Electrophysiological Effects of Imipramine on Ovine Cardiac Purkinje and Ventricular Muscle Fibers

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SUMMARY In man, therapeutic doses of imipramine suppress ventricular arrhythmias, and toxic doses can cause severe intraventricular conduction disturbances and cardiac arrest. To determine the cellular mechanisms responsible for these actions, we studied the effects of imipramine hydrochloride at concentrations from \(3 \times 10^{-8}\) to \(3 \times 10^{-6}\) g/ml on the transmembrane potentials of sheep and calf Purkinje and ventricular muscle fibers using standard microelectrode techniques. All imipramine-induced changes in transmembrane potentials became more marked with increasing concentration. At \(1 \times 10^{-7}\) g/ml (a low therapeutic concentration in man), imipramine shortened the Purkinje fiber action potential (18%) and reduced upstroke velocity slightly (6%). At \(1 \times 10^{-6}\) g/ml (a high therapeutic or toxic concentration in man), imipramine reduced the upstroke velocity of Purkinje fiber action potential by 23% and of ventricular muscle by 53%; conduction velocity fell by 26%. In Purkinje fibers, imipramine at \(1 \times 10^{-6}\) g/ml suppressed spontaneous firing elicited by isoproterenol and hypokalemia, due to a +7.9-mV shift in threshold voltage with little or no effect on spontaneous phase 4 depolarization. Depression of action potential phase 0 and action potential shortening may play a role in the antiarrhythmic effect of imipramine at therapeutic concentrations. At toxic imipramine concentrations, depression of phase 0 is marked and can account for the potentially fatal conduction defects and arrhythmias seen during imipramine toxicity in man.


TRICYCLIC antidepressant drugs are used widely to treat affective disorders and other conditions in man; about 30 million prescriptions for tricyclic antidepressant drugs are written each year in the United States alone (Blackwell, 1973). Tricyclic drugs have now replaced barbiturates as the agent most frequently taken in overdose with suicidal intent, and tricyclic overdose has rapidly evolved into a major public health problem. Even in patients with no heart disease, overdose of tricyclic antidepressant drugs can produce severe electrophysiological abnormalities, including heart block and dangerous ventricular arrhythmias (Bigger et al., 1978; Spiker et al., 1975; Vohra et al., 1975). There is concern that even therapeutic doses of tricyclic antidepressants may cause adverse cardiovascular effects in older patients or those who have known heart disease (Goodman and Gilman, 1975). On the other hand, imipramine has been shown recently to be antiarrhythmic both in animal models (Marmo et al., 1972; Schmitt et al., 1970) and in man (Bigger et al., 1977, 1978; Giardina et al., 1979). Its long half-life for elimination and relatively low toxicity have led to the proposal that it may be an advance in long-term oral antiarrhythmic therapy.

The cellular electrophysiological actions of imipramine which account for its cardiac toxicity and antiarrhythmic efficacy are uncertain. Matsuo (1967) found no change in spontaneous firing rate or action potential configuration in isolated rabbit atria exposed to imipramine at \(1 \times 10^{-7}\) g/ml, a low therapeutic concentration in man. Imipramine at \(1 \times 10^{-6}\) to \(2.5 \times 10^{-5}\) g/ml, clinically toxic levels in man, reduced upstroke velocity, diminished action potential amplitude, and slowed conduction in rabbit atria (Matsuo, 1967).

In the present experiments, we investigated the electrophysiological effects of imipramine on both ventricular muscle and Purkinje fibers, a cell type responsible for several types of experimental ventricular arrhythmias (Friedman et al., 1973; Wit et al., 1972; Bigger and Weld, 1976). We designed the present experiments to define cellular electrophysiological actions which account for the antiarrhythmic effect of therapeutic concentrations of imipramine and for the cardiotoxic effects of imipramine overdose.

