Modulation of Single Cardiac Sodium Channels by DPI 201—106

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Abstract. Single sodium channel currents were analysed in cell attached patches from single ventricular cells of guinea pig hearts in the presence of a novel cardiotonic compound DPI 201-106. The mean single channel conductance of DPI-treated Na channels was not changed by DPI (20.8 ± 4 pS, control, 3 patches; 21.3 ± 1 pS with DPI, $5 \mu mol/1$, 3 patches). DPI voltage-dependently prolongs the cardiac sodium channel openings by removal of inactivation at potentials positive to -40 mV. At potentials negative to -40 mV a clustering of short openings at the very beginning of the depolarizing voltage steps can be observed causing a transient time course of the averaged currents. Long openings induced an extremely slow inactivation. Short openings, long openings and nulls appeared in groups refferring to a modal gating behaviour of DPI-treated sodium channels. DPI-modified Na channels showed a monotonously prolonged mean open time with increased depolarizing voltage steps, e. g. the open state probability within a sweep was increased. However, the number of nonempty sweeps was decreased with the magnitude of the depolarizing steps, e. g. the probability of the channel being open as calculated from the averaged currents was voltage-dependently decreased by DPI (50% decrease at -50.7 ± 9 mV, 3 patches). Short and long openings of DPI-modified channels could be separated by variation of the holding potential. The occurrence of long Na channel openings was much more suppressed by reducing the holding potential (half maximum inactivation at -112 ± 8 mV, 4 patches) than that of short openings (half maximum inactivation at -88 ± 8 mV, 4 patches). Otherwise, short living openings completely disappeared at potentials positive to -40 mV where the occurrence of long openings was favoured. The differential voltage dependence of blocking and activating effects of DPI on cardiac Na channels as well as the differential voltage dependence of the appearance of short and long openings refers to a modal gating behaviour of cardiac Na channels.

Key words: Patch clamp — Single cardiac cells — Sodium channels — DPI 201-106 — Modes of channel gating

Introduction

Pharmacological tools are widely used in approaching of functional properties of ionic channels. Many references can be found in reviews (e.g. Catterall 1980). These tools include some new spectacular compounds which prolong the open state of different channels (e. g. dihydropyridine BayK 8644 for Ca channels, Hess et al. 1984, 1986). A novel positive inotropic agent, the diphenylmethyl-
-piperazine-indol derivative DPI 201-106 (Sandoz Ltd.) seems to be an extraordinary tool to modulate cardiac sodium channels. DPI has already been shown to drastically prolong the mean open time of cardiac sodium channels by removal of inactivation (Kohlhardt et al. 1986). However, the Na channel modulating properties of DPI seem to be much more complex than hitherto supposed. In the present paper we shall show that DPI has both blocking and stimulating effects on cardiac sodium channels. The combination on these effects might be desirable for characterizing a new class of Na channel modulating compounds with both cardiotonic and antiarrhythmic properties.

Materials and Methods

Experiments were performed on guinea pigs weighing 200—300 g. After cervical dislocation the hearts were rapidly removed and dissociated into single cells by the methods described in detail elsewhere (Nilius et al. 1986, 1987). Only ventricular cells were used for the patch clamp analysis.

The dissociated cells were stored in Eagle's minimum essential medium. After settling the cells in a glassbottomed 0.1 ml chamber they were superfused with following bath solution in mmol l/1: 140 K-aspartate, 10 EGTA, 1 $MgCl_2$, 5 Hepes, pH adjusted to 7.4 with KOH. Patch clamp recordings were performed in this medium in which the cells are nearly zeroed to a resting potential of 0mV (Hess et al 1986). The patch clamp pipette contained in mmol/1; 140 NaCl, 2.5 $CaCl_2$, 0.5 $MgCl_2$, 11 glucose, 5 Hepes, titrated with NaOH to pH 7.4. Experiments were carried out at room temperature.

The patch clamp device and pipette fabrication were standard (Hamill et al. 1981). An 8 bit A/D conversion was used. Probes were sampled at 10 kHz (2 kHz low-pass filtered), 2.5 kHz (filter 1 kHz) or 1 kHz (filter 0.5 kHz). Mean currents were determined by averaging 76 sweeps. Each sweep consisted of 320 points. Leakage and capacitive transients were corrected by subtracting the averaged sweeps without openings (nulls) from each current trace. The transitions between open and closed states of the Na channels were determined by a half-unitary current detection scheme. Open and close time distributions were fitted using the Marquardt-Levenberg algorithm. Lumped data are presented as mean $\pm$ SEM.

In all the experiments freshly dissolved S-enantiomer of DPI 201-106 was used (piperacinyl-indole (4(3-(4-diphenyl-methyl-1-piperazinyl)-2-hydroxypropoxyl)-1H-indole-2-carbonitrile), Scholtysik et al. 1985, 1986). S-DPI was kindly provided by Professor G. Scholtysik, Sandoz Ltd., Basel, Switzerland. The compound was added to the bath solution in a concentration of 5 μmol/1. The strong binding of DPI to glass surfaces accounts for substantial errors in the estimation of the real concentration. It was therefore resigned of any evaluation of concentration-response relations. All experiments were performed in the cell attached mode of patch clamping.

