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# Differential Response of DPI-Modified Cardiac $Na^+$ Channels to Antiarrhythmic Drugs: No Flicker Blockade by Lidocaine

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**Summary.** Elementary $Na^+$ currents were recorded in cell-attached patches from short-time cultured neonatal cardiocytes in order to test the hypothesis whether the open state of DPI-modified, noninactivating cardiac $Na^+$ channels is basically sensitive to blocking drug molecules such as antiarrhythmics.

Lidocaine (300 $\mu$mol/liter) effectively reduced the open probability of cardiac $Na^+$ channels and, at a stimulation rate of 1 Hz, depressed the reconstructed macroscopic peak $I_{Na}$ to 40 ± 3.5% of the predrug value. The same drug concentration failed to influence DPI-modified $Na^+$ channels. Their open state proved almost insensitive to lidocaine. $\tau_{open}$ decreased only slightly to 85 ± 2%. Still more importantly, the number of transitions between the conducting and a nonconducting configuration did not increase. At −40 mV, lidocaine may interfere with the open state with an association rate constant of $1.3 \times 10^5$ $mol^{-1}sec^{-1}$ which is about two orders of magnitude smaller than the rate constant obtained with propafenone or prajmalium. Moreover, propafenone (10–20 $\mu$mol/liter) or prajmalium (30 $\mu$mol/liter) led to a tremendous increase in the number of transitions between the open and a nonconducting configuration. Lidocaine also failed to evoke a fast flicker blockade with reaction kinetics in the microsecond range.

It is concluded that DPI-modified cardiac $Na^+$ channels discriminate between lidocaine and other antiarrhythmic drugs. As a tentative explanation, this might be indicative for multiple binding sites for those drugs in cardiac $Na^+$ channels.

**Key Words** noninactivating cardiac $Na^+$ channels · class 1 antiarrhythmic drugs · $I_{Na}$ depression · drug-induced block

## Introduction

Voltage-gated $Na^+$ channels in the sarcolemma provide the molecular substrate of excitability in normal heart muscle. Like their neuronal relatives, they react sensitively to a number of naturally occurring toxins and a group of organic compounds classified as local anesthetics. The blockade which develops in the presence of local anesthetics represents the final manifestation of an interaction of those drugs with a channel-associated binding site. Nature and location of this drug target in the $\alpha$-subunit of the $Na^+$ channel protein are not yet known. The binding site has been modeled as modulated (Hille, 1977; Hondeghem & Katzung, 1977) or as guarded drug receptor (Starmer, Grant & Strauss, 1984), but there is still some controversy whether $Na^+$ channels possess a single site capable to interfere with local anesthetics (Mrose & Ritchie, 1978). Lidocaine may be considered as a classical representative of local anesthetics and has been used together with some structure analogues in numerous biophysical studies to evaluate the properties of $I_{Na}$ depression (for review, *see* Hille, 1984). All these drugs, including the related antiarrhythmics, follow the all-or-none principle in blocking the $Na^+$ pore (Kohlhardt & Fichtner, 1988; Grant et al., 1989), thereby preventing $Na^+$ channels from attaining the conducting configuration. A depressed excitability and a hyperpolarizing voltage shift of steady-state $Na^+$ inactivation emerge from this drug action.

Inhibitory drug molecules can produce still another type of blockade. $Na^+$ channels modified by N-bromoacetamide (Yamamoto & Yeh, 1984), batrachotoxin (Wang, 1988) or DPI 201-106 (Kohlhardt et al., 1989) were reported to have a highly drug-sensitive open state, in contrast to normal $Na^+$ channels, and respond to a variety of organic compounds, including 9-aminoacridine, cocaine and several antiarrhythmics such as propafenone and prajmalium with a microscopic or flicker blockade. Flicker blockade arises from multiple transitions between the open and a blocked state as if the drug molecule would complete with the permeant cation for the permeation pathway and represents a widespread response of ionic channels to blocking molecules. In $Na^+$ channels, however, the occurrence of drug-induced flicker blockade requires channel modification (Benz & Kohlhardt, 1991), suggesting that removal of inactivation only enables $Na^+$ channels to react to blocking drugs during the open state. Since local anesthetics and the related antiarrhyth-

mics can combine their conventional inhibitory action on $I_{Na}$ with a flicker blockade, it seems tempting to assume that flicker blockade will inevitably occur when these drugs interfere with noninactivating $Na^+$ channels.

