On the Mechanism of Drug-Induced Blockade of Na\(^+\) Currents: Interaction of Antiarrhythmic Compounds With DPI-Modified Single Cardiac Na\(^+\) Channels

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In patch-clamped membranes from neonatal rat cardiocytes, elementary Na\(^+\) currents were recorded at 19° C for study of the inhibitory influence of several antiarrhythmic drugs including lidocaine, diprafenone, propafenone, and prajmalium on DPI-modified cardiac Na\(^+\) channels. Diprafenone (20 \(\mu\)mol/l) and lidocaine (300 \(\mu\)mol/l) induced a voltage- and time-dependent block of reconstructed macroscopic sodium current (I\(_{\text{Na}}\)). The drugs depressed the sustained, noninactivating I\(_{\text{Na}}\) component (which reflects the number and open probability of DPI-modified Na\(^+\) channels) effectively, in a voltage- and time-dependent fashion. Once opened, DPI-modified Na\(^+\) channels are highly drug-sensitive. Antiarrhythmic drugs (propafenone, diprafenone, and, to a lesser extent, lidocaine) provoke a flicker block, that is, the long-lasting openings are chopped into a large number of short and grouped openings. This indicates rapid transitions between a drug-associated, blocked state and a drug-free, conducting state. The latter has a unitary conductance of 12 pS, very similar to the control value in the absence of antiarrhythmic drugs. The decrease in open time of drug-treated DPI-modified Na\(^+\) channels is concentration-dependent. Hill coefficients for propafenone of about 1.0 and for prajmalium of about 0.7 were calculated. A blocking rate constant of 6.1\(\times\)10\(^7\) mol\(^{-1}\)sec\(^{-1}\) for propafenone, but of 1.5\(\times\)10\(^7\) mol\(^{-1}\)sec\(^{-1}\) for prajmalium was obtained at -30 mV. The unblocking rate constant for propafenone was, also at -30 mV, about twice as large as the unblocking rate constant for prajmalium. The open channel block kinetics are essentially voltage-dependent. The affinity of the channel-associated drug receptor increases on membrane depolarization. The blocking rate constant was inversely related to the number of Na\(^+\) ions moving through the open channel. It is concluded that the manifestation of this voltage- and Na\(^+\)-dependent flicker block is intimately related to removal of fast Na\(^+\) inactivation. (Circulation Research 1989;64:867-881)

Local anesthetics and related antiarrhythmic drugs compose a structurally heterogeneous group of organic compounds capable of interacting with Na\(^+\) channels in excitable membranes, including cardiac cells. Several lines of evidence strongly support the assumption that a channel-associated binding site exists\(^1\)-\(^3\) whose interaction or binding with these drugs will finally block the channel and hinder sodium ions from passing through the pore. The resultant decrease in excitability may be an antiarrhythmic principle in heart to suppress irregular impulses. The remarkably different manifestation of Na\(^+\) current depression, which attracted considerable theoretical interest and was well documented in numerous biophysical studies during the past years, reflects the combined influence of such factors as voltage, driving rate, and drug hydrophobicity in determination of the strength of blockade of Na\(^+\) currents. The rather complex block phenomenology finds elegant and conclusive explanations by the models of Hille\(^2\) and Hondeghem and Katzung\(^3\) on the one hand and Starmer et al\(^4\) on the other hand. The Hille-Hondeghem-Katzung model postulates a modulated receptor whose drug affinity depends on the channel state, rested or activated/inactivated, while the Starmer model assumes a guarded receptor, that is, drug access is controlled by the channel state. Such modeling of block proved valuable and indispensable in the understanding of some elementary properties of voltage-dependent Na\(^+\) channels.

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Recent patch-clamp experiments with single cardiac Na\textsuperscript{+} channels have stressed the theoretical prediction that antiarrhythmic drugs exert their blocking action in an all-or-none fashion.\textsuperscript{3} Blockade prevents the Na\textsuperscript{+} channel from attaining the conducting configuration while unblocked Na\textsuperscript{+} channels show apparently normal elementary properties, including unitary current size and open-state kinetics. This also implies that plugging and unplugging, the steps that initiate and terminate the channel blockade, remain undetectable even in single-channel studies. However, Krueger et al\textsuperscript{8} first demonstrated in batrachotoxin (BTX)-modified single neuronal Na\textsuperscript{+} channels that removal of fast inactivation may unmask these events. The blocking marine toxin saxitoxin (STX) was reported to periodically suppress the repetitive activity of BTX-modified Na\textsuperscript{+} channels, which is due to the arrival of an STX molecule at, and to its departure from, the Na\textsuperscript{+} channel and allows direct definition of the underlying molecular reaction, that is, association and dissociation rate constants, including the lifetime of the drug-occupied receptor.

This tool was applied in the present patch-clamped cardiac membranes for visualization of the blocking and unblocking of single cardiac Na\textsuperscript{+} channels by several class 1 antiarrhythmic drugs. Use of diphenylpiperazinylindole derivative DPI 201-106 as a kinetic modifier that removes fast inactivation in cardiac Na\textsuperscript{+} channels\textsuperscript{7} in this study showed that propafenone, its derivative diprafenone, prajmalium, or lidocaine may interact with open, noninactivating Na\textsuperscript{+} channels. The resultant voltage- and Na\textsuperscript{+}-dependent flicker block shares many properties with the flicker block evoked by a great variety of blocking molecules in ionic channels such as Cl\textsuperscript{-} or K\textsuperscript{+} channels that are intrinsically devoid of fast inactivation.

