Use-Dependent Features of 4-Aminopyridine Block of Transient Outward Current in Rat Ventricular Myocytes

G. CHRISTÉ[1], M. ŠIMURDOVÁ[2] and J. ŠIMURDA[2]

1 *INSERM U 121, 18 Ave Doyen Lépine, F-69500 Bron, France*
2 *Department of Physiology, Faculty of Medicine, Masaryk University, 66243 Brno, Czech Republic*

Abstract. The inhibition of the calcium-independent transient outward current (I_{to}) by the widely used blocker 4-aminopyridine (4-AP) was studied in adult Wistar rat ventricular myocytes, enzymatically isolated and voltage-clamped in the whole cell configuration using patch-clamp pipettes. 4-AP at 1 mmol/l concentration caused complete steady-state block of I_{to} at resting or hyperpolarized voltages. The block was partially removed during repeated depolarizations applied at frequencies above 0.25 Hz. Changes in the level of block during a depolarizing pulse (to +40 mV) and during a following rest period (at −90 mV) were analyzed. On depolarization, the recovery from 4-AP block (at 0.5 mmol/l) started with a delay approximately corresponding to the time constant of I_{to} inactivation and then followed a monoexponential time course (time constant of about 44 ms). The restoration of block at a holding voltage of −90 mV, after recovery of I_{to} from inactivation, followed a monoexponential time course (time constant of about 1.2 s). The results are consistent with the hypothesis that the binding site for 4-AP at the I_{to} channel is available in the closed and open states but not when inactivated. Unblocking of I_{to} at stimulation intervals shorter than approximately 1 s may occur at 4-AP concentrations below 4 mmol/l.

Key words: 4-aminopyridine — Cardiac ventricle — Wistar rat — Use-dependency — Transient outward current

Correspondence to: Georges Christé, INSERM Unit 121, Research Unit on Cardiac Electrical Activity, Bat. INSERM, salle E 18, 18 Ave Doyen Lépine, F-69500 BRON France

Introduction

4-Aminopyridine is widely used as a blocker of various types of K^+ channels in nerves (Pelhate and Pichon 1974; Ulbricht and Wagner 1976; Yeh et al. 1976; Meves and Pichon 1977; Thompson 1982), striated muscles (Stefani and Chiarandini 1982; Davies et al. 1991), smooth muscles (Boev et al. 1985; Beech and Bolton 1987), cardiac muscle (Kenyon and Gibbons 1979; Giles and VanGinneken 1985; Šimurda et al. 1988; Boyle and Nerbonne 1992) and other cells (Wagoner and Oxford 1990; Dubois and Rouzaire-Dubois 1991; Choquet and Korn 1992). Recently, the complex mechanism of interaction of aminopyridines with K^+ channels has been studied in detail in cloned channels (Kirsch and Drewe 1993; Russel et al. 1994). 4-Aminopyridine is also being considered a putative therapeutic agent in multiple sclerosis (Davis et al. 1990) and chronic spinal injury (Blight et al. 1991).

As a consequence of the diversity of 4-AP sensitive channels in various species and tissues, there are distinct differences in the detailed properties of 4-AP block. However, a common feature, confirmed in a number of delayed outward rectifiers and transient outward currents so far studied (e.g. Yeh et al. 1976; Thompson 1982), seems to be the interaction of the drug with channel gating, resulting in a tendency for depolarization to relieve the block. This is manifested in most cases as 'use dependent deblock' (termed also 'reverse use dependency'). Surprisingly, it is only recently that the reverse use dependency of the 4-AP-induced block of K^+ channels has been studied in cardiac muscle, despite the importance of the use dependency of channel inhibition for the understanding of the effects of a variety of antiarrhythmic agents. The use dependency of 4-AP block of calcium- independent transient outward current in cardiac muscle was first disclosed in dog trabeculae (Šimurda et al. 1989) and subsequently analysed in ferret (Campbell et al. 1993) and rat ventricular myocytes (Castle and Slawsky 1993).

In this paper we describe some features of the use dependent 4-AP block of I_{to} in ventricular myocytes of adult Wistar rats. Rat ventricular myocytes are extensively used in electrophysiological studies. We felt it necessary to seek for a use-dependency of 4-AP block of I_{to} both to compare its properties in this preparation to those in homologous cells in other species and to check for the validity of using 4-AP as a tool to dissect membrane currents, especially when short intervals of stimulation are used.

Materials and Methods

Cell isolation

Single ventricular myocytes were isolated from the hearts of adult male Wistar rats (260–460 g) using a protocol based on previously described procedures (Mitra and Morad 1985). After deep ether anesthesia and cervical dislocation, the hearts were quickly excised and

retrogradely perfused (at 37 °C) through the aorta in a Langendorff set-up. Nominal calcium-free modified Tyrode's solution with heparin was applied for 5 min. Then the same solution without heparin but containing collagenase (0.45 mg/ml, Sigma type I) and protease (0.15–0.3 $mg \cdot ml^{-1}$, Sigma type XIV) was recirculated through the heart for 15–30 min. After brief perfusion with a nominally calcium-free Tyrode's solution containing 1.2 $g \cdot litre^{-1}$ bovine serum-albumin (Sigma, A-4503), perfusion of KB followed. The separated ventricles were then cut with fine scissors into small fragments and myocytes were mechanically dispersed in KB solution by gentle shaking or stirring in a small Erlenmayer's flask. The cell suspension was filtered through a 100 μm nylon mesh into 10 ml plastic centrifuge tubes and stored at 6 °C. After 30 min, the cell pellet was resuspended in fresh KB solution and kept in small Petri dishes before experimental use.

