereby producing a

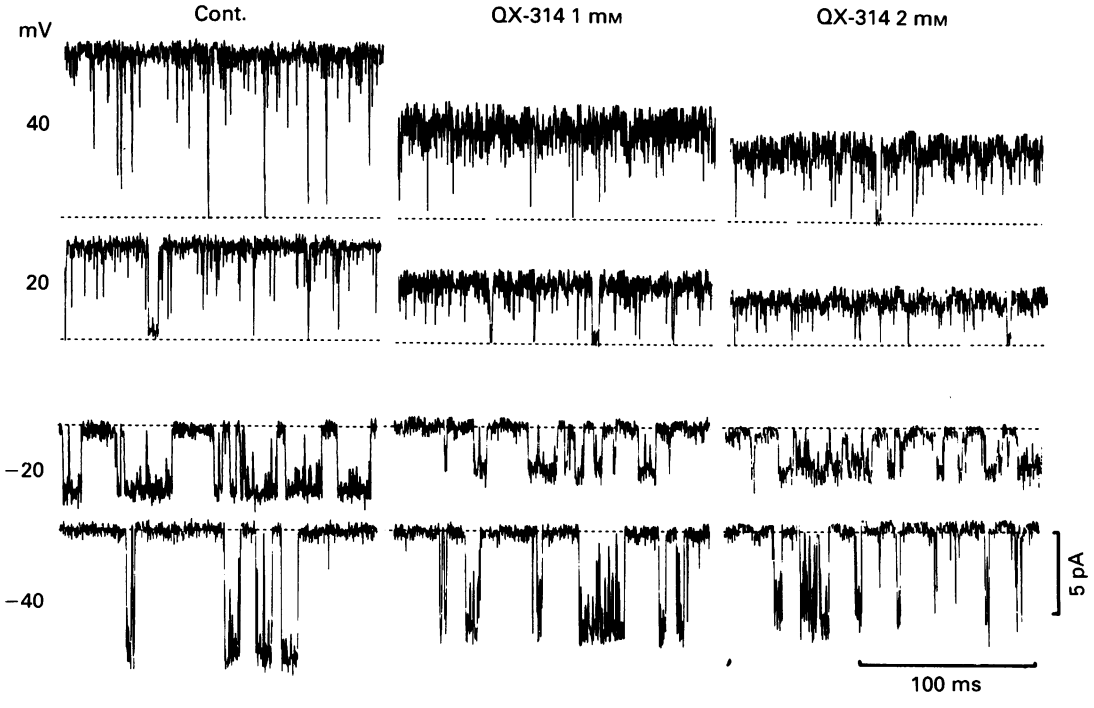

Figure 3 Effects of QX-314 1 mM or 2 mM applied to the internal surface of the inside-out patch. All records were obtained from the same Ca^{2+}-activated K^+ channel.

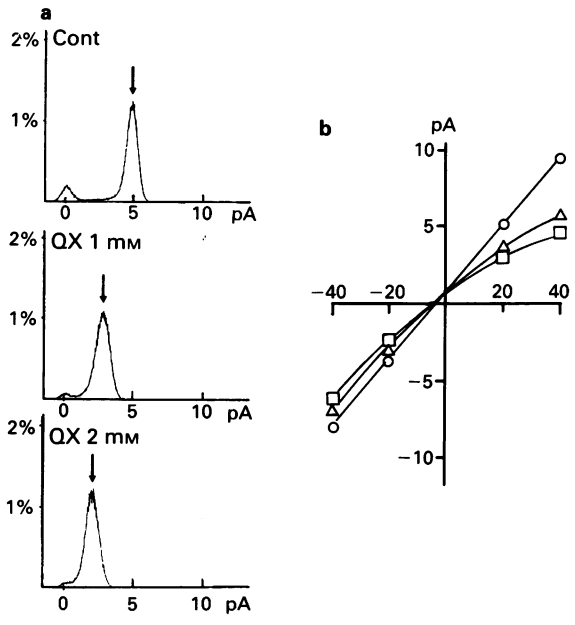

Figure 4 The amplitude histograms of the Ca^{2+}-activated K^+ channel current at +20 mV in the control and in the presence of internal QX-314 (QX) 1 mM or 2 mM. Each histogram was determined from a 10–20 s segment of the current record. The I-V curves in (b) are the control (○), QX-314 1 mM (△) and 2 mM (□). The reversal potential of −5 mV is due to an experimental error of unknown reason.