Results

DPI effects on macroscopic sodium currents

The normal gating behaviour of Na channels in heart muscle cells is characterized by a clustering of openings at the very beginning of a depolarizing step that results in a fast transient averaged current. Figure 1 shows averaged Na currents obtained from 12 sweeps at each voltage in a multichannel patch. Inward currents could be detected at membrane potentials positive to about -80 mV. A very fast inactivation was shown at positive step potentials. The duration of the inward inflection was only a few milliseconds. Outward currents through the cell attached membrane patches could be observed (Fig. 1A). After the administration of 5 μmol/1 DPI the gating behaviour of Na channels became changed. Averaged currents could already be measured at -80 mV, e. g. the activation was shifted towards hyperpolarizing potentials. The transient currents at step potentials between -80 and -50 mV decayed within less than 40 ms. At stronger depolarizations an extremely slowly inactivated current component could be observed that was overlapped by a transient phase at potentials between -70 and -50 mV (Figure 1B, -70 mV). At potentials positive to -60 mV the inactivating current was nearly completely abolished. The current at the end of a 250 ms voltage step decreased due to the increasing depolarizing steps. Also outward currents at potentials positive to $+30$ mV were observed. This type of extremely slowly inactivated currents has been already described as being Na currents through DPI modified Na channels (Fröbe et al. 1986; Kohlhardt et al. 1986).

Kinetic properties of DPI-modified single Na channels

Single channel Na currents could be observed in nearly each cell attached membrane patch. Figure 2 shows ten consecutive sweeps with openings clustering at the very beginning of the voltage step. Long lasting bursts of openings could be observed only rarely (trace 8, Fig. 2). The averaged currents decayed very fast even at a test potential of -50 mV. The administration of DPI to the same cell attached patch changed the gating of the Na channel completely within only one minute. Some depolarizing steps were answered by short openings similar to the normal gating of non-modified channels. The number of empty sweeps (nulls) was increased resulting in a decrease of the averaged current in comparison to the control current before DPI administration. The number of sweeps with long openings was also increased resulting in an extremely slow decaying averaged current. This means that in addition to the prolonga-
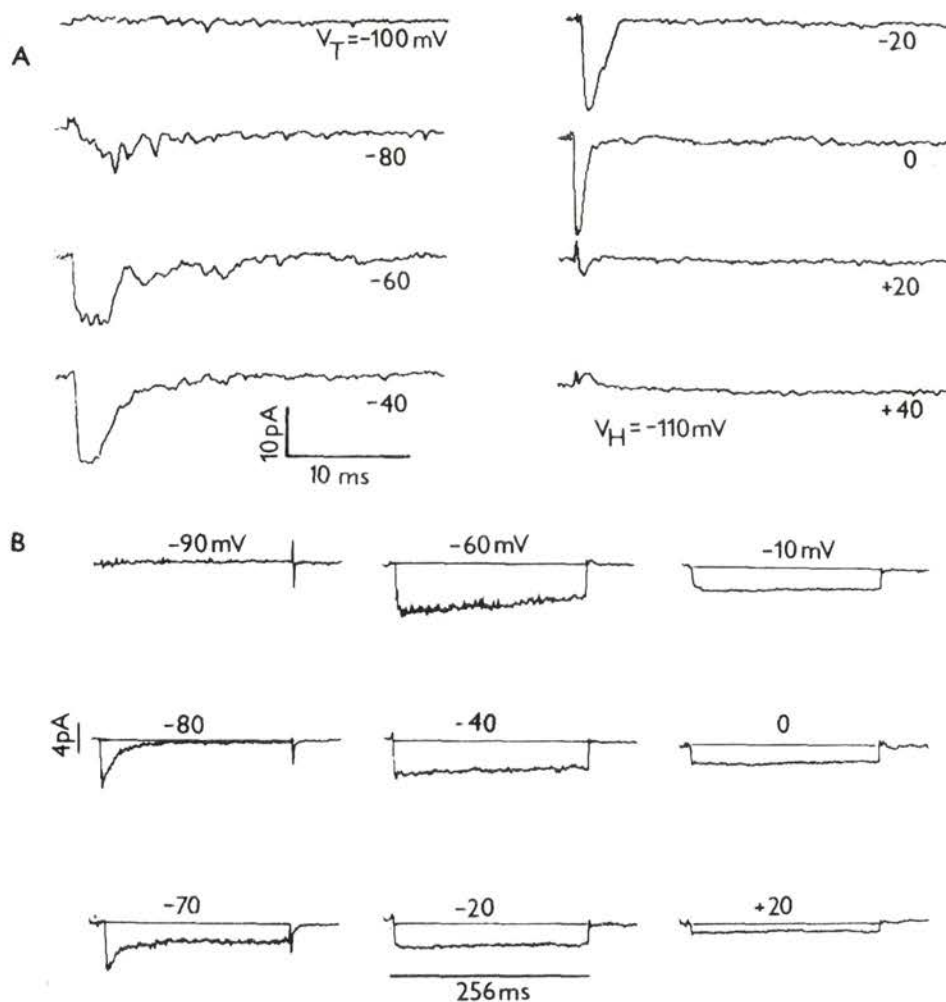
Fig. 1. *A*: Time course of the averaged sodium currents obtained from multichannel patches. The current traces were reconstructed from 15 consecutive sweeps each. Note the very fast inactivation at large depolarizing pulses (cell 220186-2, sampling rate 10 kHz, 2 kHz filter). *B*: Time course of macroscopic currents through DPI-modified channels. Averaged currents were obtained from 30 sweeps each. The holding potential was set to -120 mV, pacing frequency 0.7 Hz. Note the transient time course at negative potentials and the nearly complete lack of inactivation at potentials positive to -40 mV. A reversal could be observed at about $+30$ mV (not shown, cell 250986-5, sampling rate 1 kHz, 0.5 kHz filter).

tion of channel openings DPI also blocked Na channels by possibly stabilizing the channel in a nonavailable state (Fig. 2, right).