**Materials and Methods**

Single Na\textsuperscript{+} channel currents were recorded in cell-attached and inside-out patches from briefly (14-20 hours) cultured neonatal rat cardiocytes through the use of the standard patch-clamp technique\textsuperscript{8} and fire-polished borosilicate patch pipettes (resistance, 5-10 M\textOmega; after being filled with pipette solution). Details of the cell culture have been described elsewhere.\textsuperscript{7} The culture dishes were rinsed with modified Tyrode’s solution (solution A) to remove a small fraction of cardiocytes not yet attached at the bottom. In the cell-attached experiments, the cells, after an initial equilibration in solution A for 5 minutes, were exposed to an isotonic K\textsuperscript{+} solution (solution B) kept under these conditions during the whole experiment. The isotonic K\textsuperscript{+} solution depolarized the cells to about 0 mV, which is advantageous for two reasons: 1) Spontaneous activity eventually occurring in physiological saline is prevented, and 2) the stable membrane potential allows definition of the step membrane potential in absolute terms. In the cell-

free patch-clamp experiments, the cardiocytes were kept in solution A until a cell-attached patch had formed (seal resistances, 75-200 G\textOmega;). Before dissection of a patch, the culture dish was rinsed with isotonic Cs\textsuperscript{+} solution so that the cytoplasmic side of the membrane faced a quasi-intracellular medium.

**Data Acquisition**

Na\textsuperscript{+} channel activity was triggered by step depolarizations of the membrane from a holding potential (−110 to −130 mV under control conditions) to a suprathreshold potential delivered from a conventional stimulator at a rate of 0.2–0.5 Hz. Step depolarizations lasted 120 msec in the cell-attached experiments and 220 msec in the cell-free patches. The patch-clamp recordings were filtered at 1 kHz by an eight-pole Bessel filter, digitized by a microcomputer with a sampling rate of 5 kHz, and stored on floppy disks. A residual component of capacity transients not compensated at the level of the headstage of the patch-clamp amplifier (L/M-EPC 5, List Electronic, Darmstadt, FRG) was eliminated by averaging records without detectable channel activity (blank sweeps) and subtracting the average from the records with activity (activity sweeps). Furthermore, the records were corrected for leakage currents.

**Data Analysis**

Open time of single Na\textsuperscript{+} channels was analyzed by setting a threshold for a transition (i.e., opening or closing) at 50% of the unitary current amplitude.\textsuperscript{9} Mean open time (t\textsubscript{opcn}) was calculated from t\textsubscript{opcn} = \Sigma n\textsubscript{i}T\textsubscript{i}/n,\textsuperscript{10} where T\textsubscript{i} is the duration of n\textsubscript{i} open channels and n is a count of events. Histogram analysis was based on an unweighted fit, that is, each bin was considered to be of identical significance. To fit late, and therefore rare, events correctly, a commonly accepted procedure was used: Several bins were lumped to give a larger bin containing a certain minimum of events, arbitrarily chosen in the present analysis to be four. From the frequency distribution of the open time, t\textsubscript{closed} was calculated from the best fit of the open-time histograms by use of the least-squares (x\textsuperscript{2}) method. The closed time between events resulted also from the 50% analysis and is expressed analogously as mean closed time (t\textsubscript{closed}) or as t\textsubscript{closed}. Only activity sweeps without superpositions or overlapping events were taken for kinetic analysis. Their contribution in an ensemble was kept small (5% or less) by adjustment to an appropriate holding potential. In the presence of blocking antiarrhythmic drugs, the occurrence of superpositions indicating the simultaneous activity of more than one Na\textsuperscript{+} channel became extremely rare.

Burst analysis was essentially based on the closed-time distribution in an ensemble. Basically, the closed-time distribution of DPI-modified Na\textsuperscript{+} channels is the sum of three exponentials. At −30 mV and in the presence of propafenone (20 \textmu;mol/l), for example, the least-square method yielded values for
DPI-induced Modification of Cardiac Na⁺ Channels

Modified, burst-like Na⁺ channel activity was induced by exposure of the external surface of the membrane to racemic DPI (3 μmol/L). Figure 1 demonstrates the characteristic response and shows that an ensemble of records may consist of two types of activity sweeps differing by the open probability \( P_o \) (Figure 1B). Activity sweeps with a \( P_o<0.1 \) consistently showed a severalfold prolonged open time as compared with activity sweeps having a \( P_o>0.1 \). Therefore, \( P_o>0.1 \) activity sweeps were considered to indicate modified channel activity. Usually, the open-time histogram in DPI-treated cardiac Na⁺ channels is the sum of two exponentials because of the coexistence of normal, DPI-free and modified, DPI-associated Na⁺ channels in an individual patch. If the open-time histogram was constructed exclusively from events in activity sweeps with a \( P_o>0.1 \), the best fit consistently yielded a single exponential event distribution. This suggests that such a biased analysis eliminates most of the normal events. In the following discussion, DPI-treated patches not exposed to antiarrhythmic drugs will be referred to as "control."

The full DPI effect is considered to be attained when runs with modified Na⁺ channel activity can be triggered periodically. Similar to normal, DPI-free conditions, a run-down phenomenon appeared in about 30% of the patches, that is, the initially high Na⁺ channel activity did not persist. On repetitive stepping of the membrane to \(-40 \) mV at 0.5 Hz (the standard conditions in the cell-attached experiments), the number of samples without detectable openings (blank sweeps) gradually increased at the expense of the number of samples with openings (activity sweeps) during the first 5 minutes after patch formation. If an increase in holding potential by 20 mV proved ineffective in abolishing the run-down, the patch was discarded.

Experiments with antiarrhythmic agents were only performed if the number of blank sweeps, determined as incidence per minute, remained stable during 5 minutes. However, a significant rundown, which might develop during a later stage of the experiment, cannot be excluded, although in three cell-attached DPI-treated patches a constant channel activity during the whole-patch lifetime (45, 52, and 61 minutes) was observed.