reduced open probability. The transition between the open and blocked states might be so fast that the recorded open channel current gave a kind of average current accompanied by increased noise. In this case, the i in Equation (1) is the amplitude of the current in the absence of the blocker. In Table 1, values of p_0 were decreased by lignocaine or by increasing QX-314 concentration. It is clear that benzocaine was ineffective on the Ca^{2+}-activated K^+ channel.

Drug actions at the external surface of the Ca^{2+}-activated K^+ channel

To examine the actions of the local anaesthetics from the external side of the membrane they were added in the pipette solution. As shown in Figure 5, no significant change was noticed in either the amplitude of the single channel current or in the open-close transitions. The measurement of the i–V_m relations confirmed no significant effect of the drugs on the channel conductance in 2 experiments each for 2 mM lignocaine (212 and 218 pS), benzocaine (233 and 228 pS), or QX-314 (226 and 229 pS). Although, p_0 varied between different patches in the control, we could not detect any consistent change in the p_0 during the application of the drugs. It might be concluded that the charged form of local anaesthetics blocks the Ca^{2+}-activated K^+ channel only from the internal side of the membrane.

The open-close kinetics under the blocking effect of the drug

We constructed open time and closed time histograms in the control condition and in the presence of the local anaesthetics. The channel was activated by 12.6 μM $[Ca^{2+}]_i$ added to the internal solution. In the control, the open time histogram showed a single exponential component, and the closed time histogram was fit by a sum of two exponential components as shown in a preceding study (Figure 6 of Yoshida *et al.*, 1991). The fraction of the slow component in the closed time histogram was very small, since the channel was maximally activated by 12.6 μM $[Ca^{2+}]_i$. Furthermore, the time constant of the distribution of the fast component was less than 1 ms (0.3–0.7 ms). Thus, it was difficult to detect obvious changes in the closed time histogram induced by the local anaesthetics.

In the presence of QX-314, the increased noise in the open channel current slightly interfered with the measurement of the open time and the closed time. We arbitrarily fixed the cut-off frequency to 2.5 kHz and defined the transition between the closed and open states by giving a threshold level at the half amplitude of the current deflection. The distribution of the open time was changed by the application of QX-314. Figure 6 shows representative histograms obtained before and during the application of 2 mM QX-314 at a holding potential of +40 mV. In the control, the channel frequently showed transitions from the open state to the closed states. QX-314 induced a marked reduction in the amplitude of the open channel currents and an increase in the high frequency noise, but the number of transitions to the closed level was decreased as shown in the inset current recordings. The open time histograms were well fitted with a single exponential curve. The time constant of the distribution (τ_{open}) was 12.6 ms in the control and 17.9 ms in the presence of 2 mM QX-314.

The values of τ_{open} obtained with different concentrations of QX-314 are compared with the control in Table 2. The fractional decrease in the current amplitude is also given as the current amplitude in the presence of the drug divided by the control amplitude (i_b/i_c). At 0.2 mM QX-314 the change in the mean open time was not significant. At higher concentrations, the value of τ_{open} was increased in every experiment (τ_{cont}/τ_{drug}).

