Effects of KC 3791 on Sodium and Potassium Channels in Frog Node of Ranvier

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Abstract. Effects of a new antiarrhythmic compound KC 3791 on sodium ($I_{Na}$) and potassium ($I_K$) currents were studied in frog myelinated nerve fibres under voltage clamp conditions. When applied externally to the node of Ranvier, KC 3791 (KC) at concentrations of $10^{-5}$—$10^{-4}$ mol. l$^{-1}$ produced both tonic and cumulative (use-dependent) inhibition of $I_{Na}$. An analysis of the frequency-, voltage- and time dependence of cumulative block by KC suggested that this block resulted from a voltage-dependent interaction of the drug with open Na channels. The progressive decrease in $I_{Na}$ during repetitive pulsing was due to accumulation of Na channels in the resting-blocked state: closing of the activation gate after the end of each depolarizing pulse stabilized the KC-"receptor" complex. To unblock these channels a prolonged washing of the node had to be combined with a subsequent repetitive stimulation of the membrane; this suggested that channel could not become cleared of the blocker unless the activation gate has opened. KC also proved to be capable of blocking open K channels at outwardly directed potassium currents ($I_K$). This block increased during membrane depolarization. Unblocking of K channels after the end of a depolarizing pulse proceeded much faster than unblocking of Na channels under identical conditions. Cumulative inhibition of outward $I_K$ during high-frequency membrane stimulation was therefore readily reversible upon a decrease in pulsing frequency.

Key words: Frog node — Sodium channels — Potassium channels — Gating — Blockade

Introduction

This work represents a continuation of our previous investigations of the effects of a new antiarrhythmic drug, the compound KC 3791 (Kühl et al. 1982) on ionic channels in excitable membranes (Khodorov and Zaborovskaya 1984; Zaborovskaya and Khodorov 1984; Sokolova and Khodorov 1985). The present study was aimed to a more detailed analysis of the gate-dependent blockade of Na$^+$ and K$^+$ currents ($I_{Na}$ and $I_K$, respectively) by KC3791(KC) applied externally to the voltage-clamped node of Ranvier. The chemical structure of KC is shown in
Fig. 1 A (inset). The experiments performed suggested that KC causes use-dependent inhibition of $I_{Na}$ and $I_K$ due to its interaction with open ion channels: a molecule of the drug enters an open ion channel and remains trapped within its lumen after the end of a depolarizing pulse. This leads to accumulation of blocked channels during repetitive membrane depolarization. Similarly as N-propyl ajmaline (Khodorov and Zaborovskaya 1983), KC blocks both inward and outward $I_{Na}$, and only outward $I_K$. The blocking action of KC on $I_{Na}$ differs considerably from the effects of tertiary amine local anesthetics and some other drugs that interact preferentially with inactivated Na channels (Khodorov et al. 1976; Hille 1977; Zaborovskaya and Khodorov 1984; Courtney 1980).

**Materials and Methods**

Single myelinated fibres were dissected from *n.ischiadicus* of the frog *Rana ridibunda* and single nodes of Ranvier were voltage-clamped at 7 - 10 °C using the technique described by Dodge and Frankenhaeuser (1958) and Hille (1971) (for some details see Khodorov and Zaborovskaya 1983). To block K channels from the inside, the ends of the fibre were cut in 114 mmol/l CsF solution buffered with 5 mmol/l Tris (pH 7.3). The effects of KC on $I_K$ were examined at ends of the fibre being cut in 114 mmol/l KF solution. The composition of basic solutions used to superfuse the node is presented in Table 1.

**Symbols:** $E$ — membrane potential (internal minus external); $E_h$ — holding potential; $E_t$ — potential during the test pulse; $E_c$ — potential during the conditioning pulse; $I_{Na}$ and $I_K$ — sodium and potassium currents, respectively; $t$ — duration of the test pulse; KC — compound KC3791.

**Table 1. Composition of Solutions**

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<th>Solution</th>
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**Results**

1. **Sodium currents**

At concentrations of $10^{-5} - 5 \times 10^{-4}$ mol/l KC applied externally to the node of Ranvier produced both tonic and use-dependent (cumulative) inhibition of $I_{Na}$. 

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The tonic block was reflected in a decrease in $I_{\text{Na}}$ amplitude during the first test pulse applied 5—7 min after the start of the superfusion of the resting node by KC-containing solution. In experiments illustrated in Figs. 1 A and 3, $10^{-4}$ mol/l KC (solution No I) caused an about 30 % "tonic" decrease in $I_{\text{Na}}$. This blocking effect of KC was greatly enhanced by repetitive membrane stimulation (1 Hz, $E_{\text{r}} = -10$ mV, $t = 10$ ms); in the course of this pulsing $I_{\text{Na}}$ decreased to a new steady-state level (cumulative block). Neither tonic nor cumulative inhibition of $I_{\text{Na}}$ was accompanied by changes in current kinetics (see Fig. 1 A).