The present patch-clamp experiments with single DPI-modified cardiac $Na^+$ channels concentrated on this problem. As found with lidocaine under conditions that effectively depress the macroscopic $I_{Na}$ significantly, the drug was proven to exert this inhibitory action without producting a concomitant flicker blockade. This peculiarity may well be specific for $Na^+$ channels of cardiac origin but can be barely explained with a single drug target whose occupancy will cause both macroscopic $I_{Na}$ depression and microscopic blockade.

## Materials and Methods

Elementary $Na^+$ currents were recorded in cell-attached patches from cultured neonatal rat cardiocytes with an L-M/EPC-5 amplifier by employing the standard patch-clamp technique (Hamill et al., 1981). Details of the cell culture and the handling of the short-time (18–24 hr) cultured myocytes have been already described in detail elsewhere (Kohlhardt, Fröbe & Herzig, 1986). To avoid spontaneous activity and, thus, unpredictable changes in membrane potential, the cultured cardiocytes were kept in an isotonic $K^+$ solution to bring their resting potential to approximately 0 mV. Since depolarized cardiocytes frequently show the morphological signs of $Ca^{2+}$ intolerance, the isotonic $K^+$ solution contained 2 mmol/liter EGTA as $Ca^{2+}$ buffer. This stabilized the cellular structure usually for 1–2 hr. Nevertheless, the cellular structure was periodically monitored to detect an eventual development of cytoplasmic granulation. In this case, the cell-attached experiment was discarded since morphological signs of cellular deterioration can be accompanied by a decrease of $NP_o$ of $Na^+$ channels ($N$ means the number of $Na^+$ channels and $P_o$ open probability). After patch formation, an equilibrium period of at least 10 min was found to be necessary to achieve stable $Na^+$ channel activity. During the subsequent control period of about 20 min, a sufficiently large ensemble of sweeps was collected, thereby carefully monitoring the activity mode of the $Na^+$ channels. Just after perfusing the cardiocyte with a drug-containing solution, a second registration period began to analyze the influence of lidocaine, propafenone, diprafenone or prajmalium.

$Na^+$ channel activity was triggered by depolarizing the membrane from a holding potential ($-110$ to $-130$ mV) to a test potential ($-40$ to $-30$ mV) for 120 msec at a rate of 0.5 or 1 Hz. The patch-clamp recordings were filtered at 1 kHz with an 8-pole Bessel filter, digitized with a sampling rate of 5 kHz and stored on floppy disks. The dead time of the recording system was 0.2 msec.

Idealized patch-clamp recordings were obtained from a subtraction of leakage and residual capacity currents. Open times of and closed times between nonoverlapping single events were analyzed by setting a threshold at 50% unitary current amplitude (Colquhoun & Sigworth, 1983). Probability density functions in individual experiments before and after drug treatment yielded $\tau_{open}$ and $\tau_{closed}$, respectively, and were based on an unweighted fit, meaning that each bin was considered to be of identical significance. Late and, therefore, rare events were fitted by lumping several bins with a certain minimum of events arbitrarily chosen to be four. The best fit of the probability density functions resulted from the least-square method.

DPI 201-106 ($3–6 \times 10^{-6}$ mol/liter) added to the pipette solution was used as $Na^+$ channel modifier. Modified $Na^+$ channel activity was analyzed with a biased $P_o$ method (Kohlhardt et al., 1989): activity sweeps with a $P_o > 0.1$ showing bursts and several-fold prolonged openings were considered to represent the noninactivating mode. Burst analysis was based on the bimodal closed-time distribution. Gaps within bursts can be discriminated from gaps between bursts by introducing a certain critical gap time. The latter depends on the ratio between $\tau_{closed(1)}$ and $\tau_{closed(2)}$ and was calculated from the geometric mean of both time constants.