Figure 3 shows the voltage dependence of single channel openings. At

negative potentials short openings could be resolved which appeared in bursts. At stronger depolarizations the duration of single channel openings was dramatically prolonged.
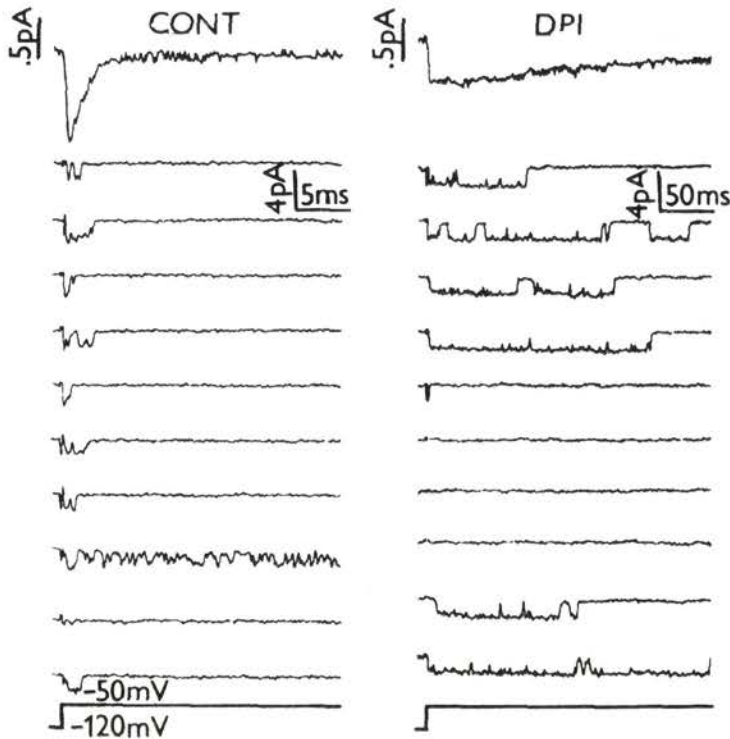
Fig. 2. Effects of DPI on the opening behaviour of cardiac sodium channels. Single channel recordings are from 10 consecutive sweeps. DPI was applied from the outside to the same patch. *Left*: control before DPI administration. Na channels open in clusters at the very beginning of the depolarizing pulse. Sweep 8 from the top shows a long lasting burst followed by a null. The averaged current from 76 sweeps is fastly inactivated (sampling rate 10 kHz, 2 kHz filter). *Right*: channel openings after treatment with DPI. The peak averaged mean current is clearly decreased due to an increased number of nulls. The single channel openings are long (note the different time scales). The single channel current is unchanged in size (sampling rate 1 kHz, 1 kHz filter, cell 031086-2).

The long openings provide an intriguing tool to use voltage ramps for recording of single channel current-voltage relationships. This method proved very reliable for the evaluation of the single channel conductance. Figure 4 shows an example: voltage ramps from -130 to $+50 \, \text{mV}$ were applied to cell-attached membrane patches. At negative potentials again a clear indication for short living channel openings could be observed. Outward currents through

single Na channels were measured constantly. The averaged current showed a clear reversal (24.7 ± 5.1 mV, $n = 3$). From three patches using the same protocol of voltage ramps a single channel conductance of 21.3 ± 1 pS could be measured which nicely matched the values obtained from measurements of single channel currents through non-modified channels (20.8 ± 4 pS, 15 measurements in three patches). These conductances are close to values obtained by other authors (e. g. 25 pS by Worley et al. 1986, 20 pS by Kunze et al. 1985). However, the single channel conductance is very sensitive to external Ca and Mg concentrations, the pipette solution used has to be thus taken carefully into account (Nilius 1987).
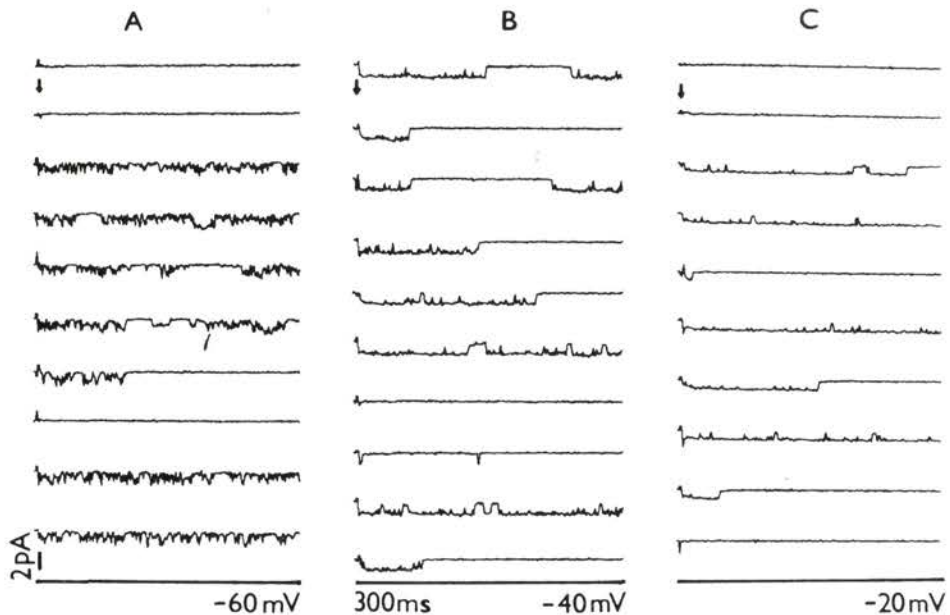

Fig. 3. Voltage dependence of single channel openings of DPI treated Na channels. At −60 mV the channels open in bursts. The single openings at −40, −20 mV are dramatically prolonged. At −20 mV within one opening closings can be rarely observed (sampling rate 1 kHz, 0.5 kHz filter, cell 281086-4).