**Fig. 1 A.** Effects of the compound KC 3791 (KC) on $I_{\text{Na}}$ in the node of Ranvier. The chemical structure of the drug is shown in the inset. Current records are grafically superimposed. 1, in the control Ringer solution; 2, 3 and 4, during the first, 20-th and 60-th pulses in the train, respectively, at a frequency of 1 Hz. The train was started after a 5-min superfusion of the resting node by $10^{-4}$ mol/l KC solution (No. 1). A decrease in the peak value of $I_{\text{Na}}$ revealed by the 1-st pulse characterizes the extent of the tonic KC block. $E_{\text{r}} = -110$ mV, $E_{\text{i}} = 0$, mV, $t = 7$ ms. Temperature 7 °C. Fibre 3.01.84.

**B.** Test for the ability of KC to induce slow inactivation of Na channels. A 1.3-s depolarizing step from $E_{\text{r}} = -90$ to $E_{\text{r}} = -10$ mV was applied to the Ranvier node pretreated with $5 \times 10^{-5}$ mol/l KC. Turning on of this depolarization elicited $I_{\text{Na}}$; its peak value was by about 20 % lower than the initial one (not shown) measured before KC application (tonic block). 100 ms after the end of the depolarizing step ($E_{\text{r}}$) a train of short (1.5 - ms) depolarizing pulses was turned on ($E_{\text{r}} = -10$ mV) at a frequency of 10 Hz. KC did not produce slow inactivation. Bottom: pulse protocol; upper traces $I_{\text{Na}}$, amplitude. Temperature 10 °C. Fibre 6. 12. 84.

In contrast to repetitive pulsing, a single long-lasting (0. 3 s) depolarizing step did not enhance the blocking action of KC, as shown in Fig. 1 B: $I_{\text{Na}}$ elicited by the first short (1.5 ms) depolarizing pulse applied 100 ms after the end of a long step was by only 5% lower than the control value of $I_{\text{Na}}$. This indicated that KC, unlike local anesthetics, was incapable of slowing down reactivation of Na channels. The tonic block produced in this experiment is not shown: prior to the long (1.3 s) depolarization, $I_{\text{Na}}$ was decreased by about 20% due to a treatment of the resting nodal membrane with $5 \times 10^{-5}$ mol/l KC.

Fig. 2 shows enhancement of the cumulative inhibition by KC of $I_{\text{Na}}$ resulting from an increase in stimulation frequency from 2 to 10 Hz. Note, however, the independence of the saturating level of the block of the pulsing frequency:
a frequency decrease from 10 Hz to 0.1 (0.2) Hz was not followed by $I_{Na}$ recovery, suggesting a very slow clearing of Na channels from KC in the interpulse intervals. The reason for such a slow unblocking of Na channels is suggested by the result of the experiment shown in Fig. 3: a repetitive membrane stimulation (2 Hz) in the presence of $10^{-4}$ mol/l KC in the solution produced a pronounced cumulative inhibition of $I_{Na}$. An about 10-min washing of the node with the control solution after pulsing has been turned off resulted in an only relatively small increase in $I_{Na}$ amplitude. However, turning on of a train of repetitive pulses (2 Hz) in the course of a continuous washing of the node produced a very fast unblocking of channels.

**Fig. 2.** Effects of repetitive stimulation frequency on KC-induced cumulative inhibition of $I_{Na}$. Abscissa: time of stimulation (s); ordinate: relative values of inward $I_{Na}$ during n-th pulse ($I_n$). The amplitude of the first inward $I_{Na}$ in series ($I_1$) is taken for unity. The node was treated with $10^{-4}$ mol/l KC. Pulsing frequency: • = 2 Hz (only each 4-th peak of $I_{Na}$ is shown); □ = 10 Hz (each 20-th $I_{Na}$ is shown); ■ = 0.1 Hz; ○ = 0.2 Hz. $E_i = -100$ mV; $E_r = 0$ mV; $t = 7$ ms. Temperature 7 °C. Fibre 13.01.84.