Ensemble averaging of 120–180 sweeps yielded the macroscopic $I_{Na}$ whose peak was taken as an index for $NP_o$. Activity sweeps refer to samples with channel openings, and blanks refer to samples without detectable channel openings.

Whenever possible, the data are given as mean ± SEM.

### Solutions (Composition in mmol/liter)

A. Isotonic $K^+$ solution: KCl 140; $MgCl_2$5; Na-pyruvate 2.5; glucose 20; EGTA 2; HEPES 10; pH 7.4. B. Pipette solution: NaCl 200; $CaCl_2$ 0.2; $MgCl_2$ 5; HEPES 10; pH 7.4. Freshly dissolved DPI 201-106 was added to give a final concentration of 3–6 μmol/liter. Temperature (controlled by a Peltier element): 19 ± 0.5°C.

### Drugs

All drugs were freshly dissolved before use in solution A. Propafenone hydrochloride and diprafenone hydrochloride were provided by Helopharm KG, Berlin; lidocaine hydrochloride was purchased from SIGMA Chemie, Munich; prajmalium bitartrate was a gift of Kali-Chemie, Hannover.

## Results

DPI 201-106 was chosen to be applied as $Na^+$ channel modifier (Kohlhardt et al., 1986) mainly for two reasons. (i) In contrast to many protein reagents and proteolytic enzymes, DPI 201-106 is also effective to remove $Na^+$ inactivation upon external application and, much more importantly, has no additional influence on the channel availability. (ii) DPI-modified cardiac $Na^+$ channels show long-lasting burst activity and are, therefore, particular suitable to detect drug-induced blocking events and to analyze the reaction kinetics of antiarrhythmics.

Lidocaine treatment (300 μmol/liter) of the cardiocytes had the expected effect and blocked DPI-modified cardiac $Na^+$ channels within a few seconds if the cell-attached patches were repetitively depolarized with an appropriate rate. As shown in Fig. 1*A*, this blockade became manifest as an increase of the fraction of blank sweeps, at the