The simple sequential model for open channel blockade (Neher, 1983) indicates a constant time integral of current during a burst, which is due to a train of transitions between the open state and the blocked state. To test this model, the relative amplitude was multiplied by the fractional mean open time (A × B in Table 2). The value is close to 1 at 0.5 mM QX-314, but tends to decrease with increasing drug concentration at +40 mV. This finding seems to be similar to that obtained in the acetylcholine (ACh)-induced single channel current of muscle cells on application of QX-222 (Neher,

Table 1 Effects of local anaesthetics on the current amplitude of the Ca^{2+}-activated K^+ channel

	i	*m.p.c.*	p_0 *I/iN*	p_0 *I/i*N*
Cont	6.33 ± 0.42 (23)	5.70 ± 0.64 (18)	0.90	
Bzc 1 mM	6.50 ± 0.26 (4)	6.46 ± 0.57 (4)	0.99	1.03
Lig 1 mM	5.41 ± 0.21 (7)	4.85 ± 0.30 (7)	0.90	0.77
QX-314 1 mM	4.00 ± 0.22 (9)	3.48 ± 0.51 (9)	0.87	0.54
QX-314 2 mM	3.24 (2)	3.01 (2)	0.93	0.48

The apparent unitary amplitude of the single channel current (i) was measured in the control or in the presence of 1 mM benzocaine (Bzc), lignocaine (Lig), QX-314, or 2 mM QX-314. The mean patch current (m.p.c.) is the time average of the current determined from record segments of 10–20 s in duration. The channel open probability (p_0) was calculated as m.p.c. divided by the apparent unit amplitude (m.p.c./i) or as m.p.c. divided by the unit amplitude obtained in the control (m.p.c./i_{cont}). Data are given as mean ± s.d. (number of experiments).

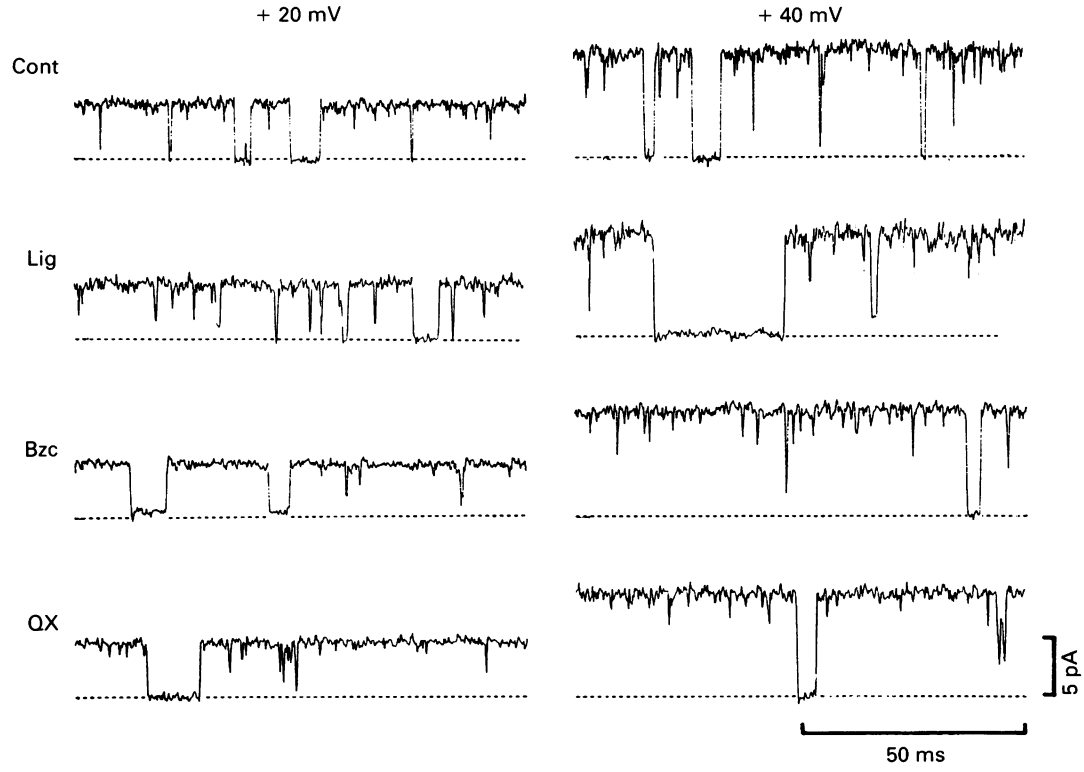

Figure 5 Effects of local anaesthetics applied in the external solution. The current records obtained at +40 mV and +20 mV were compared. The pipette solution contained 2 mM lignocaine (Lig), benzocaine (Bzc) or QX-314 (QX). The channel was activated by 10 μM Ca^{2+} in the standard internal solution.

