Shortening of the Action Potential and Reduction of Pacemaker Activity by Lidocaine, Quinidine, and Procainamide in Sheep Cardiac Purkinje Fibers

An Effect on Na or K Currents?

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SUMMARY The ionic mechanism underlying the shortening of the action potential and the reduction in pacemaker activity by lidocaine, quinidine, and procainamide in sheep cardiac Purkinje fibers was investigated using the two-microelectrode voltage clamp technique. In the presence of lidocaine (1.85 to \(3.7 \times 10^{-5}\)M), steady state currents were shifted outward over a broad range of potentials. In contrast, instantaneous currents (holding potential -40 mV) and steady state currents in 20 mM Cs-Tyrode were not affected at potentials negative to -60 mV, the outward shift being restricted to potentials positive to this level. This outward shift of the current at the plateau level by lidocaine was not observed in the presence of a maximal dose of tetrodotoxin (TTX); in Na-free medium, the effect of lidocaine was totally suppressed. On the pacemaker current, the major effect of lidocaine was to reduce the magnitude of the activation curve with no change in time constants or in the shape of the fully activated current-voltage relation. Quinidine and procainamide exerted similar effects on instantaneous currents. Quinidine affected the pacemaker in the same way as lidocaine, whereas procainamide had no effect on this current. The results indicate that local anesthetics do not increase the K inward rectifier current and have no effect on inward background current at potentials negative to -60 mV; the shift in steady state currents at these levels is due to a change in pacemaker current. Because the lidocaine-sensitive pacemaker current does not reverse at the presumed equilibrium potential for K ions, and the effect of lidocaine in the presence of 1 mM Ba is similar to that in normal Tyrode, it is concluded that the drug inhibits a Na current, sensitive to TTX. The shortening of the action potential in sheep cardiac Purkinje fibers by local anesthetics is not due to an increase in outward current but can be explained by a block of an inward current, through the TTX-sensitive channel. Circ Res 50: 257-272, 1982
rent, which was attributed to an increase in time-independent outward current (i\text{Kt}) and a decrease in background inward current.

The present experiments confirm that lidocaine and quinidine reduce the magnitude of the pacemaker current but do not support the suggestion by Weld and Bigger (1976) of an increase in i\text{Kt} and a decrease of background inward current. The lidocaine- and quinidine-induced effects cannot be explained in terms of a pacemaker current carried by K ions, but support the hypothesis of a pacemaker current activated on hyperpolarization and mainly carried by Na ions (DiFrancesco, 1981). Preliminary results appeared in communication form (Saikawa and Carmeliet, 1981).

Methods

Experiments were performed on short (<1 mm) Purkinje fibers from the left ventricle of sheep hearts, prepared according to the procedure described by Aronson et al. (1973). The two-micro-electrode technique was used to measure currents under voltage clamp conditions and was described in detail in a previous paper (Carmeliet and Ramon, 1980).

Two different voltage clamp protocols were used (Noble and Tsien, 1968). In one type, test clamps to different levels were preceded by a conditioning pulse to —40 mV. The duration of the test clamp varied between 5 and 10 seconds; the conditioning clamp was usually 3 seconds long. Clamps were applied at a frequency of 1/min. In between clamps, the potential was held at the resting potential. By measuring the currents at the beginning and the end of the test clamp to different voltage levels it was possible for us to construct current-voltage relations for potential levels negative to —60 mV provide information on changes in background current; the conditioning clamp to —40 mV completely deactivates the pacemaker current (hypothesis of DiFrancesco, 1981). Steady state current-voltage relations provide information on time-dependent and time-independent currents.

In a second type of protocol, the membrane potential was clamped at different levels starting from a holding potential corresponding to the resting potential (about —75 mV). This type allowed us to measure steady state currents and to analyze the kinetics and rectifier properties of the pacemaker current. The steady state activation relation for the pacemaker current was determined by measuring the magnitude of the tail currents on return to the holding potential. Time constants were determined either from the time-dependent changes during the voltage steps or from the tail currents obtained by repolarizing clamps to various membrane potentials after a sufficiently large depolarization (first type of protocol). Information on the ion transfer mechanism was obtained by measuring the rectifier ratio, i.e., the ratio of the time-dependent current during and after a voltage step (see arrows in Figs. 8 and 12). By multiplying the rectifier ratio with the total amplitude of the steady state activation curve, the fully activated current-voltage relation can be constructed.

Each experiment consisted of three experimental periods: a control period, a test period during which the drug was applied, and a second control period after washout of the drug. Sufficient time was allowed to obtain steady state condition for drug action (15–30 minutes). Between each experimental period, the preparation was stimulated at 60/min to record the drug effect on the action potential or to check reversibility.

The composition of normal Tyrode was as follows, in mM: NaCl, 126; KCl, 5.4; CaCl\(_2\), 3.6; MgCl\(_2\), 0.5; NaHCO\(_3\), 24; glucose, 5.5. The solution was gassed by 95% O\(_2\), 5% CO\(_2\), pH 7.4. Na-free solution was made by substituting Tris-Cl for NaCl and NaHCO\(_3\); the solution was gassed by 100% O\(_2\). Some experiments, 1 or 20 mM CsCl was added to block the pacemaker current and the K inward rectifier, respectively (for specificity of these effects see Isenberg, 1976). In experiments related to the analysis of the pacemaker, the effect of local anesthetics was studied in normal Tyrode as well as in the presence of 1 mM Ba. Ba ions, in contrast to Cs, block the inward K rectifier without affecting the pacemaker current (DiFrancesco, 1981). The following drugs were used: lidocaine hydrochloride (Federa), 1.85 and 3.7 × 10\(^{-5}\) M; quinidine sulfate (Boehringer), 0.67 to 2.68 × 10\(^{-5}\) M; procainamide hydrochloride (Siegfried), 3.7 × 10\(^{-5}\) to 3.7 × 10\(^{-4}\) M; TTX (Sankyo), 3 × 10\(^{-6}\) to 3 × 10\(^{-5}\) M.