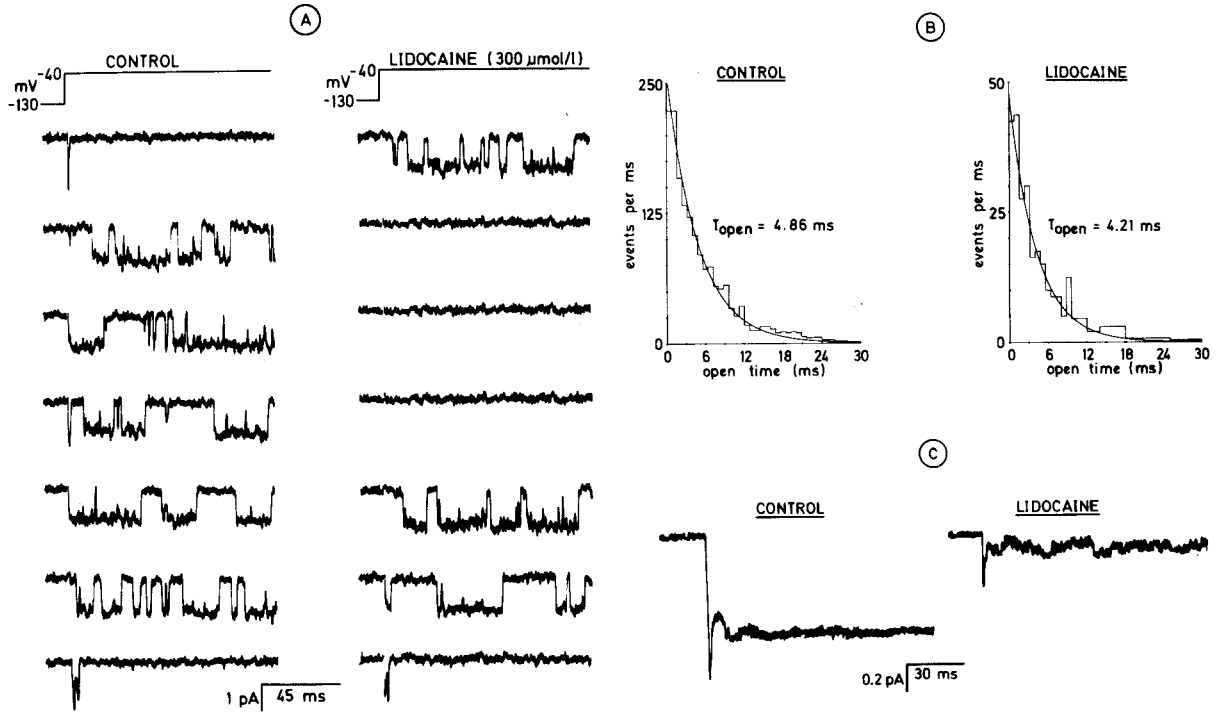


**Fig. 1.** The influence of lidocaine (300 µmol/liter) on DPI-modified cardiac $Na^+$ channels. (*A*) Recordings of elementary $Na^+$ channels before and after drug treatment. (*B*) Open-time probability density functions. The histograms were constructed from 1316 nonoverlapping events under control conditions and from 288 nonoverlapping events in the presence of lidocaine. By disregarding the first bin of 0.4 msec, they could be best fitted by $N(t) = 262\exp(-t/0.00486)$ and $N(t) = 48\exp(-t/0.00421)$, respectively. (*C*) Reconstructed macroscopic $I_{Na}$ before and after lidocaine treatment. Ensemble averages from each 128 sweeps triggered at 1 Hz. Patch 325CA; stimulation rate 1 Hz; holding potential −130 mV; test potential −40 mV.

expense of the fraction of activity sweeps. Consequently, the reconstructed macroscopic $I_{Na}$ declined (*see* Fig. 1*C*): peak $I_{Na}$ decreased in this cell-attached experiment from 1.0 p to 0.38 pA and the sustained $I_{Na}$ component (measured at the end of the 120-msec lasting membrane depolarization and reflecting $NP_o$ of noninactivating $Na^+$ channels) decreased from 0.65 to 0.08 pA at a stimulation rate of 1 Hz. It is well established from conventional $I_{Na}$ measurements in several excitable tissues, including heart muscle, that the blocking efficacy of lidocaine is strongly use dependent. To produce a significant $I_{Na}$ depression in papillary muscles, for example, stimulation frequencies of 1 Hz or higher are required (Kohlhardt & Seifert, 1985). In the present patch-clamp experiments, the $NP_o$ depression seen at 1 Hz became strongly attenuated or even disappeared when the stepping rate of the patches was reduced to 0.5 Hz. At 1 Hz, peak $I_{Na}$ (i.e., the maximum $NP_o$ attained during membrane depolarization) declined to 40 ± 3.5% ($n$ = 4) of the control value under predrug conditions.

As calculated from

$$K_D = [\text{lidocaine}] \times \frac{I_{Na(drug)}}{I_{Na(control)} - I_{Na(drug)}}$$

lidocaine would block at 1 Hz $Na^+$ channels with an apparent dissociation constant of 180 µmol/liter.

Surprisingly, the open state of DPI-modified $Na^+$ channels proved only less sensitive to the lidocaine treatment. As evaluated from $P_o > 0.1$ activity sweeps, lidocaine (300 µmol/liter) reduced $\tau_{open}$ only slightly, in the experiment illustrated in Fig. 1 from 4.86 to 4.21 msec (at −40 mV; *see* Fig. 1*B*). Three other experiments yielded a very similar result: at −40 mV, $\tau_{open}$ of DPI-modified $Na^+$ channels declined to 85.5 ± 2.1% of the control value.