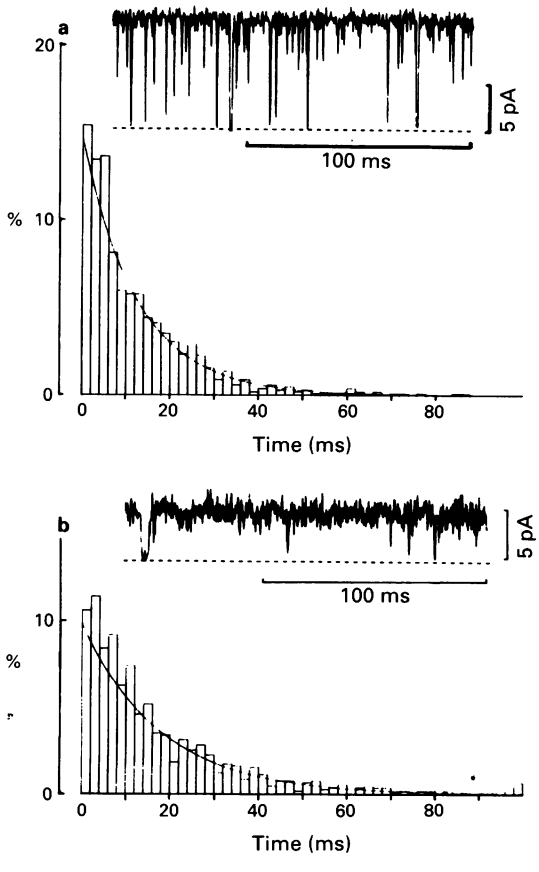

Figure 6 The open time histograms in the control (a) and presence of QX-314 (b) applied to the internal surface. The inset shows the original recordings obtained at +40 mV. The open time histogram was composed of a single exponential component with time constants of 12.6 ms and 17.9 ms in the control and 2 mM QX-314, respectively. The bin width was 2 ms and the superimposed curves were the best fits of a single exponential curve.

1983). We have no ready explanation for the large increase in τ_{open} produced by 1 mM QX-314 at +20 mV.

Based on the above findings, it might be speculated that the increased noise reflected the transitions between the open state and the blocked state with very fast kinetics, and that the apparent open time represented the 'bursting time' in the presence of the drug.

Dose-response relationships for local anaesthetics

Because of the difficulty in separating the blocked state from the closed states, the dose-response relationship could not be determined from simple measurements of p_0. The dissociation constant (K_D) can only be determined if a simple kinetic model is assumed to apply (Benham *et al.*, 1985; McCann & Welsh, 1987).

$$\text{Closed—Open—Block} \qquad (2)$$

where,

$$K_D = [D]p_0\, p_b/(p_0 - p_b) \qquad (3)$$

Here, [D] indicates the concentration of the blocker, and p_0 and p_b the open probabilities in the absence and presence of the blocker, respectively. It was assumed that channel conductance remained constant in the open state, and null in the blocked state. To get a linear relation Equation (3) is modified as,

$$(p_0 - p_b)/p_0 \cdot p_b = [D]/K_D \qquad (4)$$

The value of p_b was determined from Equation (1). It seems that Equation (4) holds true in the present study as shown by the linear relationship in Figure 7a. It may indicate that the block is occurring on a basis of a one to one binding reaction. The values of K_D obtained from the slopes of the linear relationship were plotted against V_m in Figure 7b. The smooth curve superimposed is drawn according to the equation (Woodhull, 1973),