Results

Effect of Lidocaine on Steady State and Instantaneous Currents

When lidocaine (3.7 × 10\(^{-6}\) M) is added to the perfusion solution, the shortening of the action potential in steady state is entirely due to an acceleration of the repolarization during the plateau, with no change in the initial plateau level (Fig. 1, inset). At higher concentrations, a similar phenomenon is seen during the first minutes of perfusion; later on, \(V_\text{max}\) and amplitude of the upstroke decrease and the initial plateau phase becomes more negative (Fig. 1); rate of repolarization during phase 1, however, is practically unchanged. For these reasons, the analysis of ionic currents in the majority of experiments was restricted to potentials negative to —20 mV. The description of the voltage clamp results will be limited to lidocaine concentrations that evoked near maximal effects; lower concentrations, corresponding to more “therapeutic” levels, caused qualitatively similar changes.

Examples of current records obtained in normal Tyrode and in the presence of lidocaine 1.85 × 10\(^{-5}\) M are shown in Figure 2. The voltage clamp protocol consisted of a conditioning step of 3 seconds to —40
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FIGURE 1 Steady state current-voltage relation (A) and instantaneous current-voltage relation (B) in normal Tyrode (○) and in the presence of lidocaine 1.85 × 10⁻⁵ M (×). Steady state currents were measured at the end of a 10-second clamp pulse (see Fig. 2). Instantaneous currents were estimated from current records following a conditioning clamp to −40 mV and measured by extrapolation from the slow current changes to the beginning of the test clamp (see Fig. 2). Sheep Purkinje fiber. Inset: progressive shortening of action potential by lidocaine 3.7 × 10⁻⁶, 1.85 × 10⁻⁵ and 3.7 × 10⁻⁴ M. Calibration: 20 mV and 100 msec.

mV followed by a test clamp of 10 seconds to different levels. From these examples, it is clear that steady state currents measured at the end of the 10-second test clamp are shifted in the outward direction after the addition of lidocaine. The shift was not uniform over the whole range of potentials; the current-voltage relation in Figure 1A illustrates that the shift was minimal at potentials close to the resting potential and more pronounced at depolarized and hyperpolarized levels. The effect on steady state currents was dependent on the concentration of lidocaine. At 1.85 × 10⁻⁵ M lidocaine, the effect at levels corresponding to the plateau of the action potential was practically maximal; higher concentrations of 3.7 × 10⁻⁵ M were needed to obtain maximal effects at hyperpolarized levels.

A different picture is obtained for instantaneous currents. Instantaneous currents were measured by extrapolation from the slow time-dependent phase in the current record (see arrows in Fig. 2). This means that depletion currents (for hyperpolarizing clamps) or fast inward currents (for depolarizing clamps) were neglected. Results for the same preparation as in Figure 1A are shown in Figure 1B. The main difference consists of the absence of a positive shift for potential levels negative to −70 mV; for potentials positive to −60 mV, the lidocaine-induced shift in the outward direction is still present or is even more pronounced. As illustrated in Figure 2, a hyperpolarizing step to −85 or −105 mV results in an instantaneous current step followed by a slowly increasing inward current, i.e., the pacemaker current. Addition of lidocaine only reduces the amplitude of the pacemaker current without change in the initial step. The change in steady state current by lidocaine in the range of potentials negative to −70 mV is thus due to an effect on the pacemaker current. This was further corroborated by experiments performed in the presence of 1 mM Cs.

The addition of 1 mM Cs is known to block the time-dependent changes in the pacemaker current (Isenberg, 1976; Carmeliet and Ramon, 1980; DiFrancesco 1981). This is illustrated by the examples of Figure 3. Compared to normal Tyrode, the holding current in 1 mM Cs is slightly more outward while the time-dependent increase in inward current at −85 mV and the tail current on return to −70 mV are absent; these phenomena reflect a block of the pacemaker current by Cs. Addition of lidocaine still shifts the current in the outward direction during the conditioning pulse to −40 mV but does not affect the holding current or the current during the hyperpolarizing step.

Experiments Related to the Shortening of the Action Potential

Lidocaine Does Not Affect the K Inward Rectifier

For potential levels corresponding to the resting potential, the only time-dependent current in Purkinje fibers is the pacemaker current. When the pacemaker current is blocked by 1 mM Cs, the current measured under these conditions provide
information on eventual changes in background current. An important component of the background current is carried by K ions and shows inward-going rectification; i.e., the channel conductance increases for net inward K movement and decreases for net outward movement. The experiments described in the previous section demonstrated that lidocaine had no effect on the steady state currents in 1 mM Cs. Therefore, this finding does not favor the hypothesis of Bigger and Mandel (1970) that the K inward rectifier conductance is changed by this drug, unless one assumes that the effect on the inward rectifier is exactly balanced by an effect on other background currents. A more direct test of an eventual effect on the inward rectifier was therefore performed using 20 mM Cs. This concentration of Cs ions is known to block the inward rectifier (Isenberg, 1976; Vereecke et al., 1980). By comparing the effect of lidocaine on the instantaneous currents in normal Tyrode and in the presence of 20 mM Cs, it is possible to decide whether lidocaine affects the inward rectifier or other background currents. Such a comparison and examples of currents are shown in Figure 4. In normal Tyrode, the results confirm the description presented in Figure 1B: instantaneous currents are shifted in the outward direction for potential levels positive to —70 mV (Fig. 4, • and X; and A,B,E,F). In the presence of 20 mM Cs, the current-voltage relation becomes linear for potentials negative to —40 mV due to a block of the K inward rectifier; addition of lidocaine has no effect on the currents at potential levels negative to —70 mV, but a shift in the outward direction is observed for potentials positive to —60 mV, similar to that in normal Tyrode (Fig. 4, I and +; and C,D,G,H). The absence of a lidocaine effect in 20 mM Cs at potentials negative to —70 mV indicates that lidocaine does