Methods

General

Sheep hearts obtained at a slaughterhouse were carried to the laboratory in gassed (95% O₂, 5% CO₂) physiological solution. Calf hearts were used when sheep hearts were not available (see Results). Free-
running left ventricular Purkinje fibers (0.5-1.0 mm in diameter, 5-30 mm long) and right ventricular myocardium (both epicardial and endocardial preparations each less than 2 mm thick) were mounted and superfused by test solutions at 36°C. Transmembrane potentials were recorded as the difference in voltage between an intracellular microelectrode (3 M KCl) and an Ag-AgCl pellet electrode in the bath. The electronic circuitry, and display and photographic techniques, have been described previously (Bigger et al., 1968; Weld and Bigger, 1975).

Tissue preparations were stimulated at a rate of 30/min through bipolar extracellular electrodes (Annex Research) to study membrane responsiveness, effective refractory period, and threshold. Action potential characteristics were measured in fibers driven at 60/min. We measured the following action potential characteristics: upstroke amplitude, membrane activation voltage (the intercept of a line through the terminal portion of phase 3 or 4 and a line through the subsequent premature phase 0), maximum diastolic voltage, overshoot, maximum diastolic voltage and membrane activation time. Action potential duration at 90% repolarization (APD90), slope of phase 4 depolarization was defined as the slope of a straight line between maximum diastolic voltage and membrane activation voltage. We determined the minimum drive cycle with a stimulus of three times threshold intensity by gradually decreasing the drive cycle length until the fiber no longer could follow the stimulus with a 1:1 response. We studied membrane responsiveness by driving at a 2000-msec basic cycle length and scanning the cycle with premature stimuli (Weld and Bigger, 1975). We defined effective refractory period as the longest coupling interval of a premature stimulus (three times threshold intensity) which failed to produce a propagating response.

To evaluate the response of spontaneous automaticity to imipramine, we selected Purkinje fibers exposed to potassium at 3.0 mM and isoproterenol at 1 X 10^{-7} M. After Purkinje fibers had attained a stable firing rate for 30 minutes, we added imipramine at 1 X 10^{-7} and, 1 hour later, 1 X 10^{-6} g/ml to the superfusate.

Threshold current and voltage were determined by inserting current-passing and voltage-measuring microelectrodes within 0.1 mm of each other, and then injecting progressively stronger 100-msec depolarizing constant current pulses at a frequency of 0.5/sec until a regenerative upstroke occurred. Threshold current was defined by the minimum depolarizing current which produced a regenerative response. Threshold voltage was defined as the most negative membrane voltage at which a regenerative response occurred. To measure conduction velocity, we stimulated long Purkinje fibers at one end with external electrodes and inserted two microelectrodes near the middle and distal thirds of the fiber; we divided interelectrode distance (measured with an ocular micrometer) by the time between the V_{max} for the action potential upstrokes to obtain conduction velocity.

Unless otherwise specified, our physiological solution contained the following (in mM): NaCl, 137; NaHCO_3, 12; NaH_2PO_4, 1.8; dextrose, 5.5; CaCl_2, 1.8; MgCl_2, 0.5; KCl, 4.0; when equilibrated with 95% O_2-5% CO_2 the solution had a pH of 7.3. Imipramine HCl (kindly provided by Ciba-Geigy) was dissolved in water and added to test solutions to provide concentrations of 0.03, 0.1, 0.3, 1.0, and 3.0 X 10^{-6} g/ml (0.1, 0.3, 1.0, 3.2, and 9.5 X 10^{-6} M). Purity of imipramine was determined by thin layer and gas chromatographic techniques (Perel et al., 1973), and the presence of a single homogeneous component was confirmed by mass spectrometry (Belvedere et al., 1975). We measured electrophysiological effects after 60 minutes of superfusion with imipramine, and each fiber was exposed to each drug solution in the order of increasing concentration.

In experiments on Purkinje fibers, impalements were maintained throughout all test solutions. This was not possible in ventricular muscle; for muscle the mean of data for five different cells was determined in each test solution. In Purkinje fiber experiments, data were analyzed by the t-test for paired samples (Snedecor and Cochran, 1967). In ventricular muscle, data were analyzed by the t-test for unpaired samples. Findings at each drug concentration were compared to the control measurements. Because multiple comparisons were made, the critical value of the t-statistic (alpha = 0.05) was obtained using Bonferroni's method (Miller, 1966). Data are presented in the text as mean values ± standard error. All data are from sheep preparations unless otherwise indicated.