$$K_D = K_D(0) \cdot \exp(-\delta zFV_m/RT), \qquad (5)$$

Table 2 Effects of QX-314 on the time constant in the open time histogram

	+20 mV					+40 mV				
	τ_{open}		A τ_b/τ_c	B i_b/i_c	A × B	τ_{open}		A τ_b/τ_c	B i_b/i_c	A × B
	Cont	Drug				Cont	Drug			
QX-314										
0.2 mM	9.84	10.27	1.04	0.91	0.95	13.78	14.20	1.03	0.85	0.88
0.5 mM	9.84	11.34	1.15	0.82	0.94	13.78	15.98	1.15	0.77	0.89
	12.02	15.23	1.26	0.81	1.02	14.67	18.43	1.25	0.69	0.86
1.0 mM	6.04	10.36	1.72	0.67	1.15	12.21	16.00	1.31	0.57	0.74
	5.72	11.50	2.01	0.62	1.25	12.76	16.30	1.27	0.58	0.74
	6.37	14.18	2.22	0.66	1.46	7.69	15.12	1.96	0.59	1.16
2.0 mM	13.59	15.41	1.13	0.50	0.57	15.51	18.66	1.20	0.48	0.58
						12.58	17.86	1.42	0.43	0.61
						12.52	21.75	1.73	0.46	

The open time histograms were constructed as shown in Figure 6, and their exponential time constant of distribution (τ_{open}) were determined in the control (cont) and in the presence of the QX-314 (drug) at the membrane potentials of +20 and +40 mV. The ratio of τ determined in the presence of the drug (τ_b) divided by the control τ (τ_c) was calculated (A). The ratio of the unit amplitudes (i_b/i_c) were also calculated (B). See text for the description of the value of A times B.

where $K_D(0)$ is the dissociation constant at 0 mV, σ a partition parameter, and z, F, R and T having their usual meanings in thermodynamics. The value of δ was 0.51, suggesting that the blocking site for QX-314 lies about half-way across the membrane electrical field.

Discussion

The present study demonstrates that the hippocampal Ca^{2+}-activated K^+ channel is not blocked from the external side of the membrane by lignocaine, QX-314 or benzocaine, but is blocked by lignocaine and QX-314 applied in the internal solution. These findings clearly indicate that the Ca^{2+}-activated K^+ channel is blocked by the drug through the 'hydrophilic pathway' but not via the 'hydrophobic pathway', which were suggested for the blocking of the Na^+ channel (Schwarz *et al.*, 1977). The positively charged quaternary amine may enter the channel pore through the internal mouth of the K^+ channel to block the channel. It has been shown that internal QX-314 produces an inhibition of K^+ currents in frog myelinated nerves (Strichartz, 1973), suggesting a similar blocking mechanism for the delayed rectifier K^+ channel. When QX-314 was applied internally to pyramidal neurones in the guinea-pig hippocampal slice preparation (Connors & Prince, 1982), the large K^+ conductance remained. It may be speculated that the blockade of the K^+ channel was relatively small compared with the block of the Na^+ channel.

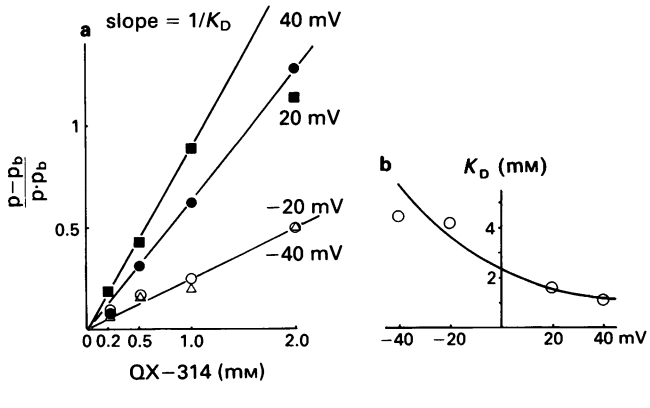

Figure 7 (a) Determination of the dissociation constant (K_D) for the blocking effect of QX-314 at +40 (■), +20 (●), −20 (○) and −40 mV (△). See text for explanation. (b) The voltage-dependency of the K_D, which were determined in (a).