**Figure 2** Examples of current records obtained in normal Tyrode (Tyr) and in the presence of lidocaine (Lidoc) 1.85 x 10^-5 M. Holding potential: —75 mV. The voltage clamp protocol consisted of a conditioning step of 3 second to —40 mV followed by a test-clamp of 10 second to different levels, indicated at the bottom of the figure. Arrows indicate way of measuring instantaneous current. Current calibration of 25 nA applies to A, B, C, D, E, and F; calibration of 50 nA to G and H. Sheep Purkinje fiber.

**Figure 3** Examples of current records in normal Tyrode (A), in 1 mM Cs (B), and in 1 mM Cs + lidocaine 1.85 x 10^-5 M (C) in a sheep Purkinje fiber. Voltage clamp protocol shown at the bottom of the figure. In the presence of Cs 1 mM, lidocaine shifts the current in the outward direction at —40 mV, but has not effect on the currents at the holding potential (—70 mV) or —85 mV (test potential). In Cs, the holding current is shifted slightly outward; note also a pronounced depletion current during the step to —85 mV and an outward tail on return to —70 mV (disappearance of depletion).
not affect background currents remaining after subtraction of the K inward rectifier. The fact that 20 mM Cs, apart from blocking the K inward rectifier, also slightly stimulates the Na pump and generates an extra outward current (Isenberg, 1976) does not invalidate this argument. Since, on the other hand, lidocaine does not change instantaneous currents in normal Tyrode at the same potentials, it is concluded that the inward K rectifier component also is not modified by lidocaine.

**Lidocaine Inhibits a TTX-Sensitive Na Current during the Plateau**

The results presented in the previous section indicate that the shortening of the action potential cannot be explained by a conductance increase of the inward K rectifier. The hypothesis that lidocaine reduces an inward Na current at the plateau level was tested in the following experiments.

In a first type, the effect of lidocaine or TTX or a combination of both drugs was studied to determine whether lidocaine acts on the same receptor system as TTX. In a second type, the effect of lidocaine was compared in media containing the normal Na concentration and in media from which the Na ions were omitted.

Figure 5 (bottom) shows the steady state current-voltage relation for potential steps to levels between —70 and —10 mV from a holding potential of —75 mV. Data are for normal Tyrode, TTX in a large concentration (3 × 10⁻⁵ M), and TTX + lidocaine (1.85 × 10⁻⁵ M). TTX alone shifts the current-voltage relation in the outward direction; the addition of lidocaine to a TTX-containing solution does not increase this shift, although lidocaine alone has a similar effect (not shown in this figure). A similar observation has been made recently by Colatsky (1981) in rabbit Purkinje fibers.

A more detailed analysis of the current changes can be made from the examples given in Figure 5 (top). Under control conditions, a clamp from —75 to —40 mV results in a net initial inward current followed by an outward current which slowly increases during the 5-second clamp. On return to —75 mV, an outward tail is recorded. For the clamp to —90 mV, an instantaneous inward current is followed by a slowly increasing inward current, i.e., the pacemaker current. In the presence of lidocaine,
TTX, and TTX + lidocaine, the steady state currents during a depolarizing clamp to -40 mV are comparable: the shift in the outward direction is slightly larger for TTX alone than for lidocaine, but—what is more important—the current in TTX + lidocaine is not increased, but rather is reduced compared to that in TTX alone. Steady state currents at the holding potential and during hyperpolarizing clamps, and tail currents on return to the holding potential, are affected differently by TTX and lidocaine. In the presence of lidocaine, holding current at -75 mV and steady state current at -90 mV are more outward, whereas tail currents are reduced for depolarizing as well as hyperpolarizing pulses. TTX is without effect on these currents.

The fact that changes in "plateau" currents by lidocaine and TTX are similar suggests that these drugs act on the same channel, i.e., the fast Na channel. If lidocaine were to affect a current different from the fast Na current, the effect of TTX and lidocaine should be additive. The results further show that the "fast" Na current contributes to the currents during the plateau of the action potential. This can be explained by an important overlapping of activation and inactivation curves (Attwell et al., 1979) and to the existence of slow inactivation of the fast Na channel.

A second approach in our study of the effect of local anesthetics on the Na current was the use of Na-free solution. Examples of current records for a clamp from -75 mV to -40 mV are shown in Figure 6 (inset). In the absence of the drug, the current initially is net inward and approaches the zero level at the end of the clamp in Na Tyrode, whereas, in Na-free medium, an outward current is obtained which decreases with time (Fig. 6B). In the presence of the drug, the current is shifted in the outward direction in Na-Tyrode, but no change is seen in Na-free solution. Steady state currents for potentials between -90 and -20 mV are illustrated by the graphs. In normal Tyrode, lidocaine (1.85 x 10^{-5} M) shifts the current in the outward direction over the whole range of potentials, the effect at this concentration being largest at depolarized levels (Fig. 6A). In Na-free medium, currents were identical whether lidocaine was present or not (Fig. 6B). It should be noted that lidocaine also had no effect at hyperpolarized levels in Na-free medium; this is due to the absence of the pacemaker current in Na-free medium (Deck and Trautwein, 1964).