The obvious voltage dependent prolongation of the duration of the open state of DPI-treated Na channels is summarized in Figure 5. Here, the distribution of the open times was fitted by a single exponential. The fits are very reliable at negative potentials but are difficult to obtain at stronger depolarizations. Therefore, at −20 mV test potential the mean open time was described by

$$\tau_0 = \sum_{i=1}^{N} n_i T_i / N \tag{1}$$
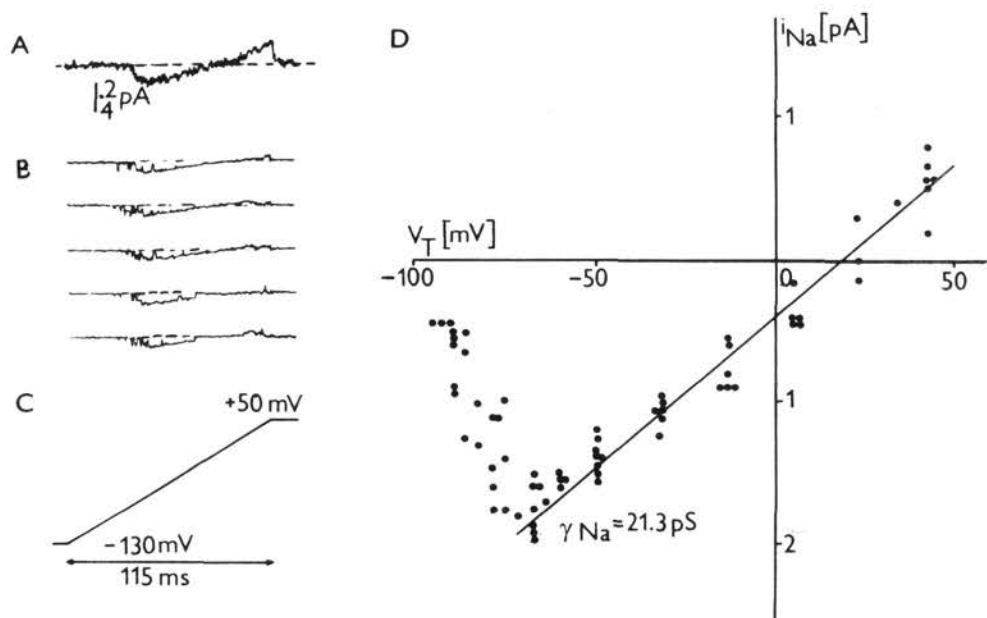
Fig. 4. Evaluation of the single channel conductance of DPI-treated Na channels by the use of voltage ramps. *A*: averaged mean current from 76 voltage ramps. Note the linear part of the averaged current with a clear reversal. *B*: single channel currents evoked by voltage ramps from -130 to $+50\,mV$. The single channel currents reversed. Long lasting openings are reflected by linearly decaying single channel currents (cell 160986-11). *C*: 115 ms ramps applied with a rate of 0.5 Hz. *D*: reconstructed single channel current voltage relationship from 115 ms ramps. Points represent individual current measurements obtained from 5 ramps each at the respective voltage. The straight line was fitted from these mean values unveiling a single channel conductance of 21.3 pS. Note the strong rectification at negative potentials, probably due to Ca block (Nilius et al. 1987b).

where T_i is the duration of n_i open channels, and N means the number of openings. Only traces without overlapping events were analysed. Figure 5*D* unveils an increase in the mean open time of single Na channels up to more than 50 ms. A monotonous voltage dependence of the mean open time can be detected.

As already shown in Fig. 2 DPI decreased the peak of the averaged current in comparison to non-treated control patches in spite of the increase in the duration of the single channel openings. This block was due to an increased number of nulls. Figure 6 summarizes the strong voltage dependence of the appearance of nulls in DPI-treated cells. In contrast to the behaviour of normal Na channels where the number of empty sweeps was nearly constant between -60 and $-20\,mV$, the number of nulls was increased due to stronger depolarizations with DPI. Boltzman functions were used to describe the voltage

dependence of the appearance of nulls, e. g. non-empty sweeps. From curve fitting a half maximal decrease of the relative number of non-empty sweeps was calculated (-50.7 mV $\pm$ 8.9 mV, 3 patches). In this respect DPI induced a voltage dependent block of single Na channels.
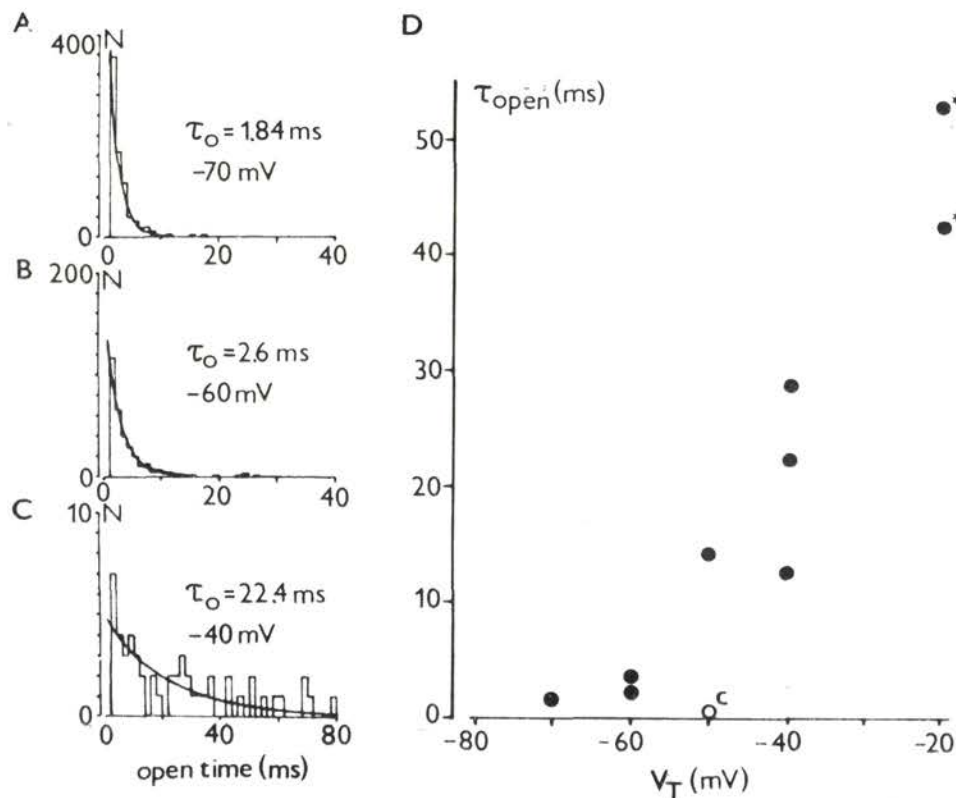