No attempt was made to select areas where cells have been reported to possess largest I_{to} (epicardial layer, e.g. Litovsky and Antzelevitch 1988). However, as our aim was to study I_{to}, only cells displaying a sizeable fast component of outward current were used in this work.

Solutions

The nominally calcium-free Tyrode's solution contained (mmol/l): NaCl 120; KCl 5.4; $MgCl_2$ 3.5; taurine 20; NaH_2PO_4 1.5; glucose 10; HEPES 10; pH was adjusted to 7.2 with NaOH. The KB solution contained (mmol/l): K-glutamate 70; KCl 25; KH_2PO_4 10; $MgCl_2$ 5; oxalic acid 10; taurine 20; glucose 20; pyruvic acid 5; HEPES 10; pH was adjusted to 7.2 with KOH. The pipette-filling solution was a nominally calcium-free solution containing (mmol/l): K-glutamate 130; KCl 5; $MgCl_2$ 5; glucose 10; EGTA 5; HEPES 10; K_2ATP 3; Na_2-creatine phosphate 5; Na-pyruvate 5; pH was adjusted to 7.2 with KOH. All chemicals were of reagent grade. 4-AP (Sigma Company, A-0152) was dissolved just before an experiment in the proper solution, pH was checked and adjusted if needed. Exposure of 4-AP containing solutions to light was avoided throughout the experimental procedure.

At the beginning of an experiment the cells were maintained in a medium containing (mmol/l): NaCl 120; KCl 5.4; $MgCl_2$ 1.2; $CaCl_2$ 1.8; NaH_2PO_4 0.4; glucose 10; HEPES 10; pH was adjusted to 7.4 with NaOH. Once intracellular recording conditions were achieved, the cell under study was superfused with the same medium, but in most experiments NaCl was replaced by choline-Cl in order to exclude the fast sodium current (I_{Na}) and the possible contribution of sodium-activated transient currents (Kameyama et al. 1984). Atropine (0.01 mmol/l) was added to eliminate choline-activated muscarinic potassium channels. This standard superfusion medium contained (mmol/l): choline chloride 120; $MgCl_2$ 2; $CaCl_2$ 1.8; glucose 10; HEPES 10; pH adjusted to 7.2 with KOH. It was supplemented with either 2 mmol/l $CoCl_2$ or 0.5 mmol/l $CdCl_2$ to completely block the calcium current (I_{Ca}).

Electrophysiological recording

A small aliquot of cells was transferred to a perfusion chamber placed on the stage of an inverted microscope (Metaval, Zeiss Jena). Macroscopic current recording was obtained with the whole-cell voltage-clamp method, no compensation for capacitance or leak currents was used. Pipettes were pulled from borosilicate glass (Kavalier, Czech Rep.) and were heat-polished before use. Their ohmic resistance, when filled with the pipette-filling solution, was between 0.8 and 2 MΩ. Most experiments were performed in the summer time and at room temperature of 27 to 29 °C.

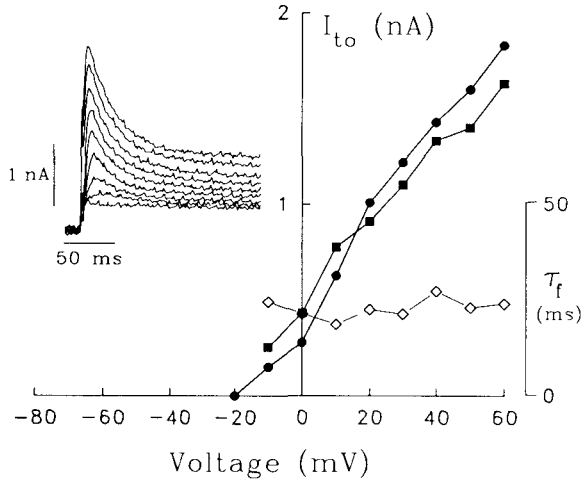

Figure 1. Current-voltage relationship for I_{to} established from a cell in the absence of Na^+ ions in the extracellular solution (replaced by choline) and with 0.5 mmol/l $CdCl_2$. Two types of measurements are compared: circles are peak I_{to} currents after subtraction of the slowly decaying outward current component as extrapolated by eye, and squares are the recalculated values (at 10 ms after pulse onset) of the rapidly decaying components of outward current, after least-squares adjustment of a double exponential decay to the current traces. The time constants of decay (τ_{fast}) of I_{to} are plotted versus voltage (diamonds). The inset shows superimposed current responses to depolarizations between −20 and +60 mV from a holding voltage of −85 mV. For clarity, capacitive currents were subtracted at time of analysis.