**Does Lidocaine Affect the Slow Inward and Positive Dynamic Currents?**

Although the description of results in the preceding section was restricted to current changes for potentials negative to -20 mV, we should mention that lidocaine in concentrations of 1.85 to 3.7 x 10^{-5} M affected currents at potential levels up to -10 and 0 mV. This raises the question as to whether the slow inward and positive dynamic currents are changed by lidocaine. Information from experiments in which lidocaine or TTX alone or in combination were used suggests that the effect on these currents is minimal or non-existent and that the outward shift in current is due mainly to an inhibition of the fast Na current. For example, during a clamp to -20 mV, at which a prominent positive dynamic current was present, lidocaine at 1.85 x 10^{-5} M shifted the current in the outward direction from the beginning of the clamp. In the presence of a large concentration of TTX, which also increased net outward current, lidocaine had no additive effect. This suggests that the whole lidocaine effect can be ascribed to its effect on the Na current. At 3.7 x 10^{-5} M lidocaine, we observed a small reduction in the positive dynamic current; this effect, however, is contrary to what is needed to explain an increase in the repolarization rate, and might be
secondary to a reduction in slow inward current (see Fig. 5, top). According to Siegelbaum and Tsien (1980), the positive dynamic current in calf Purkinje fibers is a K current triggered by an inflow of Ca ions through the slow channel. A reduction of the slow inward current by high concentrations of lidocaine thus may be responsible for a decrease of the positive dynamic current.

Results

Effect of Quinidine and Procainamide on the Current-Voltage Relation

In sheep Purkinje fibers, the addition of quinidine or procainamide to the superfusion solution results in a pronounced shortening of the action potential similar to that of lidocaine (see inset, Fig. 7). An analysis of the steady state current-voltage relation in normal Tyrode (Fig. 7A) and in the presence of 20 mM Cs shows that the effect of quinidine was quite comparable to that of lidocaine; procainamide also shifts the current-voltage relation in the outward direction at potentials corresponding to the plateau level, but had practically no effect on the current at levels more negative than -70 mV (Fig. 7B).

The shortening of the sheep cardiac action potential by quinidine is different from results described for canine Purkinje fibers (see Hoffman and Cranefield, 1960; Trautwein, 1963) and rabbit Purkinje fibers (Colatsky, 1981). The prolongation of the action potential in the dog, however, is small and due mainly to a retardation of the terminal repolarization, while the plateau is shortened (Rosen et al., 1973). In canine Purkinje fibers, the electrophysiological effect of quinidine has been shown to depend on the underlying cholinergic tone (Mirro et al., 1980). In the sheep, the shortening is not due to an anticholinergic effect of quinidine, since the effect could be reproduced in the presence of atropine.

Experiments Related to the Pacemaker Current

According to the analysis of Noble and Tsien (1968), the pacemaker is a K current activated on depolarization and deactivated on hyperpolarization; its ion transfer mechanism is characterized by inward-going rectification. In a first stage, we present a description of the lidocaine effect in the framework of the Noble and Tsien hypothesis. As will become clear, however, some observations are difficult to explain if the current is assumed to be carried by K ions. In a second stage, therefore, the effect of lidocaine will be measured in the presence of 1 mM Ba. The results will be analyzed in the framework of the hypothesis of DiFrancesco (1981), i.e., the pacemaker current is assumed to be activated on hyperpolarization and to be carried mainly by Na ions; its ion transfer mechanism is characterized by a linear current voltage relation.
The Effect of Quinidine on the Steady-State Current-Voltage Relation

The effect of quinidine sulfate $1.34 \times 10^{-5} \text{M}$ (A) and of procainamide $3.7 \times 10^{-4} \text{M}$ (B) on the steady-state current-voltage relation. The circle symbols show control measurements (mean values of data obtained before addition and after washout of drug). Crosses show measurements in the presence of the drug. Inset: progressive shortening of the action potential by quinidine (A) ($0.13, 0.67$ and $1.34 \times 10^{-5} \text{M}$) and by procainamide (B) ($0.37, 1.85$ and $3.7 \times 10^{-5} \text{M}$). Calibration: $20 \text{mV}$ and $100 \text{msec}$; horizontal line: zero potential level.

The Effect of Lidocaine on the Pacemaker Current in Normal Tyrode

Examples of current records obtained in normal Tyrode and in the presence of lidocaine ($3.7 \times 10^{-5} \text{M}$) are shown in Figure 8. Holding potential was $-75 \text{mV}$. When the membrane is clamped to $-65 \text{mV}$, an instantaneous outward current is generated, followed by a time-dependent increase, which, according to Noble and Tsien, is due to activation of a K current; on return to the holding potential an outward tail current is seen which decays with time (deactivation). For an hyperpolarizing step to $-85 \text{mV}$, the reverse phenomena are observed. In the presence of lidocaine, the holding current is more outward and the time-dependent currents are largely decreased. All these effects are reversed during washout of the drug.