Block of DPI-Modified Cardiac Na⁺ Channels by Antiarrhythmic Agents

A first series of experiments was designed for analysis of the response of DPI-modified Na⁺ channels to several class 1 antiarrhythmic drugs in the cell-attached configuration. Basically, the experimental protocol included an initial equilibration period of 10 minutes necessary for the development of the full DPI effect and, furthermore, for the detection of an eventual development of a run-down in channel activity. A superfusion of the cardiocytes with an isotonic K⁺ solution containing an antiarrhythmic drug effectively blocked DPI-modified Na⁺ channels within a few seconds. This indicated that these drugs reach the Na⁺ channel after passing the cell membrane, that is, only the neutral fraction of molecules acts in the cell-attached patch-clamp experiments on

Solution A (modified Tyrode's solution) contained NaCl 137, KCl 5.4, CaCl₂ 0.1, MgCl₂ 5, Na-pyruvate 2.5, glucose 20, and HEPES 10 (pH 7.4). Solution B contained CsCl 130, MgCl₂ 5, Na-pyruvate 2.5, glucose 20, and HEPES 10 (pH 7.4). Solution C contained KCl 140, CaCl₂ 0.1, MgCl₂ 5, Na-pyruvate 2.5, glucose 20, and HEPES 10 (pH 7.4). Solution D (pipette solution) contained NaCl 137 or 200, CaCl₂ 0.2, MgCl₂ 5, and HEPES 10 (pH 7.4). Freshly dissolved DPI was added to give a final concentration of 3 μmol/L. Bath temperature was controlled by a Peltier element device and adjusted to 19±1°C.
FIGURE 1. Low and high open probability ($P_o$) during individual activity sweeps in a DPl-treated inside-out patch. Panel A: Successive records with (activity sweeps) and without (blank sweeps) elementary Na$^+$ currents of a 1,000-sample ensemble. Stochastic analysis$^{12}$ revealed a likelihood of over 99% that sequential, long-lasting openings as appearing in the fourth and fifth sample are due to repetitive activity of one individual modified Na$^+$ channel. Panel B: Histogram of $P_o$ for activity sweeps of ensemble. Steady state $P_o$ was calculated for each activity sweep from $P_o=I/i$, where $I$ is the current averaged over the whole depolarization (in this case 160 msec) and $i$ is the unitary current. Inset shows dependence of mean open time, $\tau_{\text{open}}$, in an individual activity sweep on $P_o$ of an individual activity sweep. Horizontal bars are mean values for $\tau_{\text{open}}$ related to $P_o$ of 0.15–0.40, 0.41–0.65, and 0.66–1.0. Shaded areas are mean±SEM. Panel C: Open-time histograms of the whole ensemble (upper part; best fit [$\chi^2=0.921$] yields a biexponential distribution) and after exclusion of activity sweep with $P_o<0.1$ (lower part; best fit [$\chi^2=1.120$] gives a monoexponential distribution). Note that $\tau_{\text{open}}(11.3\text{ msec})$ of unbiased analysis agrees fairly well with $\tau_{\text{open}}(10.3\text{ msec})$ of biased analysis. (Patch 13110, 137 mmol/l external Na$^+$, -30 mV.)

bath application. Figure 2 illustrates such an experiment with diprafenone (20 μmol/l). The cell-attached patch had been kept under control conditions for 20 minutes to collect a representative ensemble of samples. High open-probability ($P_o>0.1$) samples showed repetitive channel activity and were periodically triggered on depolarization of the membrane from -130 to -40 mV; low open-probability samples ($P_o<0.1$) sometimes showed superpositions, indicating the presence of two functioning Na$^+$ channels in this patch. Diprafenone led to an increase in the number of blank sweeps at the expense of the number of activity sweeps and caused a rise in the average run length of blank sweeps from 1.8 to 5.8 blanks. Mooreover, superpositions could no longer be detected in the low-open-probability samples. The reduced likelihood that membrane depolarization activates a Na$^+$ channel also became evident from the depression of the macroscopic Na$^+$ current reconstructed from ensemble averaging (see lower part in Figure 2). Under control conditions, there was a pronounced sustained and time-independent sodium current ($I_{Na}$) component. The size of the latter was determined by the number and the open probability of DPl-modified Na$^+$ channels. Peak $I_{Na}$ declined from 0.96 pA to 0.10 pA, and sustained $I_{Na}$ declined from 0.48 pA to 0.05 pA. The evaluation of the latter was complicated by a gradual decline of the sustained $I_{Na}$, which became detectable regularly in the presence of blocking antiarrhythmic drugs. All quantitative data refer arbitrarily to a current level attained 120 msec after the command impulse. As a mean of three experiments with 20 μmol/l diprafenone, peak $I_{Na}$ decreased to 12±2% and sustained $I_{Na}$ to 10±2% of the control value. For the reason just mentioned, this apparently identical quantitative response did not indicate that peak $I_{Na}$ and sustained $I_{Na}$ possess an identical drug sensitivity.
FIGURE 2. Influence of diprafenone (20 \mu mol/l) on elementary Na$^+$ currents in a DPI-treated cell-attached patch. Successive records are from a 401-sample ensemble collected before diprafenone application and a 450-sample ensemble collected after diprafenone application. Macroscopic Na$^+$ currents reconstructed from ensemble averaging under control conditions and in presence of diprafenone are shown in traces below time scale. (Patch 322CA, 200 mmol/l external Na$^+$, -40 mV.)

As expected from the voltage dependence of drug-induced I$_{Na}$ blockade, hyperpolarization of the membrane was proven to attenuate the depressing diprafenone action. In the same experiment depicted in Figure 2, after an increase in the holding potential from -130 to -150 mV, the ensemble average gave a peak I$_{Na}$ of 0.40 pA and a sustained I$_{Na}$ of 0.20 pA. In other words, the block of both I$_{Na}$ components decreased from 90% at -130 mV to 58% at -150 mV. Two experiments with propafenone (20 \mu mol/l) confirmed this result and yielded a similar voltage sensitivity of peak and sustained I$_{Na}$.