Results

To study the 4-AP sensitive transient outward current (I_{to}) in rat ventricular myocytes, the time-dependent inward currents were suppressed: I_{Na} by substituting external Na^+ by choline, and I_{Ca} by external application of 2 mmol/l $CoCl_2$ or 0.5 mmol/l $CdCl_2$. Under these conditions, the current responses to graded depolarizing steps (Fig. 1, inset) showed characteristic waveforms as described in detail by other authors (Josephson et al. 1984; Apkon and Nerbonne 1991). Fast activation of the outward current was followed by a slower decline that could be well fitted by a function: $I_{fast} \cdot \exp^{(-t/\tau_{fast})} + I_{slow} \cdot \exp^{(-t/\tau_{slow})} + I_{ss}$.

As evidenced by Apkon and Nerbonne (1991), the slow exponential decay component (I_{slow}: amplitude at time 0, τ_{slow}: time constant) is related to slow inactivation of the 4-AP-insensitive delayed outward current (I_K) while the fast component (I_{fast}, τ_{fast}) corresponds to the inactivation of the 4-AP-sensitive transient outward current (I_{to}) · I_{ss} represents the sum of background and leakage currents.

Two approaches to the estimation of the voltage dependence of I_{to} availability are compared in Fig. 1. I_{to} was evaluated as the difference from the peak current to the slow component, as extrapolated to the time of the peak, and alternatively as the value of the rapid component 10 ms after the onset of depolarization. Both measurements gave similar current-voltage relations with a threshold of activation slightly below −20 mV. The time constant of I_{to} inactivation (τ_{fast}) did not show significant voltage-dependence. The value obtained from double exponential analysis for 86 current responses to depolarizations from $HP = -90$ mV to +20 mV in 4 cells at 27 29°C was 19.0 ± 0.6 ms (mean ± SEM)). This is twice faster than reported by Apkon and Nerbonne (1991), who worked at a lower temperature (24–25°C) and in another rat strain (Long Evans).

From this point forward, the decaying phase of the outward current response from 10 ms after the onset of a test pulse will be fitted with a sum of two exponential functions plus an offset (see above). The estimated amplitude of the fast component (I_{fast}) will be taken as a measure of the available I_{to} at the time of the pulse onset. This mode of estimation suppressed the slowly decaying and leakage currents altogether and cancelled the changes in I_{to} magnitude due to changes in activation-inactivation kinetics.

In all of the cells used in this study, a slow rundown of I_{to} was apparent. When needed, an approximate correction factor was applied to the data, based on linear extrapolation of the time course of changes of responses to identical protocols in control solution in the same cell.

Repetitive depolarizations relieve 4-AP block

The use-dependency of the I_{to} block was first tested at a 4-AP concentration of 1 mmol/l, which was sufficient to induce complete steady-state block at hyperpolarizing voltages (Fig. 2). After 20 s of rest at −90 mV, a series of 700 ms pulses to +20 mV at 0.5 Hz was applied. The fast component was absent in the current response to the first pulse (Fig. 2, '4-AP'). In contrast, in the traces corresponding to the second and next pulses, I_{to} reappeared, indicating a partial deblock due to the foregoing depolarizing pulse. Interestingly, the first and the next current responses obviously crossed-over, due to the increased slow component of the first response. To determine whether this feature was related to the effect of 4-AP, the same pattern of membrane voltage was applied under control conditions in the absence of the drug (Fig. 2, 'Control'). It is obvious that the slow component of the first response was also enhanced. The most likely explanation is that the 4-AP-insensitive delayed outward current fully recovered from inactivation before the first depolarizing pulse and partially inactivated during the following pulses, due to insufficient recovery time (1300 ms, in relation to a time constant of recovery from inactivation of 445 ms as estimated by Apkon and Nerbonne (1991)).

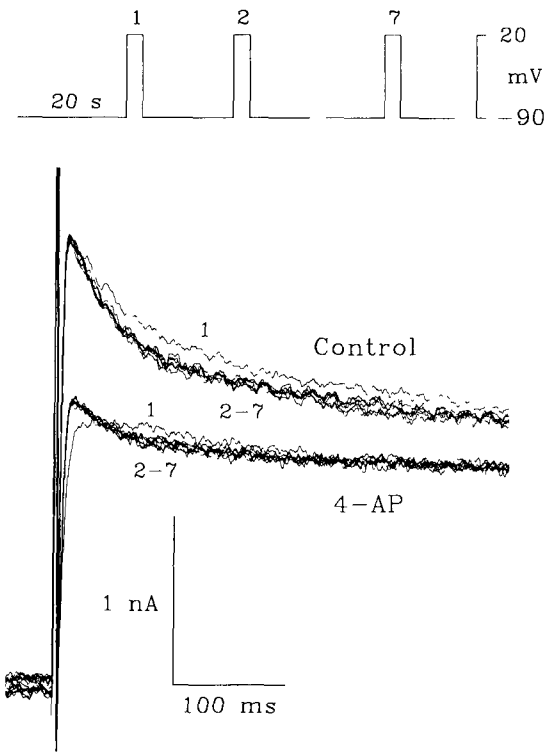

Figure 2. Use-dependent deblock of I_{to}. The voltage protocol (above) consisted of a series of 7 identical 700 ms test depolarizations (at 0.5 Hz) preceded by a 20 s period of rest. 'Control': Superimposed current responses to the depolarizing steps in Tyrode's solution. The rest period does not significantly affect I_{to} (only the slow delayed current is slightly larger in response to the first step). '4-AP': After 15 min in the presence of 4-AP (1 mmol/l), I_{to} is absent during the first pulse (full block at rest) and partly restored during the next (2–7) pulses. Only the first 400 ms of each record are shown for clarity. I_{Ca} was blocked with 2 mmol/l $CoCl_2$.