By plotting the amplitude of the tail currents ($i_B$ in Fig. 8) following depolarization and hyperpolarization to different levels as a function of membrane potential, an activation curve is obtained (Fig. 8A); lidocaine diminishes the magnitude of this activation curve but has no effect on its potential dependency (see also Table 1). The time course of the tail currents was determined at different membrane potential levels and was found to be unchanged by lidocaine (Fig. 9B). Information on the ion transfer mechanism was obtained by measuring the rectifier ratio, i.e., the ratio of the time-dependent current during and after a voltage step (see $i_A$ and $i_B$ in Fig. 8). Lidocaine did not modify the rectifier ratio (Fig. 9A). Extrapolation to very negative potentials suggests a small shift in reversal potential. In general, however, the shape of the instantaneous current-voltage relation was not changed. If the reduction in activation is taken into account, this means that the fully activated current is decreased by lidocaine.

Theoretically, a reduction of the magnitude of the activation curve (assuming that the pacemaker current is carried by K ions) can be explained in two ways: either a certain proportion of the channels is blocked and does not carry current, or a certain proportion of the channels changes from time-dependent into time-independent and is in the open, activated state at all potentials.

A distinction between these two possibilities can be made by measuring the absolute value of the tail currents (i.e., relative to the zero current level) and plotting them as a function of membrane potential. If the absolute value of the tail currents is decreased to the same degree for all potential steps, this would
mean that a certain proportion of the channels is blocked. However, from Figure 8B, it is clear that the peak amplitude of the tail current following a depolarization to $-40$ mV is not modified by lidocaine. At less depolarized levels and for hyperpolarizing steps, however, the reduction in tail current by lidocaine gradually increases. Apparently, fewer channels are deactivated and a substantial proportion remains in the activated state at hyperpolarized levels.

The same conclusion can be reached by comparing the current-voltage relation including the fully activated pacemaker current ($i$, the activation parameter, equal to 1) and the current-voltage relation without this current ($i$ equal to 0). Both current-voltage relations can be calculated from the activation curve, steady state current-voltage relation, and the rectifier ratio (Noble and Tsien, 1968). As shown in Figure 10, the current-voltage relation for $i$ equal zero is shifted in the outward direction, while the fully activated current-voltage relation is not changed, suggesting that part of the K channels remain in the open activated state in the presence of lidocaine.

The fully activated current-voltage relation can also be experimentally determined by measuring the instantaneous currents when the membrane is clamped from $-40$ mV to different hyperpolarized levels. The examples in Figures 2 and 12 show that lidocaine has no effect on the instantaneous currents.

**The Effect of Lidocaine on the Pacemaker Current in the Presence of Ba Ions.**

Although most of the effects of lidocaine can be analyzed in the framework of the K hypothesis, one observation is difficult to explain. Figure 11 shows the change in steady state current-voltage relation produced by lidocaine: the currents are shifted in the outward direction at all levels but the shift becomes more important the more negative the membrane potential. If lidocaine were to affect a K

<table>
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<th>TABLE 1 Effect of Lidocaine, Quinidine, and Procainamide on Activation Parameters of the Pacemaker Current</th>
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<tr>
<td><strong>Lidocaine</strong></td>
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<td>(1.85 × 10^-3 M)</td>
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<td>n = 6</td>
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<td>Amplitude of activation curve in %</td>
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Results are expressed as mean ± SE.
Figure 9 A: rectifier ratio in normal Tyrode (●), in the presence of lidocaine $3.7 \times 10^{-5}$ M (✕), and after washout of the drug (○). The rectifier ratio is obtained by calculating the ratio of the time-dependent currents during the clamp over the tail on return to the holding potential ($I_A$ over $I_B$ in Fig. 8). B: time constants in second of tail currents measured during clamps to different potential levels following a conditioning depolarization (3 second) to $-40$ mV. Data in normal Tyrode (●) and in the presence of lidocaine $3.7 \times 10^{-5}$ M (✕). Same preparation as Figure 8.

Figure 10 Current-voltage relation calculated for $s$, the activation parameter, equal to 1 (A) and equal to zero (B). Data in normal Tyrode (●) and in the presence of lidocaine $3.7 \times 10^{-5}$ M (✕). The fully activated current-voltage relation is not affected by lidocaine, while the current-voltage relation without the pacemaker current is shifted in the outward direction. Same preparation as Figure 8.

pacemaker current, the shift should be reduced at potential levels close to $E_K$ and reverse for more negative levels. This is not the case; the lidocaine-sensitive current does not reverse. Figure 11 further shows examples of current records for hyperpolarizations to $-93$ mV and $-104$ mV in normal Tyrode and in the presence of lidocaine. At $-93$ mV, the time-dependent inward current, which is pronounced in normal Tyrode, is largely suppressed in the presence of lidocaine. At $-104$ mV, the current pattern reverses and the inward current decreases with time. Although this current pattern is largely generated by depletion phenomena, it is not illogical to suppose that the equilibrium potential for K should be very close to this level. If lidocaine affects a K current, the effect should be minimum at this level or should even be reversed. In contrast, the example shows that lidocaine still causes the current to shift in the outward direction.

The conclusion from these experiments, therefore, is that the lidocaine-sensitive current is not a K current. This was the reason for analyzing the effect of lidocaine in the presence of Ba ions. Di-Francesco (1980b) has shown that Ba ions block the inward-going rectifier without eliminating the pacemaker current. In the presence of Ba, the pacemaker current is seen as an inward current which does not reverse even at very negative membrane...
potentials. According to DiFrancesco, the measurement of a reversal potential in normal Tyrode is due to the simultaneous occurrence of a depletion current (decreasing inward current) and an increasing inward current (the pacemaker current). Since Ba ions block the inward-going rectifier, depletion currents are less prominent and the pacemaker can be analyzed without this complication. An illustration of these phenomena is given in Figure 12. In normal Tyrode (Fig. 12, A1 and A3), hyperpolarizing steps from a holding potential of $-40 \text{ mV}$ result in large instantaneous current jumps (due to changes in $i_{k1}$) followed by a time-dependent increase of inward current. At $-95 \text{ mV}$, this time-dependent component has become small, suggesting the existence of a reversal potential. In the presence of $1 \text{ mM Ba}$ (Fig. 12, A2 and A4) holding current at $-40 \text{ mV}$ is practically zero instead of outward; the instantaneous current steps are much reduced and the time-dependent changes in inward current increase as a function of hyperpolarization without any tendency to reverse. In the presence of lidocaine, the time-dependent current changes are reduced.