In another cell-attached experiment (step potential -40 mV at 0.5 Hz) with lidocaine (300 \mu mol/l), the apparently identical responsiveness of both I$_{Na}$ components was confirmed in demonstration of the use dependence of the blocking drug action. As found in an ensemble average from 399 control samples and from 420 samples taken under lidocaine, peak I$_{Na}$ was depressed by 27% and sustained I$_{Na}$ by 30%. The ensemble average from samples collected at a step frequency of 1 Hz revealed a depression of 73% and 80%, respectively.

The open state of DPI-modified Na$^+$ channels was found to be sensitive to antiarrhythmic drugs. As demonstrated in Figure 2 with diprafenone, the open time in samples with modified activity appeared significantly shorter than under control conditions. Again, this effect occurred immediately on drug application. Open-time histograms confirmed this difference (Figure 3). $\tau_{\text{open}(2)}$, which for the reasons outlined above can be considered to reflect the lifetime of modified Na$^+$ channels, declined drastically in this experiment from 6.2 msec to 1.4 msec. In a total of three experiments with 20 \mu mol/l diprafenone, $\tau_{\text{open}(2)}$ declined from 6.5±0.4 msec to 1.5±0.3 msec. A similar result was obtained in cell-attached patches with 20 \mu mol/l propafenone, likewise analyzed at -40 mV. Here, $\tau_{\text{open}(2)}$ decreased from 7.1±0.3 msec to 1.8±0.2 msec ($n=2$). However, in striking contrast to diprafenone, $\tau_{\text{open}(2)}$ remained unaffected with propafenone. At -40 mV, $\tau_{\text{open}(2)}$ was 1.1±0.1 msec before and 1.2±0.1 msec after propafenone treatment ($n=2$). In addition, lidocaine affected $\tau_{\text{open}(2)}$ selectively but exerted a comparatively weak effect quantitatively. The open-time analysis of lidocaine patches was also based on an ensemble of 400 control samples and of 400 samples collected after drug administration at -40 mV. Lidocaine (300 \mu mol/l) reduced $\tau_{\text{open}(2)}$ only slightly from 5.3±0.2 msec to 4.5±0.1 msec ($n=3$). The drug-induced shortening of the open state of modified Na$^+$ channels was paralleled by a large increase in the number of transitions from the conducting state to a nonconducting state. Long-lasting openings, as they appeared under control conditions, were chopped into a great number of short-lasting openings. This meant that antiarrhythmic drugs cause modified Na$^+$ channels to flicker between both these configurations.

Evidence was obtained that DPI-modified Na$^+$ channels, once opened, will inevitably be flicker blocked by antiarrhythmic drugs. Ensemble analysis of a propafenone (20 \mu mol/l) and of a diprafenone (20 \mu mol/l) experiment showed that mean open time in each individual sample with modified channel activity was smaller than under control conditions in the absence of blocking drugs. This, together with an increased shut time, reduced $P_0$ in the sample and changed the frequency distribution of $P_0$ in samples. With diprafenone (20 \mu mol/l), for example, the $P_0$ histogram revealed an open probability of only 0.21, compared with a value of 0.51 under control conditions (Figure 4). As tested between -130 and -170 mV, the flicker block was insensitive to variations in holding potentials. This was found in two patches, one treated with diprafenone (20 \mu mol/l) and the other with...
propafenone (20 μmol/l). In the diprafenone experiment, \( \tau_{\text{open(2)}} \) was 1.6 ms at -130 mV and 1.7 ms at -170 mV. The same lack of response was obtained with propafenone: \( \tau_{\text{open(2)}} \) was 1.5 ms at -150 mV and 1.6 ms at -170 mV.

**Kinetic Flicker Block Analysis**

A second series of experiments exposed the cytoplasmic side of inside-out patches to antiarrhythmic drugs and concentrated on the flicker block properties in DPI-modified Na\(^+\) channels. Under these conditions, both neutral and charged drug molecules may reach the Na\(^+\) channel. The flicker block can be modeled, in the simplest scheme, by

\[
\text{closed } \xrightarrow{\beta} \text{ open } \xrightarrow{a[D]} \text{ blocked}^{11,14}
\]

Accordingly, on leaving their open state, modified Na\(^+\) channels may attain two different nonconducting configurations: 1) a closed state or, 2) after interaction with the antiarrhythmic drug, a blocked state. The rate constants \( \alpha, \beta, a, \) and \( d \) govern the transition from one state to another; \( a \) and \( d \) correspond to the blocking and unblocking rate constants of an individual drug, and \( [D] \) means the drug concentration. Consequently, the reciprocal of the mean open time, \( 1/\tau_{\text{open}} \), is the sum of \( (\beta + a[D]) \). The term \( a[D] \) can be obtained from \( (1/\tau_{\text{open}} - 1/\tau_{\text{open}}(\text{drug-free})) \) and the unblocking rate constant \( d \) from \( 1/\tau_{\text{blocked}} \). The reciprocal of open time of modified Na\(^+\) channels may be obtained either from the open time in \( P_o > 0.1 \) samples (see Figure 5) or in bursts. Burst analysis is advantageous since in the presence of a blocking drug, the shut time within the burst repre-