During this stimulation $I_{Na}$ rose pulse by pulse and it reached in about 2 min the initial value measured before the onset of repetitive pulsing (i.e. the level of the tonic block). This level proved to be very stable: no tendency towards further $I_{Na}$ recovery was observed during continuous washing of the node after the termination of pulsing (shown as a break on the abscissa). A new application of $10^{-4}$ mol/l KC to the node (■) did not affect the $I_{Na}$ value. However, in the presence of KC in the solution a train of depolarizing pulses (2 Hz) immediately caused a new fall of $I_{Na}$. Thus, two opposite effects of repetitive pulsing could be observed in this experiment: a cumulative blockade of Na channels in KC-containing solution, and a cumulative unblocking of these channels after washout of KC from the node. It is obvious that opening of the activation gate of the Na channel promoted both its blocking and unblocking.

The effect of a transient increase in the amplitude of depolarizing pulses on the time course of cumulative inhibition of $I_{Na}$ by KC is illustrated in Fig. 4. In this experiment, the nodal membrane was stimulated repetitively by short ($t = 10$ ms)
depolarizing pulses ($E_T = -15 \text{ mV}$). This caused a gradual decline in $I_{Na}$ amplitude. During the interval marked by arrows (↔) $E_T$ was suddenly augmented up to +80 mV. At this $E_T$, $I_{Na}$ got outward direction and its decrease during repetitive pulsing was enhanced. After returning $E_T$ to the initial value (-15 mV) the inward $I_{Na}$ proved to be considerably diminished (as compared to the extrapolated value showed by the dashed line), and during subsequent repetitive pulsing it rose gradually to reach a steady-state level corresponding to this $E_T$. Implications of this experiment will be considered in Discussion.
2. Potassium currents

The effects of KC were studied under conditions of blockade of Na channels by 10^{-7} mol/l TTX and a total replacement of NaCl in the external solution by KCl (solution No 2). Fig. 5 A shows a result of one such experiment. Before KC application to the node, control outward $I_k$ was elicited by a depolarizing pulse to $E_r = +90$ mV (see Fig. 5 A, trace 1). During membrane depolarization $I_k$ decayed slowly, apparently due to a partial inactivation of K channels and accumulation of $K^+$ in the perinodal space. A 7-min superfusion of the resting node with 10^{-4} mol/l KC (solution No 3) brought about a drastic decrease in $I_k$ amplitude (trace 2) and

Fig. 5. Effects of 10^{-4} mol/l KC on potassium currents ($I_k$). A — current records: (1) in K+ -rich external solution (No. 2); (2), after 7-min treatment of the resting nodal membrane by KC (solution No 3); (3) after 15-min washing of the node by a drug-free solution No 2. B — voltage dependence of $I_k$ amplitude (peak value) in the same node during the second application of 10^{-4} mol/l KC (solution No 3). The amplitudes of $I_k$ before and during KC application are represented by empty circles and squares, respectively. $E_r = -110$ mV; potassium currents were elicited by 25-ms depolarizing pulses of various amplitudes, separated by long (10 s and more) intervals. Temperature 7 °C. Fibre 20.02.84.

Fig. 6. Cumulative inhibition of $I_k$ by KC (10^{-4} mol/l). Abscissa: numbers of pulses in the train. Ordinate: relative value of $I_k$ during the $n$-th pulse. $I_k$ value during the first pulse in the train ($I_1$) was taken for unity. The figure shows both a cumulative decrease in $I_k$ during repetitive pulsing at a frequency of 10 Hz and recovery of $I_k$ after a decrease in the pulsing frequency from 10 to 0.2 Hz. $E_c = -95$ mV, $E_r = +90$ mV, $t = 10$ ms. Temperature 7 °C. Fibre 20.02.84.
appreciably accelerated the current decay during maintained membrane de-

depolarization. Subsequent 15-min washing of the node with a drug-free KCl

solution (No 2) caused only a partial recovery of $I_K$ amplitude, whereas the

KC-induced decay of the current (time-dependent inhibition) was greatly di-

minished (trace 3). Another application of $10^{-4}$ mol/l KC to the node induced

a new decrease in $I_K$: simultaneously the time-dependent inhibition of the outward

$I_K$ was considerably enhanced (not illustrated). Fig. 5 shows that KC only blocked

outward $I_K$ and virtually did not affect inward $I_K$. Similar results were obtained in

three other experiments. The frequency-dependent inhibition of outward $I_K$ by KC

is shown in Fig. 6: at 10 Hz $I_K$ gradually (pulse by pulse) declined and recovered

after the stimulation frequency decreased from 10 to 0.2 Hz.

**Discussion**

a) Na channels

Like many amine drugs with local anesthetic antiarrhythmic activity, the compound

KC produces two types of phenomenologically different inhibition of $I_{Na}$: the

so-called tonic and a cumulative (use-dependent) blockade of Na channels

(Strichartz 1973; Courtney 1975; Cahalan 1978; Khodorov et al. 1976; Khodo-

rov and Zaborovskaya 1983).