Moreover, lidocaine was found to be incapable of blocking open DPI-modified $Na^+$ channels repetitively since long-lasting openings were not chopped into multiple transitions between the conducting and a nonconducting state. This was evidenced by counting the opening events in $P_o > 0.1$ sweeps during the 120-msec lasting membrane depolarization. In the cell-attached experiment shown in Fig. 1, for example, the mean number of openings even declined,

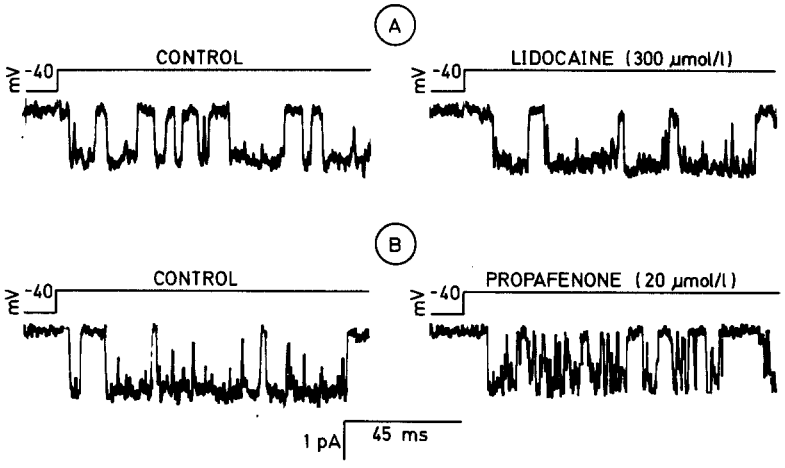


**Fig. 2.** Recordings of elementary $Na^+$ currents through DPI-modified $Na^+$ channels before and after lidocaine treatment (*A*) and before and after propafenone treatment (*B*). Holding potential: −120 mV in the experiment depicted in *A*; −130 mV in the experiment depicted in *B*. Test potential: −40 mV in both experiments. Patches 324CA (*A*) and 328CA (*B*).

from 9.2 to 6.9. Three other experiments confirmed this observation.

By comparing the response of DPI-modified $Na^+$ channels to other antiarrhythmic drugs such as propafenone or prajmalium (Fig. 2), the particular mode of the lidocaine action becomes apparent. The influence of propafenone and prajmalium was studied under the same experimental conditions, i.e., likewise in cell-attached patches at −40 mV and in concentrations (20 and 30 μmol/liter, respectively) effective to reduce $NP_o$ significantly. As expected from earlier studies with cardiac $Na^+$ channels (Kohlhardt et al., 1989), exposing the cardiocyte to a propafenone- or prajmalium-containing solution led, within a few seconds, to the occurrence of multiple transitions between the open and a nonconducting state (Fig. 2*B*). By contrast, in the presence of lidocaine (Fig. 2*A*), $Na^+$ channels can dwell some 10 msec in the open state obviously without going to be blocked. But it could still be argued that, compared with propafenone and other related drugs, lidocaine interacts with several-fold faster reaction kinetics making, for methodological limitations, channel openings and closings as the manifestations of drug unbinding and binding too short to be resolvable. Nevertheless, fast flicker blockade with kinetics in the microsecond range would be detectable under the present recording conditions as a reduction of the elementary current size. It is, therefore, important to note that lidocaine had no influence on the elementary current size (Fig. 2*A*). Propafenone and prajmalium, in interfering with DPI-modified $Na^+$ channels, increased in a concentration-dependent fashion the number of openings during membrane depolarization (*see* Fig. 3*B*). Lidocaine (300 μmol/liter) experts the opposite effect and reduced the number of openings during membrane depolarization to 75 ± 2% ($n$ = 4; *see* Fig. 3*A*).