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**FIGURE 3.** Open-time histograms of DPI-treated Na\(^+\) channels under control conditions (left panel) and in presence of diprafenone (20 μmol/l). Best fit (control, \( \chi^2 = 2.81 \); diprafenone, \( \chi^2 = 3.44 \)) gives a biexponential distribution. In fitting curves, the first bin was disregarded. (Patch 322CA, 200 mmol/l external Na\(^+\), -40 mV.)
FIGURE 5. Influence of propafenone (20 μmol/l) on modified Na+ channel activity. Panel A: Successive recordings from a control inside-out patch (13610) stepped to −30 mV at 137 mmol/l external Na+. Panel B: Successive recordings from an inside-out patch (22010) analyzed under the same conditions but exposed with cytoplasmic side to propafenone. Note different time scales in panels A and B. Biased open-time histogram analysis (panel B, lower right graph) excluding activity sweeps with P_{o}<0.1 yields a monoexponential distribution (best fit, χ²=2.1810). \( \tau_{\text{open}} \) (1.6 msec) is close to \( \tau_{\text{open}} \) (1.8 msec) of unbiased open-time analysis (best fit, χ²=1.1927) (panel B, upper right graph).

sent the blocked state. The reciprocal of the shut time, \( 1/\tau_{\text{shut}} \), yields the unblocking rate constant \( d \). Furthermore, a burst clearly indicates that an individual channel opens repetitively. This is important since, particularly at higher drug concentrations (see Figure 5), the gaps between bursts occasionally become too long for an unambiguous interpretation, that is, they can reflect either a nonconducting state of an individual channel or the gap between two bursting Na+ channels.

The validity of this block-reaction scheme proposed for DPI-modified Na+ channels was tested in noncumulative concentration-response experiments with propafenone and prajmalium. The drug-induced flicker block is concentration dependent, a result that is predicted by the reaction scheme as \( 1/\tau_{\text{open}}=(B+a[D]) \). The data from the experiments with propafenone (10, 20, and 30 μmol/l) and prajmalium (30, 45, and 60 μmol/l), which are compiled in Figures 6 and 7, show that \( \tau_{\text{open}} \) decreases with increasing drug concentration. Similarly, as found in a lumped analysis from three propafenone experiments, burst duration decreases on increasing drug concentration: \( \tau_{\text{burst duration}} \) was 28.4 msec at 10 μmol/l, 10.3 msec at 20 μmol/l, and 7.8 msec at 30 μmol/l. Transformation of the concentration-\( \tau_{\text{open}} \) relations into the Hill form revealed straights with correlation coefficients greater than 0.990. For propafenone, a slope factor of −1.2 or, after correction of the data for missed events, of −0.99 was calculated. A different value was obtained in the prajmalium experiments, in which the Hill coefficient amounted to −0.7. This difference is of interest as the apparent 1:1 stoichiometry with which propafenone interacts with modified Na+ channels seems to be not generally representative for antiarrhythmic drugs.

The interaction of prajmalium with a channel-associated binding site, for example, could be a negatively cooperative process.

The propafenone and prajmalium concentration-response experiments also showed that \( 1/\tau_{\text{open}} \), the reciprocal of open time of DPI-modified Na+ channels, varies linearly with drug concentration and perfectly fits a straight (\( r^2 \geq 0.99 \) in both sets of experiments) (Figures 6B and 7B). The ordinate intercept of this relation as it results from linear extrapolation to zero drug concentration yields the value for \( 1/\tau_{\text{open}} \) under drug-free conditions. As can
FIGURE 6. Dependence of mean open time ($t_{\text{open}}$) of DPI-modified Na$^+$ channels on propafenone concentration at cytoplasmic membrane surface. Panel A: Concentration dependence of $t_{\text{open}}$. Inset shows percentage of decline. Curve was drawn by eye. Panel B: Concentration dependence of reciprocal of mean open time ($1/t_{\text{open}}$) (same data as presented in panel A). Data points (not corrected for missed events) fit a straight line ($y = 65 + 18.6x$) with a correlation coefficient ($r^2$) of 0.99. Data points are mean values of three control patches, six patches at 10 $\mu$mol/l, seven patches at 20 $\mu$mol/l, and 11 patches at 30 $\mu$mol/l. Horizontal bars are SEM. (137 mmol/l Na$^+$, -30 mV.)

be seen in Figures 6B and 7B, the extrapolated value corresponds to the value for $1/t_{\text{open}}$ obtained experimentally in three control patches at the same membrane potential, -30 mV. A statistical analysis revealed a nonsignificant difference. The slope factor of the $1/t_{\text{open}} = [D]$ relation is of importance as it represents the parameter $a$ of the block reaction scheme, that is, the blocking rate constant. It should be emphasized, however, that uncertainties in the effective drug concentration may complicate the interpretation of the experimentally obtained values since the blocking drug molecule, neutral or charged, remains to be determined. This problem can be illustrated with propafenone (pK$_a$ 9.0), in which about 2% of the drug molecules are uncharged and 98% are in the cationic form at pH 7.4, the H$^+$ concentration facing either membrane surface in the present inside-out patch experiments. Should it be only the neutral fraction that blocks Na$^+$ channels, also in the inside-out patches, the calculation of the blocking rate constant from the plot $1/t_{\text{open}}$ versus drug concentration in the perfusate may yield a misestimated value depending on the degree of drug accumulation in the membrane phase, a factor that remains unknown at present.

The present study showed that the kinetics of the open-channel blockade by propafenone and prajmalium are not identical. After correction for missed events, a value for $a_{\text{propafenone}}$ of $6.1 \times 10^7$ mol$^{-1}$ sec$^{-1}$ was obtained; the value for $a_{\text{prajmalium}}$ was $1.5 \times 10^7$ mol$^{-1}$ sec$^{-1}$. Despite methodological limitations arising from reduced bandwidth of 1 kHz, this difference seems large enough to be valid for several reasons: 1) With correction of the experimental data for missed events, the error resulting from unresolved openings and closings should be minimized. Moreover, the remaining deviation from the true value is identical in both sets of experiments because of the identity of the recording conditions. 2) The relation between $1/t_{\text{open}}$ and drug concentration fits a straight line equally well in both the propafenone and prajmalium experiments. Since it is unknown whether these two agents block the Na$^+$ channel in their charged or uncharged form, it cannot be definitely excluded that the observed differences in blocking rate constants reflect differences between effective and given drug concentration.