Repetitive depolarizations cause more unblock at higher frequencies

Another manifestation of the use dependency of 4-AP block of I_{to} was a partial deblock at higher frequencies under steady-state at regular stimulation (Fig. 3). I_{to} was completely blocked at 0.125 Hz and progressively deblocked upon increasing the rate of stimulation with 300 ms pulses to 0.5 and 1.5 Hz. The recovery from block was first visible at 0.25 Hz. Here also, the cross-over of traces in Fig. 3*A* was attributable to frequency-dependent cumulative inactivation of the delayed outward current; therefore the current traces were shifted (Fig. 3*B*) to compensate for this effect.

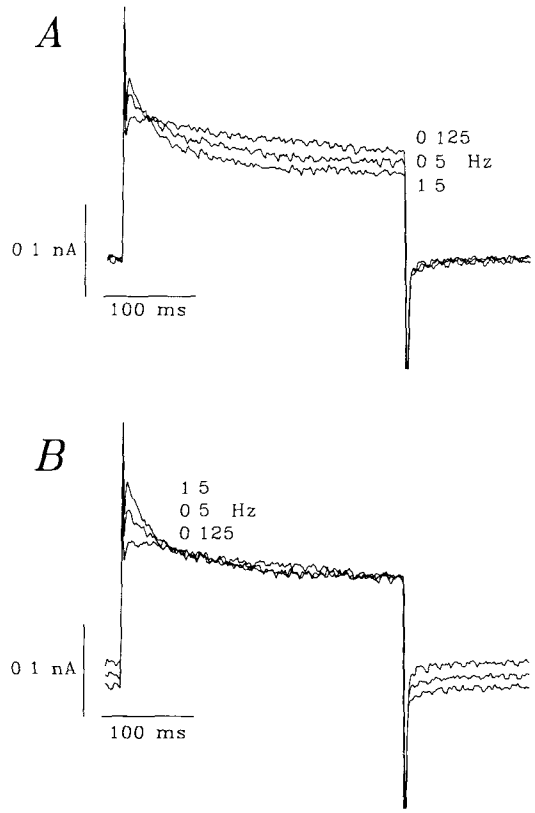

Figure 3. Frequency-dependence of I_{to} block by 4-AP (1 mmol/l). 300 ms depolarizing pulses from $HP = -90$ mV to +20 mV were applied at different frequencies in the presence of 2 mmol/l $CoCl_2$. *A*: Superimposed current responses recorded at steady state at 0.125, 0.5 and 1.5 Hz. *B*: For clarity, the same records were shifted to match the current values at the end of pulses, to compensate for decreased delayed outward current. Note that I_{to} is absent at 0.125 Hz and recovers gradually at higher frequencies.

Analysis of the time course of changes in block during a long depolarization and subsequent rest

To visualize the time courses of deblock during a depolarizing step and of restoration of the block following repolarization, two modifications of a double-pulse protocol were used. Under the effect of 0.5 mmol/l 4-AP, the membrane voltage was maintained at a hyperpolarized level of −90 mV for 20 s, to allow the block to reach its steady level, as tested by a standard 300 ms test pulse to +40 mV.

Unblock during a depolarization. A voltage clamp paradigm, composed of a conditioning depolarizing pulse to +40 mV of gradually increasing duration and a 1 s