Considering the lidocaine-induced decrease of $\tau_{open}$ as a block manifestation of open DPI-modified $Na^+$ channels, this blockade can be described, in the simplest case, by the reaction scheme

$$\text{closed} \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} \text{open} \underset{d}{\overset{a[D]}{\rightleftharpoons}} \text{blocked}.$$

The rate constants $a$ and $d$ correspond to the association and dissociation rate constants of the drug. $a$ was calculated from $(1/\tau_{open(drug)} - 1/\tau_{open(control)})$, and $d$ was calculated from the reciprocal of the mean shut time within bursts. At −40 mV, values for $a$ and $d$ of $1.3 \pm 0.12 \times 10^5$ $mol^{-1}sec^{-1}$ and 1002 ± 269 $sec^{-1}$ were obtained. Since $K_D = d/a$, the mean $K_D$ amounted to $7.7 \pm 2.7 \times 10^{-3}$ mol/liter. By contrast, $a_{propafenone}$ and $a_{prajmalium}$ are approximately two orders of magnitude larger.

DPI-modified $Na^+$ channels, like other noninactivating $Na^+$ channels, can attain at least two nonconducting states, one of them being in the submillisecond range and the other closed state being several-fold longer. Lidocaine (300 μmol/liter) increased the latter (Fig. 4*A*) to 158 ± 8.5% ($n$ = 4) of the control value. It is not yet clear whether this prolongation reflects a blocking event or whether lidocaine simply acts to stabilize DPI-modified $Na^+$ channels in that particular closed configuration. Other antiarrhythmic agents like diprafenone having a much higher affinity to open DPI-modified $Na^+$ channels can exert the opposite effect, thereby causing a decrease of $\tau_{closed(2)}$ (Fig. 4*B*).

## Discussion

The present patch-clamp experiments with neonatal rat cardiocytes demonstrate that DPI-modified, noninactivating cardiac $Na^+$ channels can tolerate the

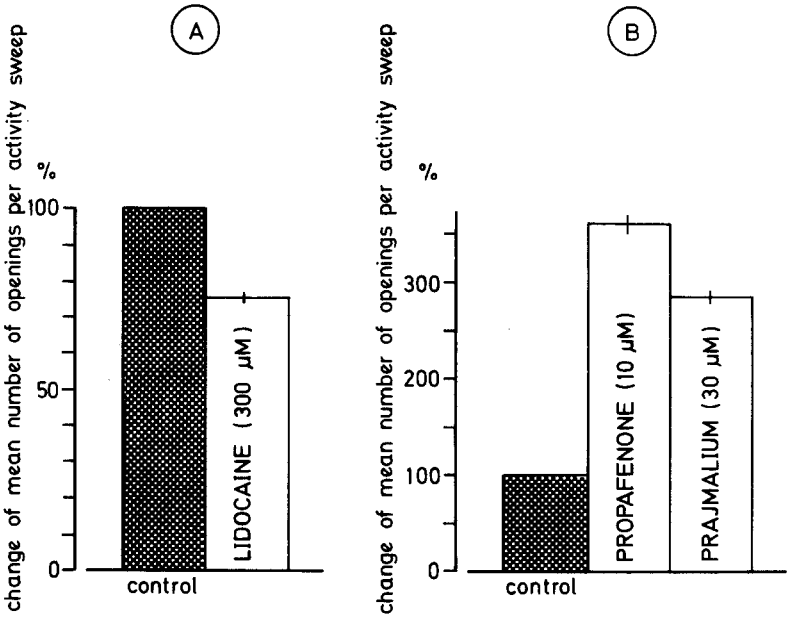


**Fig. 3.** The influence of lidocaine (*A*) and propafenone or prajmalium (*B*) on the number of openings of DPI-modified $Na^+$ channels during membrane depolarization. 100% refers to the control value under drug-free conditions. The mean number of openings was obtained from the whole number of nonoverlapping events in $P_o > 0.1$ activity sweeps divided by the number of $P_o > 0.1$ sweeps in an individual patch. Each column represents the mean of four experiments; vertical bars indicate SEM. Step potential in the lidocaine experiments was −40 mV, and in the other drug experiments step potential was −30 mV.