The unblocking rate constants, $d_{\text{propafenone}}$ and $d_{\text{prajmalium}}$, differed also to some extent. In three propafenone experiments at $-30$ mV, $\tau_{\text{burst}}$ in bursts was found to be 1.4 msec irrespective of the drug concentration (10, 20, or 30 $\mu$mol/l). This concentration independence is easy to reconcile with the block-reaction scheme. A burst analysis in two prajmalium experiments revealed a mean value for $\tau_{\text{burst}}$ of 2.4 msec. Thus, after correction for missed events, $d_{\text{propafenone}}$ was 833 sec$^{-1}$ and $d_{\text{prajmalium}}$ was 409 sec$^{-1}$.

Interestingly and in contrast to the cell-attached experiments, lidocaine produced no detectable flicker block in the inside-out patches. Even on exposure of the cytoplasmic membrane surface to a concentration as high as 300 $\mu$mol/l, open time of DPI-modified Na$^+$ channels remained unaffected. This was observed consistently in five inside-out patches, a discrepancy with the cell-attached patches that needs to be further clarified.
Control patches studied between −45 mV and −15 mV yielded the open-state characteristics of DPI-modified Na⁺ channels in drug-free conditions. An increase in the signal-to-noise ratio, which was achieved by elevating the external Na⁺ concentration to 200 mmol/l, improved the accuracy of the kinetic analysis. The concomitant increase in osmolarity exerted no detectable effect on Na⁺-channel kinetics. As previously shown15 and consistent with BTX-modified16 or N-bromoacetamide (NBA)-modified17 Na⁺ channels, the lifetime of DPI-modified Na⁺ channels is an exponential function of membrane potential (see Figure 8A, left part) as long as blocking drug molecules are absent. After open-channel blockade with propafenone (20 μmol/l), DPI-modified Na⁺ channels have lost this voltage dependence. Their open state becomes voltage independent (Figure 8A), which agrees with results in NBA-modified neuronal Na⁺ channels treated with the blocking drug 9-aminoacridine.14

Voltage Dependence of Flicker Block

It was recently shown that DPI-modified cardiac Na⁺ channels may occasionally switch between the main and a substate current level, a phenomenon that is most likely due to a loss of Na⁺ selectivity.15 Such a substate is illustrated in Figure 9. This figure compares two inside-out patches that were kept under identical conditions, that is, exposed with their cytoplasmic side to 10 μmol/l propafenone and stepped to −30 mV at 0.2 Hz. While the unitary current size was 0.7 pA (the usual level at −30 mV) in one patch (Figure 9B), it amounted to only 0.3 pA in the other (Figure 9E). Surprisingly, the fully sized elementary Na⁺ current was never reached during the whole observation period of 20 minutes. Another interesting finding was that the open time was shorter than expected from all the other propafenone experiments with the fully sized elementary Na⁺ current, $T_{\text{open}}$, amounted to only 3.4 msec instead of 4.7±0.3 msec (n=6), the value obtained with 10 μmol/l propafenone (see also Figures 9C and 9F). Although the reduced signal-to-noise ratio in the small-sized current may complicate the interpretation of this difference, the abbreviation may well be a relevant result and could indicate a more strongly pronounced flicker block. As sodium ions are known to interfere with blocking drugs and to modulate the $I_{\text{Na}}$ block,18,19 it is tempting to causally relate the more strongly pronounced flicker block to the small-sized elementary Na⁺ current.
This Na⁺ hypothesis was tested by comparison of propafenone experiments performed at different driving forces for Na⁺ across the membrane. The propafenone-induced flicker block was analyzed at −30 mV in presence of 137 mmol/l external Na⁺ and, in a separate set of experiments, in presence of 200 mmol/l external Na⁺. Amplitude histograms revealed an I_{max} of 0.75 ± 0.05 pA (n = 3) at 137 mmol/l Na⁺ and of 1.19 ± 0.1 pA (n = 3) at 200 mmol/l Na⁺. In fact, d_{propafenone} was Na⁺ dependent and decreased from 18.5 ± 1.8 x 10⁶ mol⁻¹ sec⁻¹ at 137 mmol/l Na⁺ to 12.3 ± 2.1 x 10⁶ mol⁻¹ sec⁻¹ at 200 mmol/l Na⁺; that is, a larger I_{open} was combined with a smaller blocking rate constant, the result expected from the substate experiment just presented. In contrast, d_{propafenone} was 833 sec⁻¹ (corrected for missed events) irrespective of the external Na⁺ concentration.

Time-Dependent Block of Open DPI-Modified Na⁺ Channels

It is a characteristic feature of DPI-modified cardiac Na⁺ channels that, during depolarization, their open probability remains time independent for several hundred milliseconds, that is, a channel once opened cycles between the open and a closed state monotonically until repolarization is initiated (Figure 10A). This may change in the presence of antiarrhythmic drugs. Some relaxation of the sustained I_{Na} already becomes apparent in the diprafenone experiment demonstrated in Figure 2 and was studied in greater detail in macroscopic Na⁺ currents reconstructed from 220-msec-lasting step depolarizations. Figure 10 shows that the sustained I_{Na} becomes clearly time dependent in the presence of antiarrhythmic drugs. During the 220-msec lasting pulse, a considerable fraction (40% in the demonstrated ensemble average) of the initial sustained I_{Na} may undergo relaxation. This drug-induced process obeys first-order kinetics and operates slowly with time constants in the range of several hundred milliseconds (see Figure 10B).