The tonic block develops during a prolonged treatment of the resting

membrane by KC. It can therefore be suggested that this type of block results from an

interaction of the drug with closed Na channels. Such an interaction may
decrease the number of Na channels capable of opening during the first test pulse after a 5—10 min exposure of the resting node to KC.

The cumulative inhibition of $I_{Na}$ by KC results from its binding to the open Na

channels. This conclusion is derived from the fact that a train of short depolarizing

pulses, in contrast to a single long-lasting depolarizing step (see Fig. 1.B) caused a considerable supression of $I_{Na}$ in KC-treated membrane.

To discuss some peculiarities of KC-induced cumulative inhibition of $I_{Na}$ it is reasonable to use the well known kinetic scheme of drug-channels interaction


\[ \text{Scheme I} \]

Here, R, O and I are the resting, open and inactivated states of Na channels, respectively. $R^*$, $O^*$ and $I^*$ are the corresponding states of KC-blocked Na
channels. \(k\) and \(l\) are respective blocking and unblocking rate constants of interaction of KC with open Na channels.

During a depolarizing pulse a certain fraction of normally activated Na channels interact with KC and go over to open-blocked (O*) and then to inactivated-blocked (I*) states. After the end of a depolarizing pulse Na channels return from I to R and from I* to R* states.

We have seen that a 100-fold decrease in the pulsing frequency (from 10 to 0.1 Hz) did not diminish the depth of the cumulative KC block (see Fig. 2). This indicated that the rate of unblocking of channels during interpulse intervals (i.e. at the holding potential) is very low.

Most investigators believe that inactivation plays a central role in the mechanism of drug-induced inhibition of \(I_{\text{Na}}\) irrespective of whether these drugs interact preferentially with normally inactivated Na channels, or whether they block originally open channels. In the latter case binding of the blocker to its "receptor" in the axoplasmic channel mouth promotes closing of the inactivation gate; this manifests itself as a shift in the voltage dependence of \(h^*\) (steady-state fraction of blocked non-inactivated Na channel) towards more negative potentials (Courtney 1975; Hille 1977; Schwarz et al. 1977; Cahalan 1978). However, the results of the present experiments (see Fig. 3) suggest that the main reason for the cumulative inhibition of \(I_{\text{Na}}\) by KC is an accumulation of Na channels in the "resting-blocked" (R*), but not in the "inactivated-blocked" (I*) state. Indeed, it was observed that cessation of pulsing combined with a 10-min washing of the node by a drug-free solution resulted in a relatively small increase in \(I_{\text{Na}}\). Na channels became rapidly unblocked when repetitive pulsing was turned on during a continuous washing of the node. This clearly indicates that it is the closed activation (m-) gate but not the inactivated (h-) one that impedes clearing of KC from Na channels in the interpulse intervals. If this is the case it may be concluded that both binding and unbinding of KC to and from the channel proceed via the O \(\rightarrow\) O* pathway.

In the presence of KC in the solution both blocking (R \(\rightarrow\) O \(\rightarrow\) O* \(\rightarrow\) I*) and unblocking (R* \(\rightarrow\) O* \(\rightarrow\) O) events occur during each depolarizing pulse. At the beginning of stimulation, when R \(\gg\) R*, the blocking process predominates, and the fraction of blocked channels becomes gradually smaller, and finally equilibrium is established with the number of blocked and unblocked Na channels during each depolarizing pulse becoming equal. This corresponds to the steady-state of the KC-induced cumulative block.

The suggestion that sodium inactivation does not appreciably contribute to KC-induced cumulative inhibition of \(I_{\text{Na}}\) is supported by our earlier observation that the abolishment of fast sodium inactivation by chloramine-\(T\) treatment of the nodal membrane resulted in an only relatively small reduction in this cumulative block (Khodorov and Zaborovskaya, 1984; Zaborovskaya and Khodorov 1984).
The interaction of KC with open Na channels was voltage-dependent: an increase in the magnitude of depolarizing pulses during repetitive membrane stimulation reversibly enhanced the cumulative block (see Fig. 4). To explain this fact in the framework of the kinetic scheme presented above one or both rate constants \((k, l)\) of KC-channel interaction must be assumed to be voltage-dependent. The ratio \(k/l\) increases with \(E_r\) resulting in an increase in the fraction of blocked channels. This effect is reversible since a decrease in \(E_r\) results in a reduction of \(k/l\), which, in turn, leads to a change in the balance of newly blocked and unblocked Na channels during each depolarizing pulse: less channels become blocked and/or more channels unblocked. As a result \(I_{Na}\) increases pulse by pulse until a new level of the cumulative block is reached (see Fig. 4).