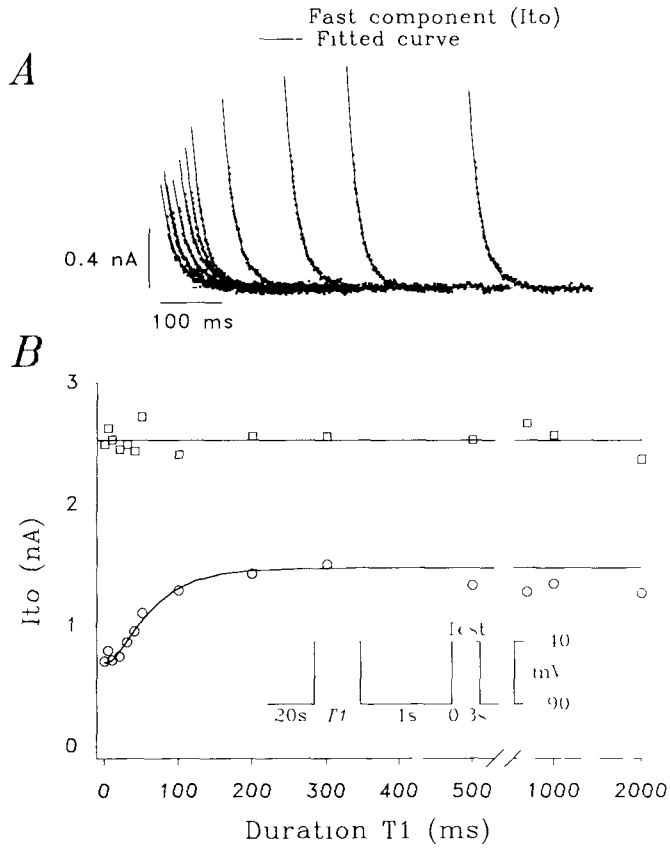

Figure 4. Time course of relief of the 4-AP block of I_{to} during a depolarizing step. Experimental protocol: a 20 s period of rest was followed by a conditioning pulse of variable duration (T1), a 1s rest interval and a uniform test pulse (inset). *A*: The currents of test pulses affected by 4-AP (0.5 mmol/l) were approximated by a function: $I_{fast} \cdot \exp^{(-t/\tau_{fast})} + I_{slow} \cdot \exp^{(-t/\tau_{slow})} + I_{ss}$, after omitting the first 10 ms of each record. I_{to} was evaluated as the fast exponential component ($I_{fast} \cdot \exp^{(-t/\tau_{fast})}$) after subtraction of the slow and constant components from the current records. The extracted time courses of I_{to} (points) with exponential fits (solid curves) are shifted along the time axis by intervals equal to the duration of the conditioning pulse T1 to mimic the development of block during depolarization. *B*: Dependence of I_{to} represented by I_{fast} on the duration of preconditioning pulses T1 under control conditions (squares) and in the presence of 4-AP (circles). Control measurements started 25 min after those in the presence of 4-AP, which allowed some rundown to take place (about 20% in 20 min in this cell). Thus a factor of 1.34 was applied to the control I_{to} amplitudes. The control solution was the same as for Fig. 1.

constant repolarized time gap, was interpolated between the resting period and the test pulse (Fig. 4, inset to panel *B*). I_{to} of the test pulse, as represented by the

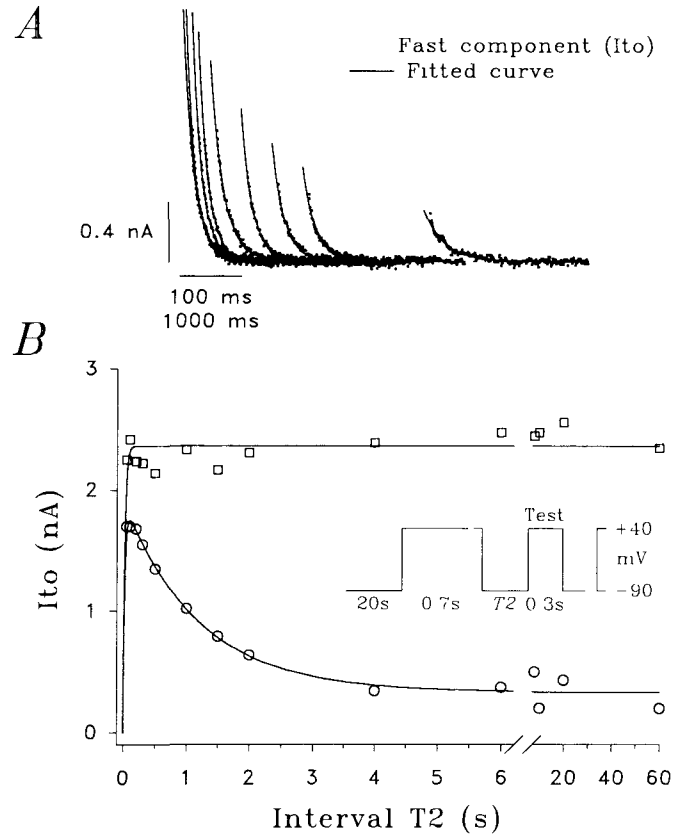

Figure 5. Development of the 4-AP block of I_{to} at rest after a depolarizing pulse. Experimental protocol: a 20 s period of rest was followed by a 700 ms depolarizing conditioning pulse separated from a test pulse by variable resting intervals (T2, see inset). *A*: I_{to} of the test pulse was evaluated by the procedure described in the legend to Fig. 4. The extracted time courses of I_{to} (points) and exponential fits (solid curves) are shifted along the time axis by intervals proportional to T2 (on a 10-fold compressed time scale) to mimic the restoration of block at rested voltage. *B*: Dependence of I_{to} (amplitude of the fast component of the current record) on the rest interval preceding the test pulse (T2) under control conditions (squares) and in the presence of 0.5 mmol/l 4-AP (circles). As in Fig. 4*B*, control measurements started 26 min after those in 4-AP. A multiplying factor of 1.39 was applied to control I_{to} amplitudes to compensate for rundown of I_{to}. The control solution was the same as for Fig. 1.

fast component of the recorded current (Fig. 4*A*) grew with the gradually increasing duration of the conditioning pulse. Interestingly, short conditioning pulses up to about 25 ms did not produce any unblock. Only longer pulses were effective

and the increase of I_{to} magnitude as a function of the duration of a conditioning pulse longer than 30 ms was well approximated by an exponential function with a time constant of 44 ms. The same protocol was applied in the absence of the drug (squares in Fig. 4*B*). As expected with regard to the fast recovery of I_{to} from inactivation, the preconditioning had no effect on the magnitude of I_{to} activated by test pulses.