Fig. 5. Voltage dependence of the mean open time of DPI-treated Na channels. Open time distributions from the same patch at three different potentials are shown at the left (A, B, C). The mean open time was evaluated by a monoexponential fit of the histogramms and increased from 1.84 ms at -70 mV to 22.4 ms at -40 mV (cell 281086-12). Short openings with a mean open time around 0.4 ms were not resolved or are mainly included in the first but not considered bin (single point events were excluded from the analysis). D: voltage dependence of the mean open times from three patches. Asterisks mark open times calculated by equation (1). The open point marked c is from a control patch defore DPI administration ($\tau_0 = 0.32$ ms). The holding potential was -120 mV in all cases.

Differential voltage dependence of Na channel modulation by DPI

From a simple inspection of sequential sweeps showing single channel openings in DPI-treated membrane patches similarities to the modal gating behaviour of

Fig. 6. The blocking effect of DPI. The number $(1 - \frac{nulls}{sweeps})$ of non-empty sweeps was decreased. The relative number of non-empty sweeps was fitted from 3 patches by $P = 1/(1 + \exp(V_T - V_h)/s)$ (equat.(2)) with $V_h = -50.7$, $s = 16.7$ mV. At membrane potentials positive to $+10$ mV Na channels were completely blocked in 5 of 7 patches.

$V_h = -50.7$ mV

$s = 16.7$ mV

V_T [mV]
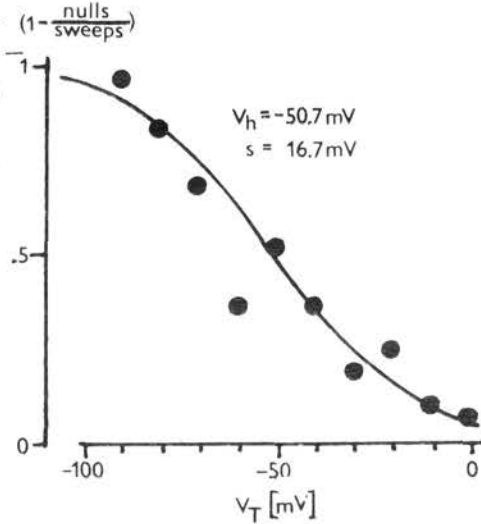
1,4-dihydropyridine modified Ca channels (Hess et al. 1984) cannot be overlooked. Figure 7*A* shows 10 consecutive sweeps with i) long openings that appear in groups of bursts, ii) single short living openings that cluster at the very beginning of a depolarizing voltage step, and iii) nulls. The sweeps showing these different types of openings appeared in groups. At membrane potentials between -80 and -50 mV this typical behaviour could be observed resulting in an averaged current with a clearly biphasic decay: a fast decay was followed by an extremely slow inactivating current. At depolarizations beyond -50 mV short living openings were exceptional. Nulls and long openings characterized the gating behaviour at these potentials.

The same gating behaviour can be shown by the use of voltage ramps. Linearly rising voltage ramps from -110 to $+90$ mV within 115 ms evoked typical single channel openings: the majority of openings appeared at negative membrane potentials, being short living (S). This gating behaviour is reflected in a clear peak of the averaged current at negative potentials (Figure 7*B*, peak S). With a lower probability of occurrence long lasting openings (L) could be observed at more positive potentials resulting in a linear decay of the averaged current with a clear reversal.