A complete analysis of the pacemaker current and its change by lidocaine in $1 \text{ mM Ba}$ compared to normal Tyrode is presented in Figures 12, 13, 14, and 15. Fig. 12B shows that depolarization from a holding potential of $-80 \text{ mV}$ results in a time-dependent increase in outward current, followed by a tail on return to the holding potential, while hyperpolarizing clamp steps result in the opposite changes. Addition of lidocaine shifts the holding current in the outward direction and reduces the amplitude of the time-dependent currents and tail currents. When tail currents are plotted as a function of membrane potential, a sigmoidal curve is obtained, whose amplitude is reduced by lidocaine (Fig. 13B); no significant change occurs in the potential dependency. Comparison with Figure 13A shows that the results in normal Tyrode and in $1 \text{ mM Ba}$ are essentially the same. Only small differences exist: in Ba, the total amplitude of the "activation" curve is slightly smaller and shifted in the hyperpolarized direction. These differences were consistently observed in four experiments. Time constants were not modified by lidocaine and in the range of potentials from $-70 \text{ mV}$ to $-100 \text{ mV}$ they were similar in normal Tyrode and in $1 \text{ mM Ba}$-Tyrode (Fig. 14). In the range of potentials positive to $-70 \text{ mV}$, we were unable to measure accurately the time course of the pacemaker current, because of the small amplitude of the current. A verification of the existence of a bell-shaped relationship between time constants and membrane potential was thus impossible.

A full description of the pacemaker process in-
FIGURE 12  A: Effect of lidocaine \((1.85 \times 10^{-5} \text{ M})\) on instantaneous and time-dependent currents in normal Tyrode \((A_1, A_2)\) and in 1 mM Ba-Tyrode \((A_3, A_4)\). Superimposed current records; currents in the presence of lidocaine indicated by (○). Voltage clamp protocol indicated below the figure. In Ba-Tyrode, the instantaneous currents are largely suppressed. In normal Tyrode as well as in Ba-Tyrode, lidocaine had no effect on the instantaneous currents and only reduced time-dependent currents. Note the absence in Ba-Tyrode of any tendency of the pacemaker current to reverse. B: Effect of lidocaine \((1.85 \times 10^{-5} \text{ M})\) on the pacemaker current in 1 mM Ba. Current records in the absence (○) and in the presence (●) of lidocaine are superimposed; \(i_A\) and \(i_B\) are time-dependent current changes used to calculate the rectifier ratio (see Fig. 15). Voltage clamp protocol at the bottom of the figure. Holding current at \(-80 \text{ mV}\) is negative, due to the block of the inward-going K rectifier. As in Figure 8, lidocaine shifts the holding current in the outward direction and reduces the amplitude of time-dependent currents during the voltage step and on return to the holding potential.

FIGURE 13  Effect of lidocaine \((1.85 \times 10^{-5} \text{ M})\) (●) on the amplitude of tail currents in normal Tyrode (A) and in 1 mM Ba-Tyrode (B). Data before addition of lidocaine (○) and after washout (○). Holding potential was \(-75 \text{ mV}\) in A and \(-80 \text{ mV}\) in B. Same preparation as Figure 12B.
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3

1

-100 -50 mV -100 -50 mV

FIGURE 14 Effect of lidocaine 1.85 \( \times 10^{-5} \text{ M} \) (\( \times \)) on time constants of tail currents in normal Tyrode (A) and in 1 mM Ba-Tyrode (B). Full circles indicate data in the absence of lidocaine. Tail currents at different potentials levels were obtained after a conditioning clamp to -40 mV (3 sec). Same preparation as Figure 12B.

cludes an analysis of the ion transfer mechanism or fully activated current-voltage relation. The shape of the current-voltage relation or rectifier ratio can be obtained from the time-dependent current changes during a clamp and the tail currents following the clamp (\( i_A \) and \( i_B \) in Fig. 12B). In the examples of Figure 12B, this ratio is less than unity for the depolarizing step to -70 mV and greater than unity for the hyperpolarizing steps to -90 mV and -100 mV. Measurement in Ba-Tyrode for intermediate voltage steps between -70 mV and -100 mV shows that this ratio increases linearly with hyperpolarization, a result quite different from that in normal Tyrode (Fig. 15, A and B). Addition of lidocaine did not change the shape of the fully activated current-voltage relation. When the reduction in activation by lidocaine is taken into account, this means that lidocaine does not change the linear behavior of the fully activated current-voltage relation but reduces it slope. Lidocaine thus blocks a certain proportion of the pacemaker channels.

Effect of Quinidine and Procainamide

Experiments were made in normal Tyrode and in Ba-Tyrode. Quinidine (1.34 \( \times 10^{-5} \text{ M} \)) reduced the magnitude of the pacemaker activation by 15% (Table 1 and Fig. 16) and shifted the steady state current-voltage relation in the outward direction. Procainamide (3.7 \( \times 10^{-4} \text{ M} \)) had no effect on the pacemaker current.