Similar voltage-dependence of the cumulative inhibition of \(I_{Na}\) is typical of many antiarrhythmic drugs which interact preferentially with open Na channels (Khodorov and Zaborovskaya, 1983; Zilberter et al. 1983; Bolotina 1984).

What may be the reason of the inability of KC to interact with inactivated Na channels?

According to Hille's hypothesis (Hille 1977), the lipid-solubility of the drug is a major factor that determines its pathway to the channel receptor: “lipid-soluble drug forms pass via hydrophobic region of the membrane, while charged and less lipid-soluble forms come and go from the receptor via hydrophilic region (the inner channel mouth)”. The data presented in this paper seem to be at variance with this notion. Indeed, KC has a much higher lipid solubility than many other tertiary amine local anesthetics that prefer to interact with inactivated Na channels. Thus, for example, the value of \(\log P\) (n-octanol/water partition coefficients) calculated according to the theoretical scheme of Hansh and Leo (1979) for procaine, lidocaine (see Courtney 1980) and KC (this paper) are 2.00, 2.76 and 4.39, respectively. Nevertheless, KC is unable to interact with inactivated Na channels and blocks them mainly after the opening of the activation gate. We may therefore conclude, that lipid solubility of a drug itself does not define the major pathway of the drug to (and from) the receptor.

The inability of KC to escape readily from the resting-blocked Na channels (i.e. to undergo \(R \rightarrow R^*\) transition) after the end of a depolarizing pulse may be explained by assuming that the closed activation gate stabilizes the drug-receptor complex in the resting channel.

b) K channels

KC, similarly as N-propyl ajmaline or ajmaline (Khodorov and Zaborovskaya 1983) or quinidine (Revenko et al. 1981, 1982) can block K channels along with Na channels. There is, however, a considerable difference in the blocking action of KC on these two types of voltage-sensitive channels: KC inhibits both inward and
outward $I_n$ but only outward $I_k$ (see Fig. 5 B). The resistance of inward $I_k$ to KC may be explained by an interaction of KC and K+ within a single pore with multiple occupancy. The mechanism of such a current-dependent blockade of K channels by organic cations has been analyzed by Armstrong (1975).

K channels in the nerve membrane are known to lack fast inactivation. In spite of this, KC produces a clear-cut cumulative inhibition of outward $I_k$. This strongly supports the notion that inactivation is not a prerequisite for cumulative blockade of ion channels. To explain the cumulative inhibition of $I_k$ it should be assumed that the closing of the activation gate at the end of a depolarizing pulse delays KC escaping from the pore; as a result blocked K channels accumulate during repetitive membrane depolarization.

There is a considerable difference in the rate of unblocking between K and Na channels after the termination of repetitive pulsing. Thus, a decrease in the pulsing frequency from 10 to 0.2 Hz resulted in a relatively fast recovery of $I_k$ from the KC block (see Fig. 6), whereas under the same conditions $I_n$ remained practically unchanged (see Fig. 2). This difference could be understood by postulating that KC binds loosely to the blocking site in the K channel while being much more tightly bound to the corresponding site in the Na channel.

There is little doubt that during each depolarizing pulse KC interacts with open K channels. This conclusion follows from the dependence of the KC block on the direction of $I_k$ (see Fig. 5 B), and from the time course of the inhibition of outward $I_k$ during a long-lasting depolarizing step (see Fig. 5 A).

The question that remains to be solved is what is the pathway by which KC leaves the blocked K channels in the interpulse intervals: via hydrophobic region of the membrane or via hydrophilic route upon rare spontaneous openings of K channels. In the latter case, the unequal rate of unblocking of K and Na channels can be explained by assuming different probabilities of spontaneous opening of these channels at the holding potential.

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Note added in proof

After this paper was submitted the report of Yeh and Tanguy (1985) appeared. These authors have shown in experiments on internally perfused squid giant axons that membrane hyperpolarization (over the range from −70 to −120 mV) induced a considerable increase in the time constant for slow recovery from use-dependent block by drugs interacting with open Na channels (compounds QX-222, QX-314, 9-aminoacridine). The conclusion was drawn that during repetitive pulsing, the drug molecules were trapped by the activation gate of the channel ("m-gate trapping hypothesis"). This conclusion is in agreement with that inferred in the present paper from other experimental data.
References


Hille B. (1971): The permeability of the sodium channel to organic cations in myelinated nerve. J. Gen. Physiol. 58, 599—619


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