Figure 8 and 9 show the voltage dependences of both types of gating. From an at least 3 channel patch averaged currents and also single channels currents were measured at different test potentials (Fig. 8). At -90 mV only a transient current could be evoked from a holding potential of -120 mV. Between test
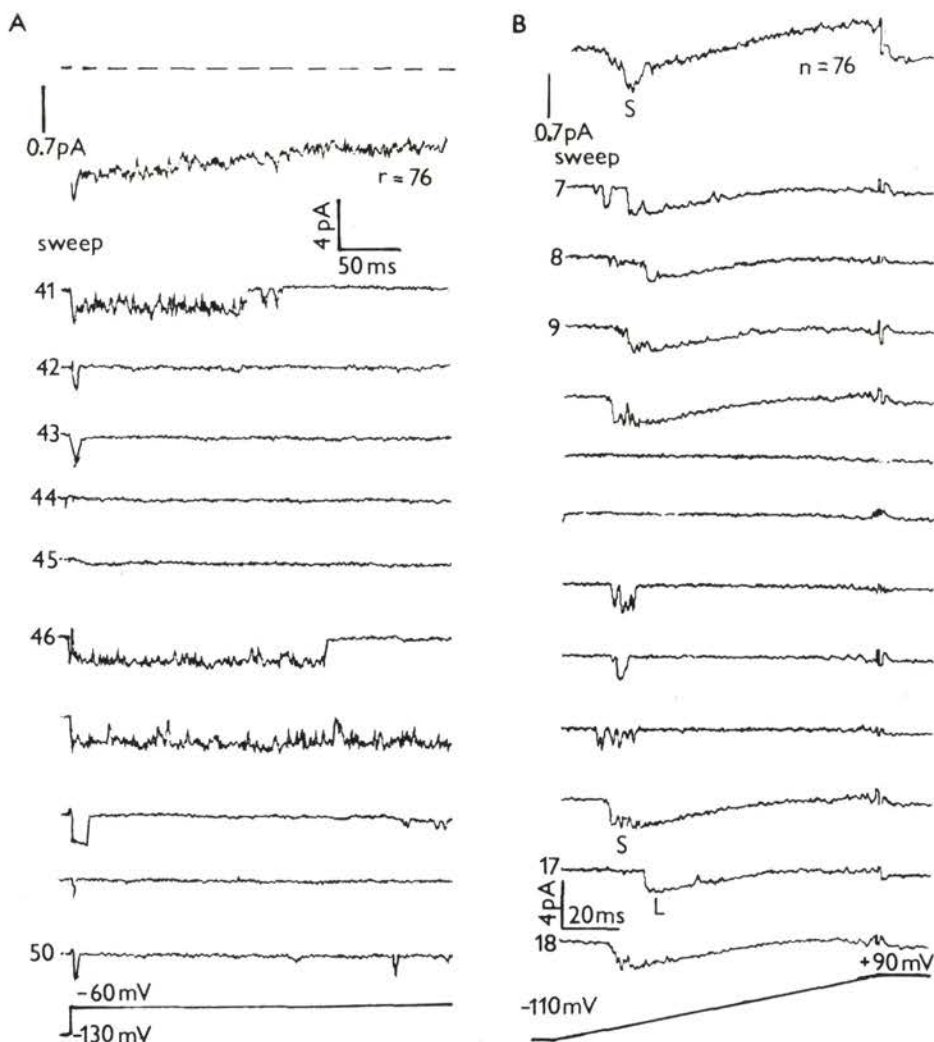
Fig. 7. *A*: Single channel openings of DPI-modified Na channels exhibit different types of gating behaviour. Ten consecutive sweeps show long lasting openings which are grouped as are the short openings and the nulls. The averaged mean current is characterized by a biphasic decay (sampling rate 1 kHz, 0.5 kHz filter, cell 221086-31). *B*: Gating behaviour of DPI treated sodium channels elicited by 115 ms voltage ramps from −110 to +90 mV. At negative membrane potentials the channels are short living. Short openings are followed by long openings in sweeps 7 to 10 followed by 2 nulls. Sweep 13, 14, 15 only show short openings at negative membrane potentials followed again by grouped long openings. The averaged current from 76 sweeps clearly shows that short living openings appear with a higher probability than long openings as indicated by a pronounced hump at negative potentials (cell 221086-32, 2.5 kHz sampling rate, 1 kHz filter).

potentials of −80 and −50 mV there was again an overlap of a transient and a long-sustained current component. At potentials positive to −40 mV short openings could rarely be seen and the transient current disappeared. The measurement of single channel currents unveiled the same voltage dependence and the same size of short and long lasting events (Fig. 8B). The current-voltage relationship of the averaged current summarizes the differential dependence of short (clustering at the beginning of the pulse and reflecting the clear cut peak of the averaged current) and long openings (reflecting the well maintained averaged current, Figure 8C). Short openings and transient currents appeared in a voltage range between 10 and 20 mV more negative than long openings.
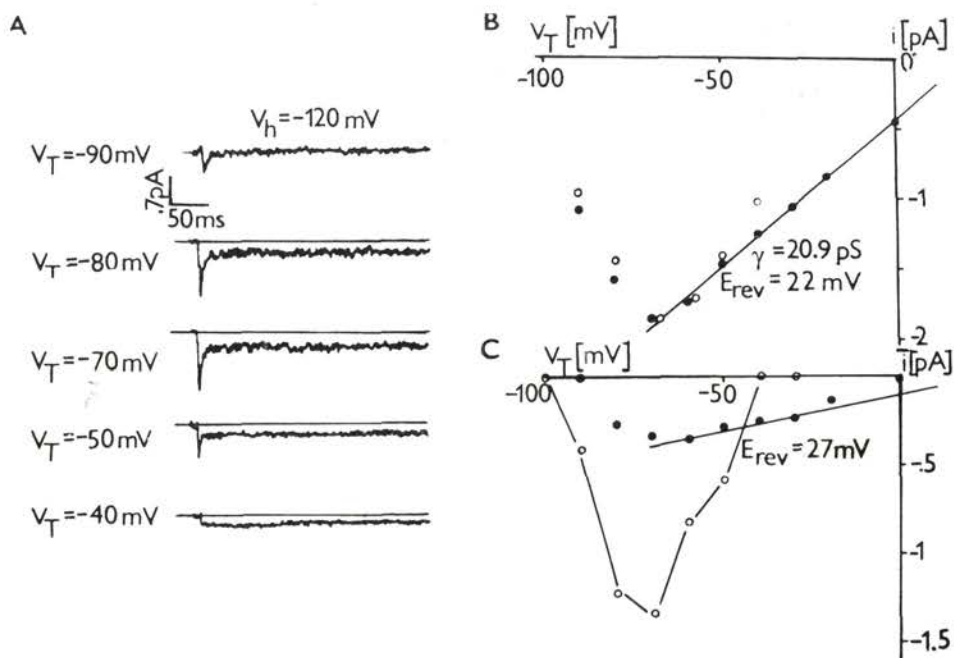