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FIGURE 16  Effect of procainamide $3.7 \times 10^{-4}$ M (A) and quinidine $1.34 \times 10^{-5}$ M (B) on the amplitude of the tail currents in the same preparation. Measurements before addition of the drug (○), in the presence of the drug (×), and after washout (□).

Discussion

The present experiments show that the shortening of the action potential by lidocaine is not due to an increase in K conductance but, rather, to a block of Na current through the TTX-sensitive fast Na channel. At potentials corresponding to the dia
istolic membrane potential, the effect of lidocaine consists in blocking the pacemaker current; no evidence is found for an increase in the inward K rectifier current or a decrease in inward background current.

Mechanism for the Current Changes at Potentials Positive to $-60$ mV

The conclusion that the lidocaine-induced outward current at plateau levels is due to a block of the "fast" Na current is based on the finding that the effect disappears in Na-free medium, and that the effect of TTX and lidocaine is the same and not additive, when large concentrations are used. Both drugs interfere with the same channel, and since TTX is generally accepted to be specific in its action (see Narahashi, 1974), it is concluded that lidocaine blocks the fast Na channel. The question of how a channel with fast kinetics affects steady state currents can be answered if it is assumed that activation and inactivation curves overlaps significantly (Attwell et al., 1979). A supplementary mechanism whereby block of the Na channel can affect late currents is to accept a slow phase of inactivation. Slow inactivation indeed may be the reason for the increasing outward current during clamps to about $-40$ mV. During a long clamp to $-40$ mV, the block by TTX as judged from the difference between control currents and currents in the presence of TTX, is greater at the beginning (100-200 msec) than at the end (5 sec) of the clamp. This effect cannot be explained by overlapping of activation and inactivation curves but suggests the existence of a slow inactivation process in normal Tyrode.

Lidocaine (Bigger and Mandel, 1970; Weld and Bigger, 1975) or TTX (Coraboeuf et al., 1979) shortens the action potential in concentrations that have no effect on $V_{max}$ during the upstroke. This observation is not contrary to the hypothesis that these drugs affect only the fast Na current. During the upstroke, unidirectional inward and net inward currents are very large and a small reduction will be hardly detectable. During the plateau, unidirectional inward as well as outward currents are not negligible but practically in balance, and a small reduction of inward current will result in a comparatively large change of the net current and, therefore, of the potential course.

In this respect it should be mentioned that $V_{max}$ measurements are only an indirect estimation of the Na current. A comparison between TTX-induced changes in $V_{max}$ and in Na current (voltage clamp) has shown that the dose-effect curve for both parameters is not identical, $V_{max}$ being much less sensitive than the direct measurement of the Na current (J. Cohen, personal communication). Another phenomenon which may be important in explaining the difference in sensitivity of the upstroke and plateau to a block by these drugs is an increasing binding to the channel during depolarization. The block by local anesthetics is dependent on the state of the channel [use-dependency and potential-dependency (Strichartz, 1973; Courtney, 1975; Hondeghem and Katzung, 1977)]. In heart, the block by TTX is also use-dependent (Reuter et al., 1978; Cohen et al., 1979); evidence for a voltage-dependent effect is controversial: $V_{max}$ sensitivity to TTX block is strongly voltage dependent (Baer et al., 1976), but direct measurements of $i_Na$ do not show a voltage-dependent block by TTX (Bean et al., 1980).

The fact that lidocaine and TTX block the Na current does not mean that both drugs interfere with the same receptor in the Na channel. Rather, the following differences suggest that the site of
activity is not the same: local anesthetics, to be effective, have to diffuse in the lipid phase of the membrane and eventually must reach the intracellular phase before they can interfere with the Na channel (Hille, 1977); it is known that TTX acts from the outside (Narahashi et al., 1966).

Finally, one should mention that the present experiments cannot exclude small effects of local anesthetics on the slow inward current, the positive current from the outside (Narahashi et al., 1966). It is known that TTX acts on the sheep Purkinje fiber is on the fast sodium channel (Hille, 1977); it is known that TTX acts on the sheep Purkinje fiber is on the fast sodium channel, and the slow outward current, since a detailed analysis of these currents was not made. In the rabbit, for instance, a reduction of i, by quinidine may explain the small lengthening of the action potential (Colatsky, 1981). We feel, however, that the major effect of local anesthetics in the sheep Purkinje fiber is on the fast sodium current; this effect is sufficiently pronounced to provide an explanation for the shortening of the action potential.

**Mechanism for the Current Changes at Potentials Negative to —60 mV**

In a voltage-clamp analysis of the lidocaine effect, Weld and Bigger (1975) found that “in most fibers the steady state current-voltage relationship for lidocaine was lying positive to the control curve over the entire voltage range from the reversal potential to the threshold voltage for the early inward transient current” and concluded that “the lidocaine-induced increase in outward current is caused by an increase in background current iK, and to a decrease in background inward current iNa.” As far as the steady state current-voltage relation is concerned, our results confirm these observations. Our conclusion, however, is different: we did not find evidence for an effect on background current, and we explain the positive shift of the current-voltage relation by the lidocaine-induced block of the pacemaker current.

The evidence for the absence of an effect on background current is as follows: (1) lidocaine did not affect the currents in 20 mM Cs-Tyrode at potentials negative to —60 mV; this indicates that the background current remaining after subtraction of the inward rectifier is not affected; this background current is net inward at these potentials; (2) this information, combined with the absence of an effect of lidocaine on the instantaneous current in normal Tyrode for the same range of potentials, further shows that the inward rectifier component of the K background is not changed. In this context, we should mention the results of experiments with lidocaine and aprindine (a local anesthetic with lidocaine-like effects on the action potential) in which we could not find any evidence for an increase in radioactive K efflux in normal Tyrode and, rather, a reduction of K efflux in ouabain-Tyrode (Carmeliet and Verdonck, 1974).