Reblock at rest after unblock. The time course of restoration of the resting block after the end of a long depolarization was tested by a modified double-pulse protocol (Fig. 5, inset to panel *B*). The interval between a conditioning pulse of constant duration (0.7 s) and the standard test pulse was progressively increased in the range between 50 and 4000 ms. After recovery from inactivation, the magnitude of I_{to} decreased asymptotically to a resting level following an exponential course (time constant 1.2 s). At shorter interpulse intervals (up to 50 ms) the I_{to} magnitude increased, due to the recovery from inactivation, as tested in other cells (not shown). The recovery from inactivation was fast with a time constant of exponential increase around 30 ms (not shown) which is in accord with observations of other authors (Josephson et al. 1984; Apkon and Nerbonne 1991).

Discussion

The results of this work provide evidence for use-dependent effects of 4-aminopyridine-induced block of transient outward current in ventricular myocytes of adult Wistar rats. We may infer that the block depends strongly on membrane voltage even though the experimental protocols used involved two voltage levels only. The results of measurements of the dependence of I_{to} magnitude on the duration of conditioning pulses (Fig. 4) clearly demonstrate much deeper steady block of I_{to} in hyperpolarized than in depolarized membranes. Surprisingly, in the presence of 0.5 mmol/l 4-AP, the time constants related to the processes of I_{to} unblocking at +40 mV and reblocking at −90 mV (0.044 and 1.12 s respectively) were several times shorter than those estimated by Castle and Slawsky (1993) also in rat ventricular myocytes (approximately 0.42 and 5.2 s). The time constants estimated hitherto for other species, i.e. dog ventricle (0.23 and 4.7 s (Šimurda et al. 1989)) and ferret ventricular myocytes (0.24 and 1.38 s (Campbell et al. 1993)) were intermediate between the values found for rat. Such considerable differences in the apparent kinetics of drug-channel interaction in the same species might be related to differences in experimental conditions: e.g. temperature (about 4 °C higher in this work), glutamate replacing chloride ions in the pipette solution, and also to differences in the basic kinetic characteristics of the I_{to} current: (inactivation and recovery from inactivation time constants were respectively 57 ms at +40 mV and 143 ms at −70 mV for Sprague-Dawley rat (Campbell et al. 1993) and 19 ms at +20 mV and 30 ms at −90 mV for Wistar rat (present work)). Further compara-

tive experiments in native myocytes and probably also comparison of the channel structures in both rat strains are needed to confirm and explain these differences.

Very likely, the apparent voltage dependency of the block is mediated by voltage dependent channel gating. There is growing evidence in support of the idea that the binding sites for 4-AP molecules are located close to the inner mouth of K^+ channels (Kirsch and Drewe 1993). Taking into account that 4-AP molecules in their charged form are effective from inside the cell (Kirsch and Narahashi 1983; Kasai et al. 1986), direct voltage-dependency of drug-receptor interaction within the membrane electrical field seems unlikely. The notion that the drug-receptor interaction depends on the state of the channel, in accord with the modulated receptor hypothesis (Courtney 1975; Hille 1977), is more acceptable. Within the scope of this hypothesis we interpret our data as indicating high affinity of the drug to rested channel state and very low or zero affinity to the inactivated channel.

There remains to answer the question of the block in the open state, which is more difficult because the steady-state fraction of open channels at all voltages is very low. The behavior of 4-AP block in various kinds of K^+ channels indicated very large disparity in the potency of 4-AP to interact with open channels, as illustrated in two extreme cases. On the one hand, no access of the drug to its binding site in the open channel state of transient outward current was suggested in rat melanotrophs e.g. (Kehl 1990) and ferret ventricular myocytes (Campbell et al. 1993). On the other hand, the binding site was assumed to be accessible solely in the open state of the K^+ channels in amphibian myelinated nerve fibres (Århem and Johansson 1989). One convincing indicator of open channel block is related to characteristic alteration of the kinetics of the current, namely an apparent acceleration of inactivation (Thompson 1982). This effect, however, depends on the ratio of the rate constants related to inactivation to those related to drug-receptor interaction, and can be very low even if the open channel block is operative, as we were able to simulate on a quantitative model (not shown). In the present experiments, we did not observe significant acceleration of the apparent inactivation of I_{to} in the presence of 4-AP. On the other hand, the ineffectiveness of short conditioning pulses to unblock channels (Fig. 4) indicates that no considerable unblock occurs during transitions of channels through the open state because the initial delay in the plot of I_{to} magnitude versus conditioning pulse duration (Fig. 4*B*) approximately corresponds to the time constant of I_{to} inactivation. If any unblock in the open state occurs, its rate must be slow in comparison with the time constant of inactivation. An alternative explanation of this effect is a similarity of apparent affinity of the drug to rested and open channel. In dog ventricular muscle, short conditioning pulses even appeared to increase the level of the block beyond that achieved at rest (Šimurda et al. 1989) suggesting maximum affinity in the open state. In contrast, Campbell et al. (1993) interpret their observations in ferret ventricular myocytes in terms of a quantitative model where open-state-

block is excluded. Taken together, the present results suggest that, in Wistar rat ventricular cells, 4-AP molecules bind with a higher affinity to I_{to}-channels in the resting than in the open state, but not when inactivated. Further experiments are, however, needed to clarify the drug-channel interaction in the open state.