![Figure 1](http://circres.ahajournals.org/)

**Figure 1** Time course of action potential changes during exposure to imipramine. V_{max} = maximum upstroke velocity. APD_{90} = action potential duration at 90% repolarization. Data are normalized; control values are 100%.
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Results

Purkinje Fibers

Time Course of Imipramine Effects

The time course of action potential changes in Purkinje fibers during imipramine superfusion at 1 × 10⁻⁶ g/ml is shown in Figure 1 for maximum upstroke velocity and for action potential duration at 90% repolarization. A semilogarithmic plot of the fall in $V_{\text{max}}$ and action potential duration showed that the half-time to maximum effect was 19 minutes for $V_{\text{max}}$ and 14 minutes for APD₉₀. By taking measurements after 1 hour of imipramine superfusion, we studied fibers which had achieved 85% or more of their steady state imipramine effect. The time course for recovery during washout of imipramine was similar to that for the onset of imipramine effect, although recovery frequently was incomplete even after 90 minutes.

Dose-Response Relationships

Dose-response relationships for Purkinje fibers are shown in Figure 2. Each of the action potential changes shown became more marked as the imipramine concentration was increased. The decreases in APD₅₀ and APD₉₀ became statistically significant at 1 × 10⁻⁷ g/ml. The reduction in $V_{\text{max}}$ in imipramine at 1 × 10⁻⁷ g/ml was smaller (6%) than the reduction of APD₅₀ (19%) or APD₉₀ (17%), but was nonetheless significant due to the consistency of this small change. The decreases in overshoot and action potential amplitude first became significant at 1 × 10⁻⁶ g/ml.

The effects of imipramine at 1 × 10⁻⁶ g/ml on action potentials in 18 Purkinje fibers driven at 60/min are shown in Table 1. Imipramine significantly reduced action potential duration at both 50% and 90% repolarization, maximum upstroke velocity, and overshoot. Activation voltage shifted by an average of +2.3 mV ($P < 0.025$), while maximum diastolic voltage shifted by an average of +1.4 mV ($P > 0.10$).

Figure 3 shows representative action potentials from the basic drive cycle (left panels) and from premature cycles (right panels). By varying the coupling interval of premature stimuli, we obtained premature action potential upstrokes throughout the later portion of phase 3 repolarization and also during phase 4 depolarization. From this spectrum of premature action potential upstrokes, we plotted (for the fiber shown in Fig. 3) $V_{\text{max}}$ of premature upstrokes against their activation voltage ("membrane activation voltage and maximum diastolic voltage; D: action potential duration at 50% repolarization (APD₅₀) and at 90% repolarization (APD₉₀). Two of the six fibers became refractory to external stimulation at imipramine 3 × 10⁻⁶ g/ml.

brane activation voltage and maximum diastolic voltage; D: action potential duration at 50% repolarization (APD₅₀) and at 90% repolarization (APD₉₀). Two of the six fibers became refractory to external stimulation at imipramine 3 × 10⁻⁶ g/ml.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Dose-response relationships in Purkinje fibers. A: Action potential amplitude; B: maximum upstroke velocity of phase 0 depolarization ($V_{\text{max}}$); C: membrane activation voltage and maximum diastolic voltage; D: action potential duration at 50% repolarization (APD₅₀) and at 90% repolarization (APD₉₀). Two of the six fibers became refractory to external stimulation at imipramine 3 × 10⁻⁶ g/ml.}
\end{figure}
TABLE 1  Effect of Imipramine (1 x 10^{-6} g/ml) on Purkinje Fiber and Ventricular Muscle Transmembrane Potentials