A large and persistent K^+ current, activated by volatile general anaesthetics, was found in some neurones but not others (Franks & Lieb, 1988). In the squid axon clinical concentrations of general anaesthetics blocked a potassium conductance in the resting membrane (Haydon *et al.*, 1988). The former finding may explain the hyperpolarization recorded in the hippocampal pyramidal cells induced by the general anaesthetics (Nicoll & Madison, 1982). It might be speculated that the action of the local anaesthetics is different from that of the general anaesthetics.

The voltage-dependence of the action of QX-314 (Figures 4 and 7) indicates that the charged molecule indeed affects the channel. The effect of positively charged QX-314 on the Ca^{2+}-activated K^+ channel was increased when the outward driving force for the positive charge was increased by shifting the membrane potential progressively positive. This voltage-dependence was explained by assuming a binding site at an electrical distance of 0.5 across the membrane electrical field. The finding is in line with the fact that the effect of intracellular drug application on the Na^+ channel in planar lipid bilayers increases with increasing depolarizations, $\delta = 0.48$ from the internal side (Wang, 1988). Also the effect of extracellular drug application on ACh channels of endplate increases with increasing hyperpolarization, $\delta = 0.78$ from the external side (Neher & Steinbach, 1978).

The finding that benzocaine failed to affect the Ca^{2+}-activated K^+ channel is different from the blocking effect on the Na^+ channel (Hille, 1977b). If benzocaine shares a common binding site with other local anaesthetics (Schmidtmayer & Ulbricht, 1980), the molecular structure of the K^+ channel may prevent the invasion of benzocaine from the lipid bilayer into the channel complex (hydrophobic pathway). If the benzocaine-binding site is different from the binding site for other anaesthetics in the Na^+ channel (Mrose & Ritchie, 1978), this finding may suggest that the Ca^{2+}-activated K^+ channel does not have a binding site.

It is thought that the conductance of the blocked Na^+ channel is zero, and that the channel current fluctuates between the full open level and the blocked level in the presence of local anaesthetics. However, the on-off flickering block by QX-314 is too fast to be resolved by the present recording techniques, resulting in the apparent reduction in the unitary current flowing through open channels (Wang, 1988). In the present study, the current amplitude of the single Ca^{2+}-activated K^+ channel was also reduced by the application of local anaesthetics. We attribute the reduction in the amplitude to a very fast flickering block by the drug, since the current record shows a slight but significant increase in current fluctuations during the application of QX-314. It may be speculated that lignocaine may induce still faster block of

the Ca^{2+}-activated K^+ channel, so that the recorded current did not show an apparent increase in fluctuations. Clear transitions between the open and closed levels of the single channel current were demonstrated in the block of endplate channels of skeletal muscle (Neher & Steinbach, 1978; Neher, 1983). The blockade of a 47 pS K^+ channel in isolated turtle colon epithelial cells by lignocaine is also of the fast flickering type (Richard & Dawson, 1986). However, the present study does not completely exclude the possibility of a reduction in the single channel conductance.

The use-dependent block of the Na^+ channel also provides strong support for the hypothesis that local anaesthetics enter the ion pathway of the channel to induce the open channel block (Schwartz *et al.*, 1977; Strichartz, 1973). It will be necessary in the future to test whether the Ca^{2+}-activated K^+ channel is blocked by local anaesthetics before the channel is opened by Ca^{2+}.

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References

ADAMS, P.R., CONSTANTI, A., BROWN, D.A. & CLARK, R.B. (1982). Intracellular Ca^{2+} activates a fast voltage-sensitive K^+ current in vertebrate sympathetic neurons. *Nature*, **296**, 746–747.

AKAIKE, N.M., INOUE, M. & KRISHTAL, O.A. (1986). Concentration clamp study of γ-aminobutyric acid-induced chloride current kinetics in frog sensory neurons. *J. Physiol.*, **379**, 171–216.

BENHAM, C.D., BOLTON, T.B., LANG, R.J. & TATEWAKI, T. (1985). The mechanism of action of Ba^{2+} and TEA on single Ca^{2+}-activated K^+-channels in arterial and intestinal smooth muscle cell membranes. *Pflügers Arch.*, **403**, 120–127.