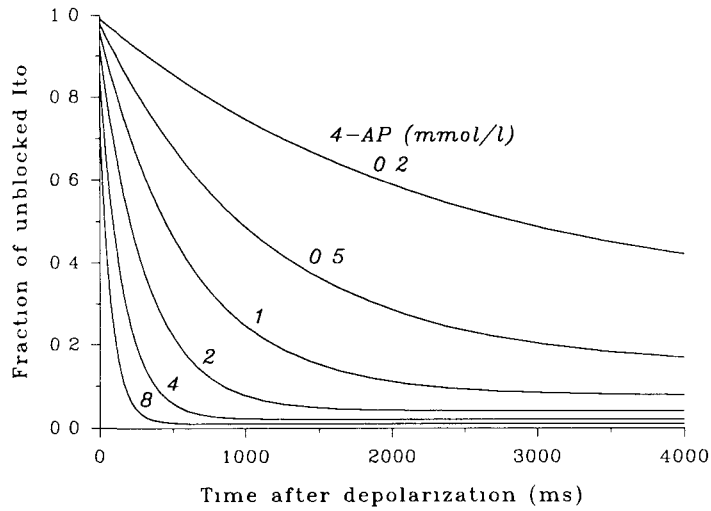

Figure 6. Model calculation of the time course of decay of available I_{to} after a depolarization long enough to cause maximal unblock. The model assumed a one to one binding of 4-AP to the I_{to} channel, the rate constants at −90 mV and +20 mV were adjusted to account for the data in Figs. 4 and 5 with 0.5 mmol/l 4-AP. Using the same values of rate constants, computations were done for several other 4-AP concentrations. The minimum time between depolarizing pulses to ensure maximal block may be read directly for each 4-AP concentration.

4-Aminopyridine is widely used in the millimolar range of concentrations to dissect membrane currents. When designing experimental protocols, the properties of use-dependent unblock (mainly the rate of deblock on depolarization and rate of restitution of block at rest) must be considered carefully. A complete steady block of I_{to} at rest would not ensure the absence of I_{to} at regular stimulation (Fig. 3). This of course may be less dramatic when larger 4-AP concentrations are used. From the knowledge of the kinetics of reblock in a given cell type, one might design protocols to ensure a sufficient degree of suppression of I_{to} throughout an experiment. One requirement for this is illustrated in Fig. 6. A simple one to one binding of 4-AP to the rested channel was used to estimate the time course of reblock at various 4-AP concentrations (Fig. 6). It was assumed that a steady level of deblock was reached at the end of a previous depolarization to +40 mV. If,

for example, more than 90% block is required, then the minimal interval between depolarizations should exceed 2.4 s at 1 mmol/l, while 0.9 s would be sufficient at 2 mmol/l 4-AP.

Acknowledgements. The authors are grateful to Mrs E. Kellnerová, Mrs B. Vyoralová, Dr. M. Nováková and Dr. Z. Nováková for their help. This work was supported by PECO programs Nos 12674, 12675 and 12676 of the Human Capital and Mobility scheme of the European Community Commission, and by funds from the Granting Agency of the Czech Republic (Projects 305/93/0169 and Z1411/3).

References

Apkon M., Nerbonne J. M. (1991): Characterization of two distinct depolarization-activated K^+ currents in isolated adult rat ventricular myocytes. J. Gen. Physiol. **97,** 973—1011

Århem P., Johansson S. (1989): A model for the fast 4-aminopyridine effects on amphibian myelinated nerve fibres. A study based on voltage-clamp experiments. Acta Physiol. Scand. **137,** 53—61

Beech D. J., Bolton T. B. (1987): The effects of tetraethylammonium ions, 4-aminopyridine or quinidine on K^+-currents in single smooth muscle cells of the rabbit portal vein. Biomed. Biochim. Acta **46,** S673—S676

Blight A. R., Toombs J. P., Bauer M. S., Widmer W. R. (1991): The effects of 4-aminopyridine on neurological deficits in chronic cases of traumatic spinal cord injury in dogs: A phase I clinical trial. J. Neurotrauma **8,** 103—119

Boev K., Bonev A., Papasová M. (1985): 4-Aminopyridine induced changes in electrical and contractile activities of the gastric smooth muscle. Gen. Physiol. Biophys. **4,** 589—595