<table>
<thead>
<tr>
<th></th>
<th>Activiation voltage (mV)</th>
<th>Maximum diastolic voltage (mV)</th>
<th>Maximum overshoot (mV)</th>
<th>Maximum upstroke velocity (V/sec)</th>
<th>Action potential duration (50% repolarization) (msec)</th>
<th>Action potential duration (90% repolarization) (msec)</th>
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<tbody>
<tr>
<td>Purkinje fibers (n = 18)</td>
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<tr>
<td>Control</td>
<td>-83.1±4.2</td>
<td>-86.2±3.5</td>
<td>38.9±5.6</td>
<td>704±224</td>
<td>247±36</td>
<td>436±120</td>
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<tr>
<td>Imipramine</td>
<td>-80.8±5.3</td>
<td>-84.8±5.0</td>
<td>39.1±6.2</td>
<td>540±235</td>
<td>119±31</td>
<td>254±35</td>
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<tr>
<td>Ventricular muscle (n = 9)†</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Control</td>
<td>-77.4±4.5</td>
<td>-78.0±4.6</td>
<td>24.9±5.2</td>
<td>192±84</td>
<td>361±30</td>
<td>439±35</td>
</tr>
<tr>
<td>Imipramine</td>
<td>-76.2±6.3</td>
<td>-76.2±6.8</td>
<td>18.2±1.8</td>
<td>91±46</td>
<td>337±47</td>
<td>418±37</td>
</tr>
</tbody>
</table>

All values are means ± SD.
* Difference between control and imipramine significant at P < 0.05.
† Difference between control and imipramine significant at P < 0.01.
‡ Five calf and four sheep preparations. Calf action potentials longer than sheep (average: 117 msec at 50% and 84 msec at 90% repolarization; P < 0.05).

brane responsiveness") in Figure 4A. The V_max from upstrokes elicited during phase 3 are represented as unfilled symbols, and those elicited during phase 4 are shown as filled symbols. In control solution, premature upstrokes during phase 3 had only slightly lower V_max than upstrokes elicited at identical activation voltages during phase 4. Imipramine decreased V_max of phase 3 upstrokes much more than V_max of upstrokes elicited from the same activation voltage in phase 4 (see arrow, Fig. 4A). Figure 4B demonstrates the time dependence of this action. In all six responsiveness experiments, imipramine caused a comparable delay in the reactivation of V_max. Reactivation of V_max was so delayed that V_max of action potentials elicited at maximum diastolic voltage were smaller than those elicited at less negative voltages later in diastole (filled symbols at less negative voltages, Fig. 4A). In these experiments on membrane responsiveness, imipramine at 1 x 10^{-6} g/ml reduced peak V_max by 30 ± 4% and shifted V_h, the membrane voltage at half-maximal V_max, by -5.5 ± 0.5 mV. We also measured conduction velocity in five Purkinje fibers (three sheep, two calf) before and after exposure to a 1 x 10^{-6} g/ml concentration of imipramine. Imipramine slowed conduction in all five fibers, from 3.5 ± 0.4 to 2.4 ± 0.3 m/sec, a decrease of 32% (P < 0.02).

Effective Refractory Period and Minimum Drive Cycle

In six Purkinje fibers driven at 30/min, the mean effective refractory period decreased from 466 ± 119 to 292 ± 15 msec (P < 0.005) during superfusion with imipramine at 1 x 10^{-6} g/ml. The decrease in effective refractory period was due to the marked shortening of action potentials which occurred at long drive cycles. In these same six fibers, imipramine at 1 x 10^{-6} g/ml increased the minimum drive cycle from 253 ± 19 to 454 ± 95 msec (P < 0.005). The increase in minimum drive cycle despite a striking decrease in effective refractory period reflects two factors: (1) a cumulative delay in recovery of excitability at short drive cycles (see also Fig. 4) and (2) the minimal shortening of action potentials which occurred at short drive cycles.

Automaticity

Imipramine had no significant effect on the slope of spontaneous phase 4 depolarization in the 18 sheep Purkinje fibers in Table 1. The slope of phase 4 depolarization was ±5.3 ± 3.4 mV/sec in control solution and +5.4 ± 4.2 mV/sec during superfusion with imipramine at 1 x 10^{-6} g/ml (P > 0.80). The shorter Purkinje fiber action potential during exposure to imipramine resulted in a correspondingly
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+2.3 mV, P < 0.05) than in maximum diastolic voltage (mean +1.4 mV, P > 0.10).