Accompanied changes were a gradual decline in burst duration (Figure 10C) from 12.6 msec during the first 55 msec to 8.4 msec during the latest 55 msec of a 220-msec-lasting step depolarization and an increase in the gaps between bursts. A count of events revealed a smaller number of openings during the late depolarization as compared with the early phase. I_{open} did not decline during membrane depolarization, indicating that no time-dependent changes of the flicker block occurred; this result was eminently important for the relevance of the kinetic-block data presented above. Thus, prolonged exposure of open channels to antiarrhythmic drugs does not influence the blocking and unblocking rates. The drug-induced relaxation of sustained I_{Na} suggests that DPI-modified Na⁺ channels may be trapped in still another blocked state that develops with time and is more stable than the flicker block.

Do Unblocked DPI-Modified Na⁺ Channels Conduct Normally?

Unblocking should be due to the departure of an antiarrhythmic drug from the binding site and is expected, according to the all-or-none theory of block, to reestablish the normal channel properties. This already has been proven in normal cardiac Na⁺ channels with intact inactivation and was further stressed by the present experiments with modified channels. In the presence of 20 μmol/l propafenone, their unitary conductance was found to be 12 pS in two patches. This is close to the control value (12.5 pS) obtained in two patches in the absence of antiarrhythmic drugs.

Discussion

The major findings of the present experiments with single DPI-modified cardiac Na⁺ channels may be briefly summarized as follows: 1) Similar to the fast-inactivating Na⁺ current, the nondecaying Na⁺ current can be blocked by antiarrhythmic drugs. Whether or not both current components have the same drug sensitivity remains to be elucidated. The drug-induced block of the nondecaying Na⁺ current retains a characteristic feature in that its strength is voltage and time dependent. This could suggest that DPI-modified Na⁺ channels are trapped, similar to normal (i.e., inactivating) Na⁺ channels, in a non-conducting configuration. 2) Open DPI-modified Na⁺ channels are also sensitive to antiarrhythmic drugs and respond to their presence with a voltage- and Na⁺-dependent flicker block. Therefore, it might be concluded that antiarrhythmic drugs evoke two types of block: 1) a "classical" block preventing modified Na⁺ channels from opening during depolarization, and 2) the flicker block.

Is There a "Classical" Block in DPI-Modified Na⁺ Channels?

Modified Na⁺ channels attracted interest for elucidation of the role of inactivation for the blocking action of local anesthetics. Removal of inactivation can be achieved by interventions such as pronase, NBA, or chloramine T that destroy this gating process irreversibly. Another group of modifiers including BTX or the diphenylpiperazinylindole derivative DPI find a receptor and, furthermore, share the common feature to bind noncovalently, that is, they exert a reversible modifying effect, in contrast to the protein reagents.

Although pronase treatment was reported in squid axons to abolish both use-dependent and voltage-dependent I_{Na} block induced by the lidocaine derivatives QX 222 and QX 314, an intact inactivation process was not essential for the blocking-drug action. QX 314 and the neutral benzocaine effectively block chloramin T-modified neuronal Na⁺ channels. Wang et al. assumed that this discrepancy in the response is related to an additional effect of pronase on the channel protein, which
could finally abolish the drug affinity during the open state.

BTX-modified Na⁺ channels, on the other hand, may partially lose their drug sensitivity. As shown by Khodorov and his group (for review, see Reference 23), the blocking efficacy of several tertiary and quaternary amines is only 10% of their potency in normal Na⁺ channels with intact inactivation. The neutral benzocaine, however, retains its usual inhibitory potency in BTX-modified Na⁺ channels. Evidence was presented that several local anesthetics may compete with BTX or the modifying plant alkaloid aconitine for a common or an allosterically coupled binding site in the Na⁺ channel.

The depression of the noninactivating I⁰Na after treatment with propafenone or lidocaine observed in the present experiments, therefore, is ambiguous in nature. DPI was proven to interfere with the binding of tritium-labeled BTX in brain synaptosomes, which is reminiscent of the BTX displacement by several local anesthetics. This suggests that DPI may well find a binding site identical to or allosterically coupled with the binding site that interacts with local anesthetics. If this holds true, diprafenone of lidocaine might displace DPI instead of blocking the noninactivating Na⁺ channel. As the result of such a displacement, a reduced size of the noninactivating I⁰Na is also expected to occur.

The observed depression of the noninactivating I⁰Na could indicate, as still another possibility, that Na⁺ channels become blocked before interacting with DPI. This hypothesis implies noncompetitive
interference of DPI with local anesthetic binding. Therefore, it would be predicted that a blocked Na⁺ channel that binds to DPI remains trapped in a nonconducting state and becomes detectable only after dissociation of the blocking drug.

**Drug-Induced Flicker Block in DPI-Modified Na⁺ Channels**

Frequent flickering between the conductive and the blocked state is a characteristic response of ionic channels to the presence of a great variety of blocking molecules. First demonstrated in single acetylcholine-receptor channels with QX 222,26 flicker block also occurs, for example, in K⁺ channels from skeletal muscle sarcoplasmic reticulum in response to several trimethylammonium derivatives27 or in cardiac Ca²⁺ channels when inhibitory cations including Cd²⁺ and Mg²⁺ compete with Ca²⁺ for the channel passage.28 Voltage-dependent Na⁺ channels in excitable membranes respond similarly to the presence of bivalent cations including Ca²⁺ in the extracellular space. However, Ca²⁺-induced flicker block is too fast to be resolved even at a recording filter frequency of 2 kHz and is presumably due to a temporary occlusion of the channel by Ca²⁺,29 possibly by interference of Ca²⁺ with Na⁺.