Fig. 8. Analysis of the voltage dependence of the two types of gating. *A*: averaged currents from 76 sweeps. The peaks result from short living openings which cluster at the very beginning of the steps. The maintained current is evoked by long openings. V_h: holding potential −120 mV, the test potential V_T is indicated at each trace. Note the complete disappearance of a transient current at −40 mV (cell 060187-3, sampling rate 1 kHz, filter 0.5 kHz). *B*: single channel current-voltage relationship. The single channel currents of the short (O) and long (●) openings have the same magnitude and voltage dependence. The single channel conductance was calculated from the positive slope (except O at −40 mV) being 20.9 pS. The extrapolated reversal potential is 22 mV (same patch as in *A*). *C*: averaged current-voltage relationship (same patch as in *A*, at least three channels are in the patch). The peak currents (O) and the currents at the end of the 300 ms step (●) were plotted against the test potential. The straight line was calculated from 4 points. Both current components are blocked at stronger depolarizations. The extrapolated reversal potential (calculated from the shown points (except −20 mV and 0 mV) was 27 mV.

The voltage dependence of the probability of the channel being open was calculated from

$$P = \frac{\bar{i}}{i \cdot N} \tag{3}$$

with $\bar{i}$ being either the peak of the averaged current to estimate P for short openings, or the current at the end of the depolarizing pulse to quantify P for long openings, i is the unitary current, N the number of channels estimated from the maximal number of simultaneously open channels. Figure 9 shows again from another patch a leftward shift of P for short openings in comparison to the long openings. At stronger depolarizations both the probability of the ap-
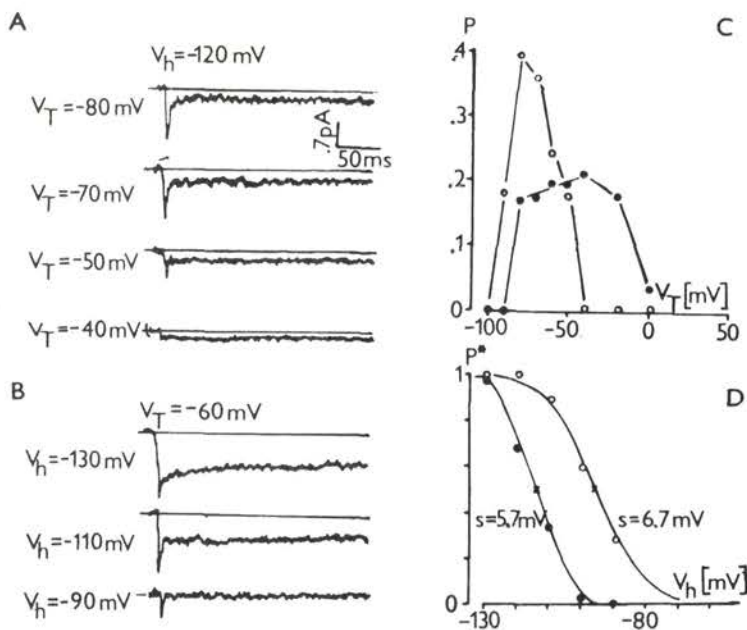
Fig. 9. Voltage dependence of activation and inactivation of DPI-modified Na channels. A: averaged currents from 76 sweeps to calculate the probability of a channel being open according to equation (3) (V_h: holding potential, V_T: test potential, patch 060187-4, 1 kHz sampling rate, 0.5 kHz filtering). B: averaged currents from 76 sweeps. The holding potentials was changed. The test step was always to -60 mV (patch 070187-2, 1 kHz sampling rate, 0.5 kHz filtering). C: voltage dependence of the probability of a channel being open calculated from the measured single channel currents. Three channels in a patch were proposed. $\bigcirc$: P calculated from the peak currents for the short openings. $\bullet$: calculated from the currents at the end of the step for the long openings. Note the different potential range of activation and block. D: normalized probability of a channel to open (measured from the peaks ($\bigcirc$) and the maintained current ($\bullet$)) was plotted against the holding potential. The plots were fitted by equation (4). Half maximal inactivation for the short openings was -93 mV, slope 6.7 mV, that for the long opening -113 mV, slope 5.7 mV.

pearance of short and long living channel states is decreased. This decrease reflects a voltage dependent block of DPI. The appearance of short and long openings have also been proved to be sensitively dependent on the holding potential. The dependence of the normalized P values (P^*) for short and long openings on the holding potential was described by

$$P^* = 1/(1 + \exp{(V - V_k)/k}) \tag{4}$$

(V_k is the potential of half maximal decrease in P^*, k is the slope parameter in mV). From four patches V_k was measured and values of -87.7 ± 7.5 mV for the short openings and -112.3 ± 7.9 mV for the long openings (see also Figure 9D) were found. No significant changes in the slope k of inactivation of both types of openings were observed (8.8 ± 3.0 mV for long, 9.5 ± 4.8 mV for short openings).
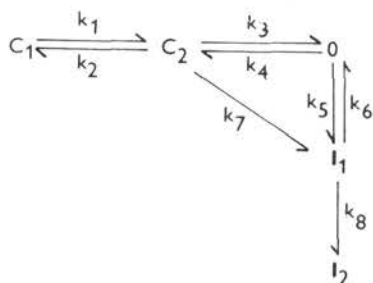
Discussion

The new inotropic compound DPI 201-106 has positive ionotropic activity, negative chronotropic effects, it induces a prolongation of action potentials combined with an antiarrhythmic action and exerts coronary dilation (Scholtysik et al. 1985, 1986; Buggisch et al. 1985). It also depresses the maximal upstroke velocity of cardiac action potentials similar by as do several antiarrhythmic drugs with local anaesthetic effects, such as lidocaine (Scholtysik et al. 1985; Fröbe et al. 1986).