To investigate the effect of imipramine on spontaneous automaticity, we established stable automatic firing in six sheep Purkinje fibers by exposing them to hypokalemia and isoproterenol (see Methods). After 1 hour of exposure to imipramine at $1 \times 10^{-7}$ g/ml, the spontaneous firing rate was unchanged (39 ± 4, control; 38 ± 6, imipramine). Exposure to imipramine at $1 \times 10^{-6}$ g/ml did not depress the magnitude of phase 4 depolarization before cessation of automatic firing, which occurred in all six preparations (e.g., Fig. 5).

The fact that imipramine stopped spontaneous firing before significantly depressing phase 4 depolarization suggested that imipramine caused a positive shift in the threshold voltage of Purkinje fibers. To test this hypothesis, we determined threshold current and voltage (see Methods) in five preparations (three calf, two sheep) before and after exposure to a $1 \times 10^{-6}$ g/ml concentration of imipramine. Imipramine increased threshold current by 34 ± 7% ($P < 0.01$) and shifted threshold voltage by +7.9 ± 2.5 mV ($P < 0.05$). Results of a representative experiment are shown in Figure 6. Imipramine did not alter the constant current-voltage relationship between resting voltage and threshold voltage in these six fibers. The positive shift of threshold voltage can fully account for the anti-automatic effect of imipramine.

**Ventricular Muscle**

Imipramine at $1 \times 10^{-6}$ g/ml reduced $V_{\text{max}}$ of nine ventricular muscle preparations (five calf, four large diastolic depolarization during the 1000-msec drive cycle. The increased magnitude of spontaneous phase 4 depolarization resulted in a slightly larger positive shift in activation voltage (mean

![Figure 4](http://circres.ahajournals.org/)

**Figure 4** A: Effects of imipramine on membrane responsiveness. Open symbols represent $V_{\text{max}}$ from action potential upstrokes elicited during action potential phase 3 (repolarization); filled symbols are $V_{\text{max}}$ from action potential upstrokes elicited during phase 4 (diastole). Arrow indicates activation voltage for points plotted from Figure 3 (same fiber). B: Time course for recovery of phase 0 $V_{\text{max}}$ (same fiber). Origin of time axis is when preceding action potential has repolarized to −65 mV. Delay in recovery of phase 0 $V_{\text{max}}$ during exposure to imipramine is responsible for "hook" configuration of responsiveness curve in Figure 4A.

![Figure 5](http://circres.ahajournals.org/)

**Figure 5** Effect of imipramine on normal automaticity in Purkinje fibers. A: Stable spontaneous automatic firing in control solution. B: After 1 hour of imipramine at $1 \times 10^{-7}$ g/ml, there was no significant change. C: After 18 minutes of imipramine at $1 \times 10^{-6}$ g/ml, regenerative upstrokes no longer occurred, even though membrane voltage became more positive than the spontaneous take-off voltages in panels A and B. One hour after wash-out of imipramine, stable automatic firing similar to panel A was again present.
sheep) driven at 60/min by 53%, compared to a 23% reduction of $V_{\text{max}}$ in Purkinje fibers (Table 1). Action potential amplitude and overshoot also fell significantly. In contrast to the decrease of Purkinje fiber action potential duration during exposure to imipramine, imipramine had no statistically significant effect on ventricular muscle action potential duration: APD$_{50}$ changed by $-23 \pm 23$ mV (range $-108$ to $+66$ msec) and APD$_{50}$ changed by $-21 \pm 26$ msec (range $-91$ to $+88$ msec) after 1 hour of exposure. Interestingly, the six preparations (five calf, one sheep) with the longest control action potentials showed abbreviation during exposure to imipramine, whereas the three preparations (all sheep) with the shortest action potentials showed prolongation during exposure to imipramine. Imipramine had no effect on activation voltage or threshold current and voltage. In each panel, top trace: transmembrane current; bottom trace: transmembrane voltage. During superfusion with imipramine, threshold current increased from 0.18 to 0.23 ma; threshold voltage was shifted by $+5$ mV.

**Action Potential Characteristics**

Imipramine at $1 \times 10^{-6}$ g/ml increased the minimum drive cycle length in these eight ventricular muscle preparations from 302 $\pm$ 32 msec to 610 $\pm$ 55 msec ($P < 0.01$), a 46% greater increase than seen in Purkinje fibers. As in the case of Purkinje fibers, the increase in minimum drive cycle reflects a cumulative delay in recovery of excitability at short drive cycles.