Arnsdorf and Bigger (1972) described hyperpolarizations in Na-deficient, low-K solutions and increases in slope conductance which they attributed to an increase in K conductance. Although we did not check current changes under the same conditions, it is possible that the presence of 13.8 mM Na in the solutions used by these authors is large enough to provide for an inward current at potentials near the plateau and for a persistence of a pacemaker current at “diastolic” potentials. Blocking of the remaining Na inward current (at plateau level) and of the pacemaker current (carried mainly by Na ions) may be sufficient to hyperpolarize the membrane. As far as the measurement of an increase in slope conductance is concerned, we would like to remark that most of the results by Arnsdorf and Bigger (1972) were obtained in 0.5 and 2.0 mM K (see their Table 2) in which changes in membrane potential by lidocaine may have complicated the interpretation of the measurements.

The positive shift of the steady state current-voltage relation can be explained by a change in the pacemaker current. The positive shift is indeed not seen in conditions which eliminate the pacemaker current, i.e., 1 mM Cs (Isenberg, 1976) or Na-free medium (Deck and Trautwein, 1964), or when the pacemaker current is not activated (instantaneous currents measured following a conditioning clamp to —40 mV).

The effect of quinidine and procainamide can be explained along the same lines. Quinidine blocks the pacemaker current, and this explains the positive shift of the steady state current in the potential range negative to —70 mV; procainamide has practically no effect on the pacemaker and does not affect the steady state current in this range of potentials.

Our analysis of the pacemaker current confirms the finding by Weld and Bigger (1976) that lidocaine reduces the magnitude of the pacemaker current.

Interpretation of the changes in pacemaker current, until recently, was given in terms of K current, deactivating on hyperpolarization. However, the fact that lidocaine shifted the steady state current-voltage relation in the outward direction (Fig. 11), even at potentials close to or at the equilibrium potential for K ions, indicates that lidocaine’s effect is difficult to explain by a change in a current carried by K ions alone. Interpretation in terms of a current activated on hyperpolarization and carried mainly by Na ions, on the other hand, explains the positive shift of the steady state current-voltage relation, and the results obtained in Ba-Tyrode.

The pacemaker current does not seem to be changed by Ba ions: the effect of lidocaine was the same whether tested in normal Tyrode or in Ba-Tyrode. Also, adrenaline has been shown to exert the same effect under both conditions (Hart et al., 1980). The only difference is that the analysis of the pacemaker current in Ba-Tyrode is simplified by the block of the inward-going rectifier which eliminates depletion currents. The absence of depletion currents indeed makes it possible to describe the fully activated current as a linear function of voltage.

The measurement of an apparent reversal poten-
tial for the pacemaker in normal Tyrode has been explained by DiFrancesco (1980a, 1980b) as being due to the simultaneous occurrence of a depletion current and activation of a pacemaker current. Our results with lidocaine in normal Tyrode lend support to this interpretation: when activation of the pacemaker current is blocked by lidocaine, the currents restored during hyperpolarizations reveal a pronounced depletion pattern.

From their analysis of the lidocaine effect, Weld and Bigger (1976) concluded that the drug exerted an anti-automatic effect, not only by blocking the pacemaker current, but also by increasing background K current iK1, and decreasing background inward current. The data in this paper show that the changes in steady state current-voltage relation in the potential range negative to ~70 mV can be explained entirely by a reduction in pacemaker current. Furthermore, data obtained in 20 mM Cs have demonstrated that lidocaine does not affect background K current or background inward current at hyperpolarized levels. Therefore, the anti-automatic effect of lidocaine must be explained by reduction of the pacemaker current and inhibition of the fast Na current. A similar explanation can be proposed for quinidine. In the case of procainamide, the decrease in spontaneous activity (Rosen et al., 1973) may be entirely due to reduction of the fast Na current.

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References


Carmeliet E, Ramon J (1980) Effects of acetylcholine on the time-independent currents in sheep cardiac Purkinje fibers. Pfluegers Arch 387: 207-216


Cohen CJ, Colatsky TJ, Tsien RW (1979) Tetrodotoxin block of cardiac sodium channels during repetitive or steady depolarizations in the rabbit. J Physiol (Lond) 296: 70P-71P

Colatsky TJ (1981) Actions of lidocaine, quinidine and tetrodotoxin on membrane currents underlying the cardiac action potential plateau. Biophys J 33: 35a


Deck KA, Trautwein W (1964) Ionic currents in cardiac excitation. Pfluegers Arch 280: 63-80

DiFrancesco D (1980a) Evidence that “iK2” in Purkinje fibres is an inward current activated on hyperpolarization. J Physiol (Lond) 305: 64P-65P

DiFrancesco D (1980b) The pacemaker current “iK1” in Purkinje fibres is carried by sodium and potassium. J Physiol (Lond) 308: 32P-33P


Isenberg G (1976) Cardiac Purkinje fibers: Cesium as a tool to block inward rectifying potassium currents. Pfluegers Arch 365: 99-106

McAllister RE, Noble D, Tsien RW (1975) Reconstruction of the electrical activity of cardiac Purkinje fibres. J Physiol (Lond) 251: 1-59


Shortening of the action potential and reduction of pacemaker activity by lidocaine, quinidine, and procainamide in sheep cardiac purkinje fibers. An effect on Na or K currents?
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