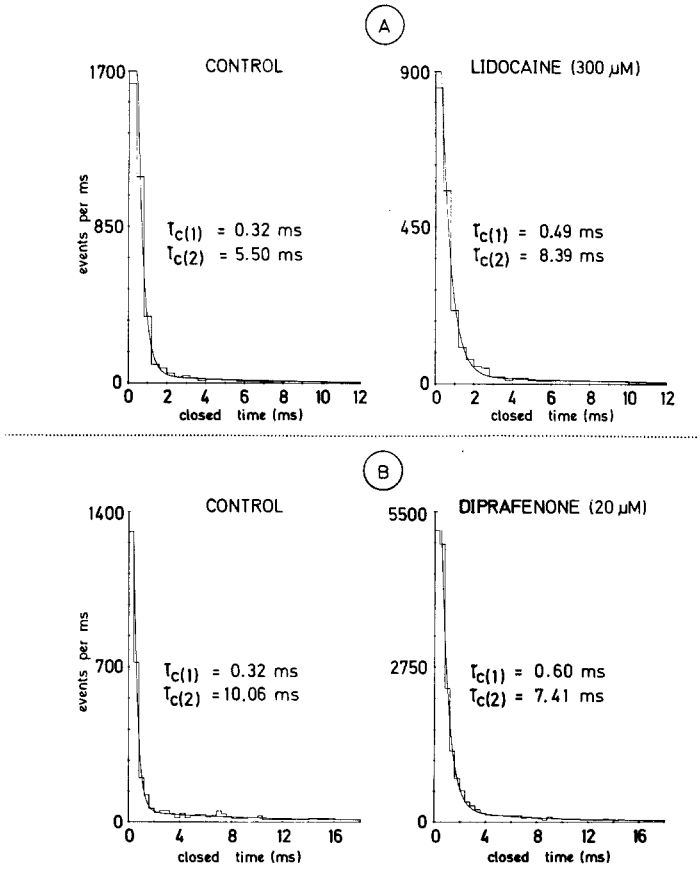


**Fig. 4.** The influence of lidocaine (*A*) and diprafenone (*B*) on the closed-state kinetics of DPI-modified $Na^+$ channels. By disregarding the first bin of 0.4 msec, the best fits were as follows: in *A*, control: $N(t) = 7101\exp(-t/0.00032) + 46\exp(-t/0.0055)$; lidocaine: $N(t) = 1714\exp(-t/0.00049) + 23\exp(-t/0.00839)$. In *B*, control: $N(t) = 4346\exp(-t/0.00032) + 49\exp(-t/0.0106)$; diprafenone: $N(t) = 12244\exp(-t/0.0006) + 223\exp(-t/0.00741)$. Patch 327CA; holding potential −120 mV; test potential −40 mV.

presence of antiarrhythmic drug molecules without responding with a flicker blockade. Lidocaine in concentrations effective to reduce $NP_o$ and to depress $I_{Na}$ significantly, failed to block DPI-modified $Na^+$ channels repetitively during their open configuration. This particular mode of action fundamentally contrasts to the effect of a number of antiarrhythmics, including propafenone, diprafenone or prajmalium which combine their depression on $NP_o$ with a flicker blockade.

Regardless of the chemical tool employed for modification, N-bromoacetamide (Yamamoto & Yeh, 1984), batrachotoxin (Wang, 1988) or DPI 201-106 (Kohlhardt et al., 1989), noninactivating $Na^+$ channels in neuronal tissues, skeletal muscle and heart share the common property of being sensitive to organic blocking molecules when dwelling in the open state. It is important to note in this context that batrachotoxin and DPI 201-106 bind, in contrast to protein reagents, noncovalently to $Na^+$ channels and eliminate $Na^+$ inactivation not irreversibly. Compounds capable of provoking a flicker blockade comprise structurally heterogeneous molecules such as 9-aminoacridine (Yamamoto & Yeh, 1984), cocaine (Wang, 1988) and antiarrhythmic drugs (Kohlhardt et al., 1989). Lidocaine, like other related drugs, are supposed to interact with a channel-associated binding site which can be reached from the surrounding lipid matrix or, after passing the latter, from the cytoplasmic side (Hille, 1977). In cell-attached experiments, this route is the only access for antiarrhythmic drugs to reach, after bath application, the $Na^+$ channels within the patched membrane. As inferred from the observed $NP_o$ depression, an effective lidocaine concentration existed in the close vicinity of the $Na^+$ channels. Moreover, the lipophilic lidocaine fraction would even reach the external face of the patched membrane simply by a retrograde permeation of the sarcolemma. This is important since Alpert et al. (1990) presented evidence in favor of a second local anesthetic binding site in cardiac $Na^+$ channels accessible for hydrophilic compounds such as QX-314 upon external application.