Boyle W. A., Nerbonne J. M. (1992): Two functionnally distinct 4-aminopyridine-sensitive outward K^+ currents in rat atrial myocytes. J. Gen. Physiol. **100,** 1041—1067

Campbell D. L., Qu Y., Rasmusson R. L., Strauss H. C. (1993): The calcium independent transient outward potassium current in isolated ferret ventricular myocytes. II. Closed state reverse use-dependent block by 4-aminopyridine. J. Gen. Physiol. **101,** 603—636

Castle N. A., Slawsky M. T. (1993): Characterization of 4-aminopyridine block of the transient outward K^+ current in adult rat ventricular myocytes. J. Pharmacol. Exp. Ther. **264,** 1450—1459

Choquet D., Korn H. (1992): Mechanism of 4-aminopyridine action on voltage-gated potassium channels in lymphocytes. J. Gen. Physiol. **99,** 217—240

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

Davies N. W., Pettit A. I., Agarwal R., Standen N. B. (1991): The flickery block of ATP-dependent potassium channels of skeletal muscle by internal 4-aminopyridine. Pflügers Arch. **419,** 25—31

Davis F. A., Stefoski D., Rush J. (1990): Orally administered 4-aminopyridine improves clinical signs in multiple sclerosis. Ann. Neurol. **27,** 186—192

Dubois J. M., Rouzaire-Dubois B. (1991): Interaction of 4-aminopyridine with normal and chloramine-T-modified K channels of neuroblastoma cells. Pflügers Arch. **419,** 93—100

Giles W. R., VanGinneken A. C. G. (1985): A transient outward current in isolated cells from the crista terminalis of rabbit heart. J. Physiol.(London) **368,** 243—264

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

Josephson I. R., Sanchez-Chapula J., Brown A. M. (1984): Early outward current in rat single ventricular cells. Circ. Res. **54,** 157—162

Kameyama M., Kakei M., Sato R., Shibasaki T., Matsuda H., Irisawa H. (1984): Intracellular Na^+ activates a K^+ channel in mammalian cardiac cells. Nature **309,** 354—356

Kasai H., Kameyama M., Yamagushi K., Fukuda J. (1986): Single transient K channels in mammalian sensory neurons. Biophys. J. **49,** 1243—1247

Kehl S. J. (1990): 4-Aminopyridine causes a voltage-dependent block of the transient outward K^+ current in rat melanotrophs. J. Physiol.(London) **431,** 515—528

Kenyon J. L., Gibbons W. R. (1979): 4-Aminopyridine and early outward current of sheep cardiac purkinje fibers. J. Gen. Physiol. **73,** 139—157

Kirsch G. E., Drewe J. A. (1993): Gating-dependent mechanism of 4-aminopyridine block in two related potassium channels. J. Gen. Physiol. **102,** 797—816

Kirsch G. E., Narahashi T. (1983): Site of action and active form of aminopyridines in squid axon membranes. J. Pharmacol. Exp. Ther. **226,** 174—179

Litovsky S. H., Antzelevitch C. (1988): Transient outward current prominent in canine ventricular epicardium but not endocardium. Circ. Res. **62,** 116—126

Meves H., Pichon Y. (1977): The effect of internal and external 4-aminopyridine on the potassium currents in intracellularly perfused squid giant axons. J. Physiol. (London) **268,** 511—532

Mitra R., Morad M. (1985): A uniform enzymatic method for dissociation of myocytes from hearts and stomachs of vertebrates. Amer. J. Physiol. **249,** H1056—H1060

Pelhate M., Pichon Y. (1974): Selective inhibition of potassium current in the giant axon of the cockroach. J. Physiol.(London) **242,** 90P—91P (Abstract)

Russel S. N., Publicover N. G., Hart P. J., Carl A., Hume J. R., Sanders K. M., Horowitz B. (1994): Block by 4-aminopyridine of a Kv1.2 delayed rectifier K^+ current expressed in Xenopus oocytes. J. Physiol.(London) **481,** 571- 584

Šimurda J., Šimurdová M., Čupera P. (1988): 4-Aminopyridine sensitive transient outward current in dog ventricular fibres. Pflügers Arch. **411,** 442—449

Šimurda J., Šimurdová M., Christé G. (1989): Use-dependent effects of 4-aminopyridine on transient outward current in dog ventricular muscle. Pflügers Arch. **415,** 244—246

Stefani E., Chiarandini D. J. (1982): Ionic channels in skeletal muscle. Annu. Rev. Physiol. **44,** 357—372

Thompson S. (1982): Aminopyridine block of transient potassium current. J. Gen. Physiol. **80,** 1—18

Ulbricht W., Wagner H. H. (1976): Block of the potassium channels of the nodal membrane by 4-aminopyridine and its partial removal by depolarization. Pflügers Arch. **267,** 77—87

Wagoner P. K., Oxford G. S. (1990): Aminopyridines block an inactivating potassium current having slow recovery kinetics. Biophys. J. **58,** 1481—1489

Yeh J. Z., Oxford G. S., Wu C. H., Narahashi T. (1976): Interactions of aminopyridines with potassium channels of squid axon membranes. Biophys. J. **16,** 77—81

Final version accepted March 31, 1995