**Discussion**

**Action Potential Characteristics**

In Purkinje fibers, the action potential shortened at low imipramine concentrations. Previous workers reported little change in action potential duration of atrial or ventricular muscle fibers during exposure to tricyclic antidepressant drugs (Auclair et al., 1966; Matsuo, 1967). We also found no significant effect of imipramine on action potential duration of ventricular muscle, but did note that action potential shortening occurred in the six ventricular muscle preparations (five calf, one sheep) with the longest control action potentials, whereas action potential prolongation occurred in the three ventricular muscle preparations (all sheep) with the shortest control action potentials. These data raise the possibility of different imipramine effects between species as well as the possibility that imipramine may exert an antiarrhythmic effect by making the time course of ventricular refractoriness more uniform (Wittig et al., 1973). The small and variable changes in ventricular muscle APD during imipramine superfusion is consistent with reports stating that imipramine either does not change or else slightly prolongs the electrocardiographic QT interval (Bigger et al., 1978; Giardina et al., 1979).

The difference between the effect of imipramine on APD in Purkinje fibers and in ventricular muscle is an interesting and unexplained finding.

The ionic basis for the shortening of the Purkinje fiber action potential by imipramine has not been identified. If imipramine increased $g_{K1}$, the instantaneous potassium conductance (Noble, 1975), the slope and magnitude of phase 4 depolarization would decrease, contrary to our findings. On the other hand, if imipramine increased $i_{\text{si}}$, the major outward repolarizing current in Purkinje fibers (Noble, 1975), the action potential could shorten without any significant effect on phase 4 depolarization, due to rapid inactivation of $i_{\text{si}}$ at diastolic voltages. Also, a decrease in the slow inward current, $i_{\text{si}}$, could abbreviate the action potential without significantly affecting the slope of phase 4 depolarization, since $i_{\text{si}}$ is also inactivated at diastolic voltages. Further experiments will be needed to distinguish between these two mechanisms.

**$V_{\text{max}}$ and Conduction Velocity**

Imipramine reduced $V_{\text{max}}$ of upstrokes elicited late in phase 4, suggesting that it decreased the voltage-dependent availability of the early inward transient current. Imipramine reduced $V_{\text{max}}$ of upstrokes elicited during phase 3 much more than it depressed $V_{\text{max}}$ of upstrokes elicited from an identical membrane voltage during phase 4 (Figs. 3 and 4). Since imipramine did not accelerate terminal phase 3 repolarization, these data suggest that imipramine depressed $V_{\text{max}}$ of premature upstrokes by time- as well as by voltage-dependent effects. The striking increase in minimum drive cycle, which occurred in Purkinje fibers despite a shortening of the effective refractory period, is further evidence in favor of time-dependent depression of $V_{\text{max}}$. It is possible that imipramine decreased $V_{\text{max}}$ by increasing outward current rather than by decreasing the early inward transient current (Cohen and Sritchartz, 1977); however, the contribution of outward current is likely to be small under our experimental conditions (Hondegem, 1978; Walton and Fozzard, in press). Regardless of the cause, the depression of $V_{\text{max}}$ and conduction velocity by imipramine has important electrophysiological implications for
rhythm and conduction: depression of $V_{\text{max}}$ is the electrophysiological action most commonly associated with antiarrhythmic drug efficacy (Hoffman et al., 1975), and marked depression of $V_{\text{max}}$ and conduction velocity is associated with conduction block, a toxic electrophysiological effect (Singer et al., 1967).