CAHALAN, M.D. (1978). Local anesthetic block of sodium channels in normal and pronase-treated squid giant axons. *Biophys. J.*, **21**, 285–311.

CONNORS, B.W. & PRINCE, D.A. (1982). Effects of local anesthetic QX-314 on the membrane properties of hippocampal pyramidal neurons. *J. Pharmacol. Exp. Ther.*, **220**, 476–481.

COURTNEY, K.R. (1975). Mechanism of frequency-dependent inhibition of sodium currents in frog myelinated nerve by the lidocaine derivative GEA 968. *J. Pharmacol. Exp. Ther.*, **195**, 225–236.

DE JONG, R.H., ROBLES, R. & CORBIN, R.W. (1969). Central actions of lidocaine – Synaptic transmission. *Anesthesiology*, **30**, 19–23.

FABIATO, A. & FABIATO, F. (1979). Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. *J. Physiol.*, **75**, 463–505.

FRANKS, N.P. & LIEB, W.R. (1988). Volatile general anaesthetics activated a novel neuronal K^+ current. *Nature*, **333**, 662–664.

FRAZIER, D.T., NARAHASHI, T. & YAMADA, M. (1970). The site of action and active form of local anesthetics. Experiments with quaternary compounds. *J. Pharmacol. Exp. Ther.*, **171**, 45–51.

HAMILL, O.P., MARTY, A., NEHER, A.E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.*, **391**, 85–100.

HAYDON, D.A., REQUENA, J. & SIMON, A.J.B. (1988). The potassium conductance of the resting squid axon and its blockage by clinical concentrations of general anaesthetics. *J. Physiol.*, **402**, 363–374.

HILLE, B. (1977a). The pH-dependent rate of action of local anesthetics on the node of Ranvier. *J. Gen. Physiol.*, **69**, 475–496.

HILLE, B. (1977b). Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J. Gen. Physiol.*, **69**, 497–515.

HONDEGHEM, L.M. & KATZUNG, B.G. (1977). Time- and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. *Biochim. Biophys. Acta.*, **472**, 373–398.

KAKEI, M. & ASHCROFT, F.M. (1987). A microflow superfusion system for use with excised membrane patches. *Pflügers Arch.*, **109**, 337–341.

KANEDA, M., NAKAMURA, H. & AKAIKE, N. (1988). Mechanical and enzymatic isolation of mammalian CNS neurons. *Neurosci. Res.*, **5**, 299–315.

KHODOROV, B.I., SHISHKOVA, L., PEGANOV, E. & REVENKO, S. (1976). Inhibition of sodium currents in frog Ranvier node treated with local anesthetics. Role of slow sodium inactivation. *Biochim. Biophys. Acta*, **433**, 409–435.

KUBA, K. (1980). Release of calcium ions linked to the activation of potassium conductance in a caffeine-treated sympathetic neurone. *J. Physiol.*, **298**, 251–269.

KUBA, K., MORITA, K. & NOHMI, M. (1983). Origin of calcium ions involved in the generation of a slow afterhyperpolarization in bullfrog sympathetic neurones. *Pflügers Arch.*, **399**, 194–202.

LATORRE, R. & MILLER, C. (1983). Conduction and sensitivity in potassium channels. *J. Memb. Biol.*, **71**, 11–30.

LATORRE, R., OBERHAUSER, A., LABARCA, P. & ALVAREZ, O. (1989). Varieties of calcium-activated potassium channels. *Ann. Rev. Physiol.*, **51**, 385–399.

McCANN, J.D. & WELSH, M.J. (1987). Neuroleptics antagonize a calcium-activated potassium channel in airway smooth muscle. *J. Gen. Physiol.*, **89**, 339–352.

McDERMOTT, A.B. & WEIGHT, F.F. (1982). Action potential repolarization may involve a transient Ca^{2+} sensitive outward current in a vertebrate neurone. *Nature*, **300**, 185–188.