Antiarrhythmic drugs do not cause a flicker block in Na⁺ channels analogous to QX 222 in acetylcholine-receptor channels that are devoid of a fast inactivation process unless fast Na⁺ inactivation is eliminated.

Most likely, intact Na⁺ inactivation prevents flicker block by induction of the characteristic short-lasting conductive state consistently reported in the litera-
Kohlhardt et al. Drug-Induced Blockade of Na⁺ Currents

Figure 10. Time-dependent block of DPI-modified Na⁺ channels. Panel A: Reconstructed macroscopic Na⁺ currents from activity sweeps in a control DPI-modified inside-out patch (patch 13110, upper trace) and from activity sweeps in a propafenone (20 μM)-treated DPI-modified inside-out patch (patch 22010, lower trace), both stepped to -30 mV at 137 mM external Na⁺. Note that amplitude difference is not indicative for blocking drug action. Continuous line indicates zero current. Panel B: Semilogarithmic plot of current against time after peak sodium current (Iₙa). Open symbols (O) refer to a propafenone-treated patch (lower average current in panel A) and are fitted by eye to curve. Panel C: Burst duration during first, second, third, and fourth quarters of 220-msec-lasting step depolarization as analyzed in patch 22010. Mean burst duration (tₑ) is a function of time, which follows (tₐ) = 14 x t⁻⁰.⁰² with a correlation coefficient (r²) of 0.81.

Charge to be close to 1.5 msec (at 19°C). Consequently, normal Na⁺ channels reach the nonconductive configuration more rapidly than blocking molecules with slow association kinetics, such as antiarrhythmic drugs, can interact with them. Flicker block in DPI- or NBA-modified Na⁺ channels seems primarily related to their severalfold prolonged channel lifetime, although it cannot be definitely excluded that channel modification per se might finally alter the drug sensitivity of the open state.

In contrast to 9-aminoacridine and the blocking antiarrhythmic drugs tested in the present experiments, STX was shown to be incapable of flicker blocking open modified Na⁺ channels. This is indicative of a difference in dissociation kinetics, which may be a reflection of the proper-
ties of either the drug molecule, the binding site, or both. It is noteworthy in this context that local anesthetics and related compounds interact with a site most likely located between the selectivity filter and the inner channel mouth. On the other hand, acts at a binding site near the outer channel mouth in front of the selectivity filter. Therefore, flicker block is no general response of the noninactivating Na⁺ channel to blocking molecules.

Properties of Antiarrhythmic Drug Binding to Cardiac Na⁺ Channels

The present flicker block data may help to further characterize the molecular interaction of blocking antiarrhythmic drugs with Na⁺ channels provided that the DPI modification per se leaves the binding properties unaffected. The blocking rate constants found with propafenone and prajmalium are in the same order of magnitude as those obtained with several local anesthetics or octylguanidine in acetylcholine-activated channels or with 9-aminoacridine in NBA-modified neuronal Na⁺ channels. Interestingly, in testing the guarded receptor model, Starmer and Grant computed from experimental use-dependent Iₘ block data in cardiac muscle a value for D₈ of 1.7×10⁻¹⁰ mol⁻¹ sec⁻¹ at -93 mV and 35°C, which is in the same order of magnitude as D₈ calculated from the open-channel blockade. The rather uniform blocking-rate constant of structurally heterogeneous blocking molecules observed in single-channel studies together with a low temperature dependence of the forward and backward reaction in blocking open channels (Q₁₀ of about 1.3⁴) were taken as arguments that diffusion controls the binding reaction.

This is not inconsistent with a drug specificity of block kinetics that is suggested by the observed differences between the propafenone-induced and the prajmalium-induced open-channel blockade. Assuming that these drugs find one and the same channel-associated binding site, the resulting difference in the Kₐ values (estimated at -30 mV to be 1.4×10⁻⁵ mol/l for propafenone but 2.7×10⁻⁵ mol/l for prajmalium) might indicate the capability of this receptor to discriminate between several drugs.

The flicker block kinetics are voltage-dependent, which is consistent with results in open acetylcholine receptor channels. Consequently, the calculated Kₐ is a function of voltage and decreases with membrane depolarization. It should be emphasized that modulation of drug affinity can only be achieved by changes in the step potential but not in holding potential. This fundamentally contrasts with the well-established voltage-dependent drug action in Na⁺ channels with intact inactivation.

The modulating role of sodium ions for the local anesthetic Iₘ block was established in voltage-clamped squid axons internally perfused with the permanently charged QX 314. In antiarrhythmic drug-treated cardiac tissue, low external Na⁺ shifts the hₐ-curve further into the hyperpolarizing direction as if the drug concentration had been increased. This is consistent with the observed Na⁺ dependence of flicker block and suggests that the charges, on passing the open pore, may interfere with block development. The question, however, arises whether the decrease of D₈ seen at elevated external [Na⁺] actually indicates a reduced forward rate and, thus, a diminished receptor affinity for the drug, or whether the decreased D₈ is related to an imbalance between the effective and the given (in the superfusate) drug concentration. Precisely, sodium ions are suspected to influence the drug concentration in the receptor pool. As proposed by Cahalan and Almers and recently by Yeh and Ten Eick, Na⁺ could control drug access, that is, the larger the number of charges moving through the open channel, the smaller might become the likelihood that a blocking molecule reaches the receptor area, and vice versa. One reason for such an antagonism might be electrostatic repulsion for the case that cationic drug molecules are going to block the Na⁺ channel. Some support for the access control hypothesis may arise from the other experimental observation that D₈ is apparently Na⁺ insensitive. Such a differential response of D₈ and D₈ is not the result that would be expected from a receptor with a Na⁺-dependent ligand affinity.

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On the mechanism of drug-induced blockade of Na+ currents: interaction of antiarrhythmic compounds with DPI-modified single cardiac Na+ channels.

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