**Automaticity**

Hypokalemia enhances Purkinje fiber pacemaker depolarization by reducing $g_{K1}$ and $g_{K2}$ (Noble, 1975) at transmembrane voltages between $-90 \text{ mV}$ and $-60 \text{ mV}$. Catecholamines shift the activation curve for $g_{K2}$ to less negative voltages (Tsien, 1974). Thus, automatic firing in Purkinje fibers exposed to potassium at $3.0 \text{ mM}$ and to isoproterenol at $1 \times 10^{-7} \text{ M}$ is due to enhanced “normal” pacemaker activity. The actions of imipramine on normal spontaneous automaticity in Purkinje fibers provide one of our most interesting experimental findings. Quinidine, procainamide, lidocaine, and phenytoin all decrease the slope of phase 4 depolarization (Hoffman et al., 1975; Bigger and Mandel, 1970; Bigger et al., 1968). In contrast, imipramine did not significantly depress phase 4 depolarization before automatic firing stopped. Imipramine suppressed automatic firing by shifting threshold voltage to a more positive value. The lack of phase 4 depression and the unchanged current-voltage relationship argue against an increase in outward current as the mechanism for the positive shift in threshold voltage by imipramine. The most likely explanation for the positive shift in threshold voltage is a decrease in the voltage-dependent magnitude of the early inward transient current during exposure to imipramine (cf. Fig. 4A).

High concentrations of quinidine and procainamide can depolarize cardiac Purkinje fibers and induce automatic firing (Weidmann, 1955). We did not observe this effect even during exposure to highly toxic imipramine concentrations (up to $3 \times 10^{-6} \text{ g/ml}$).

**Relationship of Electrophysiological Actions to Clinical Cardiac Effects**

One difficulty in extrapolation from these in vitro studies to the effects of imipramine in man is the extensive in vivo metabolism of imipramine to form desmethylinipramine (the major metabolite), 2-hydroximidipramine, and 2-hydroxidesmethylinipramine. Preliminary experiments in our laboratory suggest that these metabolites are qualitatively and quantitatively comparable to imipramine with regard to actions on the transmembrane potentials of superfused cardiac fibers; we previously have reported some of these observations (Weld et al., 1978).

Imipramine overdose provides a comparison of the clinical electrophysiological actions of imipramine and desmethylinipramine. In the early stages of imipramine overdose in man, plasma drug levels consist mainly of imipramine, whereas later during recovery from imipramine overdose, plasma drug is mainly desmethylinipramine (Spiker et al., 1975). Imipramine concentrations are reported as (imipramine + desmethylinipramine), and correlate closely with the electrocardiographic QRS duration throughout recovery from imipramine overdose (Spiker et al., 1975). These findings suggest that the clinical effects of imipramine and desmethylinipramine are comparable.

A second uncertainty in extrapolation from superfusate drug concentrations to in vivo plasma concentrations is the significance of plasma protein binding in man. In the case of imipramine, cardiac tissue concentrations are 30 or more times higher than steady state plasma concentrations (Perel et al., 1977), suggesting that cardiac tissue has a significantly higher affinity for imipramine than do plasma proteins. This finding suggests that plasma protein binding is a less important determinant of cardiac imipramine binding than the total (bound and unbound) plasma imipramine concentration. These considerations suggest that superfusate concentrations of imipramine should correlate fairly well to in vivo plasma concentrations with regard to electrophysiological effects.

At $1 \times 10^{-6} \text{ g/ml}$, imipramine depressed upstroke velocity by 23% in Purkinje fibers and by 53% in ventricular muscle. Conduction velocity in Purkinje fibers decreased by 26%. These electrophysiological findings explain the widening of the electrocardiographic QRS interval and the slowed conduction block in the His-Purkinje system seen in cases of tricyclic antidepressant drug overdose (Bigger et al., 1978; Spiker et al., 1975; Vohra et al., 1975; Kantor et al., 1975).

In view of the recent evidence that imipramine is effective against ventricular arrhythmias in man (Bigger et al., 1977; Giardina et al., 1979), it is interesting to speculate on the mechanisms of antiarrhythmic action of imipramine. In theory, a decrease in action potential duration could contribute to ventricular reentrant arrhythmias: abbreviation of the action potential might permit a shorter pathway to suffice as a reentrant circuit. In practice, some clinically useful antiarrhythmic drugs, including lidocaine (Arnsdorf and Bigger, 1972), phenytoin (Bigger et al., 1968), and mexiletine (Weld et al., 1979) do shorten the Purkinje fiber action potential. Bigger and Weld (1976) and Arnsdorf and Mehlman (1978) have suggested that normalization of repolarization in tissue with abnormally prolonged action potential plateaus could be antiarrhythmic. Wittig and coworkers (1973) have postulated that a shorter and more homogeneous action potential in the His-Purkinje system may result in more uniform distribution of refractoriness and thereby account for an antiarrhythmic effect.