The most striking effect of DPI, however, is the prolongation of single sodium channel openings as already described by Kohlhardt et al. (1986). In this respect the action of DPI resembles the effects of certain small molecules as N-bromoacetamide (Patlak and Horn 1982) or chloramine-T (Wang 1984) in modifying the inactivation of sodium channels. Unlike BTX, DPI does not reduce the single channel conductance of Na channels (for BTX, see Quandt and Narahashi 1982). The single channel conductance of DPI modified Na channels evaluated by the very reliable ramp method in this study was around 22 pS. This value agrees very well with that of non-DPI treated Na channels in the control experiments and — taking into account different concentrations of Ca and Mg in the patch pipettes — also in other studies (Cachelin et al. 1983; Kohlhardt et al. 1986; Nilius 1987). Besides the already described prolongation of the mean open time in DPI-treated Na channels, the new findings in this study are: i) a steep monotonous voltage dependence of the mean open time in DPI-modified Na channels which contrasts with control channels (Benndorf and Nilius, unpublished results), ii) the appearance of two types of channel openings (short and long living open states) with the same voltage dependence and size of the unitary currents, iii) voltage dependent blockage of DPI on both

types of channel openings but in a different voltage range, iv) a differential dependence of the two types of gating on the holding potential (inactivation), v) striking similarities to the modal gating behaviour of Ca channels as extensively described by Hess et al. (1984) and Fox et al. (1986). Clear indications to favour this idea of a modal gating behaviour of at least DPI-modified Na channels are i) long openings, short openings, and nulls always appear in groups indicating that the channel spends a longer time in a state in which a special, kinetically defined, gating behaviour is favoured, ii) the open time distributions for openings of steps from -120 to $-60\,$mV with both populations of openings together show that the mean open time of the short openings can be monoexponentially fitted with a time constant similar to the normal Na channel openings ($0.43 \pm 0.15\,$ms in control patches, Nilius et al. 1987a, $0.57 \pm 0.32\,$ms in this study from DPI-treated patches, $n = 3$). A similar concept that Na channels like Ca channels act in different modes of gating has already been proposed (Patlak and Ortiz 1985, 1986).

To explain qualitatively the observed properties of DPI-treated cardiac Na channels a kinetic scheme modified after Horn and Vandenberg (1984) was calculated

$$C_1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C_2 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} O$$

with transitions k_5, k_6, k_7 to I_1 and k_8 to I_2

where C_1, C_2 are closed states, O is the only conducting state, I_1, I_2 are inactivated states of the channel. I_1 has to be transferred into another real absorbing state with a rate coefficient k_8 which is small as compared to the other considered rate coefficients because no openings can be seen at holding potentials positive to $-70\,$mV. A recovery from the inactivated (absorbing) state I_2 has not been considered. The prolonged mean open time of the Na channels cannot be explained by an only decreased backward rate k_4 from the open into the closed state because tail currents after clamping back to holding potentials between -80 and $-140\,$mV were never observed. It means that in contrast to the effect of dihydropyridine BayK 8644 on Ca channels (Hess et al. 1984, 1986; Sanguinetti et al. 1986) the channel closing should be complete within about one sampling period. In accordance to Kohlhardt et al. (1986) the time course of the averaged currents at strong depolarizations can be described by only a decrease in the transition rate from the open to the inactivated state (k_5). However, for explanation of the increased number of nulls an increased rate coefficient k_7 for the

transition from the closed to the inactivated state by-passing the open state should be supposed. The transient component of current due to short but multiple openings which cluster at the very begining of the depolarizing pulse and which are shown to be separable from long openings by shifting the holding potential towards less negative values (Figures 8, 9) cannot be explained by this tentative kinetic scheme. It is argued that Na channels act in at least two modes. One mode is characterized by short and multiple openings at the very beginning of the depolarizing pulse between -80 and $-50\,\mathrm{mV}$. This gating behaviour can be described by a nearly unchanged rate coefficient k_5 but a moderately increased coefficient k_6 inducing reopenings. The steady state inactivation of this mode is as voltage dependent as for the DPI-untreated channels. Half maximal inactivation is observed at about $-85\,\mathrm{mV}$ similar to normal Na channels (Cachelin et al. 1983).

A second mode of channel gating exhibits long openings. The gating can be modelled by a decrease of both rate coefficients k_4 and k_5. Tis gating mode can be inactivated at more negative potentials than the mode showing short openings (half maximal inactivation around $-115\,\mathrm{mV}$). The DPI induced block can be described by an increased rate coefficient k_7 favouring the entrance into the absorbing state without sojourns in the open state. This assumption can easily explain the voltage-dependent increase in the number of nulls (Nilius et al. 1987). As a further explanation of DPI-induced block a prolonged stay in the inactivated state (e. g. a decelerated recovery from I_2 back to C_1 or C_2) can be proposed.

In this respect one of the most intriguing properties in modifying single Na channels is that DPI induces a high open state probability if the channel can open at all during a depolarizing voltage step, and also favours the stay of the channel in an unavailable state (increased number of nulls).

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