Unfortunately, the channel-associated binding site is not yet defined on a molecular level. Its nature and location within the $Na^+$ channel protein is purely hypothetical and remains to be characterized. Assuming that both the conventional $I_{Na}$ blockade as arising from the $NP_o$ depression and the microscopic blockade in noninactivating $Na^+$ channels emerge from a drug interaction with one and the same drug target, the present observations with lidocaine find no plausible explanation.

In considering the response of noninactivating cardiac $Na^+$ channels to lidocaine and other antiarrhythmic drugs, the question is crucial indeed as to whether the intrinsic drug binding properties remain conserved after chemical or pharmacological channel modification. As recently reported from studies in planar bilayers, batrachotoxin-activated $Na^+$ channels of various origins, including heart muscle, show a drastically altered stereoselectivity in that the (+)-enantiomer of cocaine and bupivacaine were found to be less effective than the (−)-enantiomer (Wang, 1990). This is opposite to the effectiveness in intact nerve fibers. Wang (1990) offers two possible explanations, a loss of an additional binding site in planar bilayers or an influence of batrachotoxin on binding properties. But it should be emphasized that the (+)-enantiomers of cocaine and bupivacaine did not principally loose their capability to evoke a flicker blockade. Using DPI 201-106 as modifier of cardiac $Na^+$ channels *in situ*, a microscopic block analysis with propafenone revealed reaction kinetics (Kohlhardt et al., 1989) which are close to the association rate constant (in the order of $10^7$ $mol^{-1}sec^{-1}$) computed by Starmer & Grant (1985) from experimental data in papillary muscles of guinea pig (Kohlhardt & Seifert, 1983). Moreover, external $Na^+$ variations which are well established from $I_{Na}$ measurements in voltage-clamped axons (Cahalan & Almers, 1979) and ventricular cardiac preparations (Kohlhardt, 1982) to modulate the local anesthetic action have the same crucial influence on the drug-induced flicker blockade in that a reduction in external $Na^+$ accentuates the blocking drug efficacy and vice versa (Kohlhardt et al., 1989).

This would not be in favor of the assumption that DPI 201-106, in interacting with cardiac $Na^+$ channels, and thereby removing reversibly their inactivation process, may simultaneously alter some fundamental drug binding properties. In other words, the failing ability of lidocaine to produce a flicker blockade still awaits a tentative explanation.

A more attractive hypothesis is based on the assumption that noninactivating cardiac $Na^+$ channels can discriminate among antiarrhythmic drugs. This postulates a separate drug receptor as being involved in the flicker blockade and having no lidocaine affinity. In fact, there is still another argument against a single drug receptor whose occupancy evokes $I_{Na}$ depression together with flicker blockade. As shown in DPI-modified cardiac $Na^+$ channels with propafenone (Benz & Kohlhardt, 1991), the dissociation rate constant was 700 $sec^{-1}$ (at −45 mV and 9°C). Recovery from $I_{Na}$ blockade, however, proceeds with a rate of about 0.2 $sec^{-1}$ (at −90 mV and 35°C; Kohlhardt & Seifert, 1985). Even a fast $I_{Na}$ recovery recently described in papillary muscles treated with quinidine operates with a rate of 10

$sec^{-1}$, as an upper limit (Snyders & Hondeghem, 1990).

Nevertheless, DPI-modified cardiac $Na^+$ channels are not definitely resistant to lidocaine since the drug decreased $\tau_{open}$ and the number of opening events during membrane depolarization. Clearly, this response is definitely distinct from flicker blockade and suggests that lidocaine may hinder noninactivating $Na^+$ channels from gating.

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