MARTY, A. (1981). Ca-dependent K channel with large unit conductance in chromaffin cells. *Nature*, **291**, 497–500.

MROSE, H.E. & RITCHIE, J.M. (1978). Local anesthetics: Do benzocaine and lidocaine act at the same single site? *J. Gen. Physiol.*, **71**, 223–225.

NEHER, E. (1983). The charge carried by single-channel currents of rat cultured muscle cells in the presence of local anaesthetics. *J. Physiol.*, **339**, 663–678.

NEHER, E. & STEINBACH, J.H. (1978). Local anaesthetics transiently block currents through single acetylcholine-receptor channels. *J. Physiol.*, **277**, 153–176.

NICOLL, R.A. & MADISON, D.V. (1982). General anesthetics hyperpolarize neurones in the vertebrate central nervous system. *Science*, **217**, 1055–1057.

PETERSEN, O.H. & MARUYAMA, Y. (1984). Calcium-activated potassium channels and their role in secretion. *Nature*, **307**, 693–696.

RICHARDS, N.W. & DAWSON, D.C. (1986). Single potassium channels blocked by lidocaine and quinidine in isolated turtle colon epithelial cells. *Am. J. Physiol.*, **251**, C85–C89.

SCHMIDTMAYER, J. & ULBRICHT, W. (1980). Interaction of lidocaine and benzocaine in blocking sodium channels. *Pflügers Arch.*, **387**, 47–54.

SCHWARZ, W., PALADE, P.T. & HILLE, B. (1977). Local anesthetics: effect of pH on use-dependent block of sodium channels in frog muscle. *Biophys. J.*, **20**, 343–368.

STARMER, C.F. (1987). Theoretical characterization of ion channel blockade: competitive binding to periodically accessible receptors. *Biophys. J.*, **52**, 405–412.

STORM, J.F. (1987). Intracellular injection of a Ca^{2+} chelator inhibits spike-repolarization in hippocampal neurons. *Brain Res.*, **435**, 387–392.

STRICHARTZ, G.R. (1973). The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. *J. Gen. Physiol.*, **62**, 37–57.

STRICHARTZ, G.R. (1976). Molecular mechanisms of nerve block by local anesthetics. *Anesthesiology*, **45**, 421–441

STRICHARTZ, G.R. & RITCHIE, J.M. (1987). The action of local anesthetics on ion channels of excitable tissues. In *Handbook of Experimental Pharmacology*, Vol. 81, ed. Strichartz, G.R. pp. 21–52. Berlin: Springer-Verlag.

TANAKA, K. & KUBA, K. (1987). The Ca^{2+}-sensitive K^+-currents underlying the slow afterhyperpolarization of bullfrog sympathetic neurons. *Pflügers Arch.*, **410**, 234–242.

TSIEN, R.Y. & RINK, T.J. (1980). Neutral carrier ion-selective microelectrodes for measurement of intercellular free calcium. *Biochim. Biophys. Acta*, **599**, 623–638.

USUBIAGA, J.E., WIKINSKI, J., FERRERO, R., USUBIAGA, L.E. & WIKINSKI, R. (1966). Local anesthetic-induced convulsions in man, an electroencephalographic study. *Anesthesia Analgesia*, **45**, 611–620.

WARNICK, J.E., KEE, R.D. & YIM, G.K.W. (1971). The effects of lidocaine on inhibition in the cerebral cortex. *Anesthesiology*, **34**, 327–332.

WANG, G.K. (1988). Cocaine-induced closures of single Batrachotoxin-activated Na^+ channels in planar lipid bilayers. *J. Gen. Physiol.*, **92**, 747–765.

WOODHULL, A.M. (1973). Ionic blockade of sodium in nerve. *J. Gen. Physiol.*, **61**, 687–708.

YOSHIDA, A., ODA, M. & IKEMOTO, Y. (1991). Kinetics of the Ca^{2+}-activated K^+ channel in rat hippocampal neuron. *Jpn. J. Physiol.*, **41**, 297–315.

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