Action potentials with low upstroke velocities may propagate slowly enough to generate reentrant
arrhythmias (Wit et al., 1972). Action potentials arising either prematurely or in depolarized tissues with partially depolarized resting transmembrane voltages have low upstroke velocities (Bigger and Weld, 1976), and may therefore participate in arrhythmogenesis. Since imipramine exerts a greater depressant effect on V_max from premature upstrokes and from upstrokes in partially depolarized tissue than its depressant effect on V_max from normal action potential upstrokes, imipramine could theoretically extinguish premature action potentials or those propagating in partially depolarized tissues, with insignificant effect on action potentials in normal tissues.

Since imipramine has potent central nervous system effects, it is quite possible that these effects can account for part of the antiarrhythmic action of imipramine in man. Although we have shown that imipramine has direct cellular electrophysiological effects similar to those of other antiarrhythmic drugs, these effects may not entirely account for its clinical antiarrhythmic efficacy.

The present experiments suggest that the cellular electrophysiological effects of imipramine are similar to those of several currently available antiarrhythmic agents. Recent studies indicate that imipramine is effective against ventricular arrhythmias in man at concentrations that are therapeutic for depression (Bigger et al., 1978; Giardina et al., 1979). Extensive clinical use of imipramine for treating depression suggests that, in therapeutic concentrations, imipramine will not be significantly cardiotoxic and will have fewer adverse extracardiac toxic effects than many currently available antiarrhythmic drugs. Also, imipramine has a long halftime of elimination (Bigger et al., 1978), a desirable feature which is unfortunately lacking in many antiarrhythmic drugs now in clinical use. In view of its potential advantages, imipramine deserves further clinical investigation to evaluate its potential in treating cardiac arrhythmias.

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POLYMORPHONUCLEAR leukocyte aggregation in the pulmonary vascular bed (pulmonary leukostasis) is known to occur during both hemodialysis and cardiopulmonary bypass (Craddock et al., 1977a, Bolanowski et al., 1977; de Bugh Daly et al., 1954; Neville et al., 1963; Kaplow and Goffinet, 1968; Toren et al., 1970). Such pulmonary leukostasis is associated with a marked but transient peripheral leukopenia, which usually recovers within 1 hour after onset of extracorporeal perfusion.

Craddock and his associates (1977a) have demonstrated that exposure of blood to dialyzer cellophane activates complement, with resulting peripheral leukopenia and pulmonary leukostasis. This mechanical obstruction of pulmonary capillaries with white cells was postulated by these investigators to be the most likely explanation for the pulmonary dysfunction observed following the onset of hemodialysis in patients, and for the rise in pulmonary artery pressure observed in sheep following infusion of autologous complement-activated plasma (Craddock et al., 1977a, 1977b, 1978).

We previously have observed a similar pulmonary response in sheep after the onset of venovenous membrane oxygenator perfusion using a silicone rubber oxygenator (Birek et al., 1976). However, our finding that pretreatment of the animal with sulfipyrazone abolished the pulmonary response without altering the leukopenic response cast doubt that leukostasis alone was an adequate explanation for the pulmonary response following complement activation. The present study was designed to investigate further the relationship between complement-mediated leukopenia and alterations in pulmonary vascular resistance and pulmonary gas exchange.

SUMMARY Pulmonary leukostasis, as a result of complement activation, has been invoked as a cause of pulmonary dysfunction. To investigate this phenomenon, we studied the pulmonary response to infusion of autologous complement-activated plasma in sheep and rabbits. Complement activation was produced by plasma incubation with zymosan. Leukopenia, with selective loss of polymorphonuclear leukocytes into the lungs, occurred in all animals immediately after the onset of plasma infusion. Complement-activated plasma infusion in sheep produced a significant fall in the arterial Po$_2$ and a marked rise in pulmonary vascular resistance, whereas no such effects were observed in rabbits. Pretreatment of the sheep with sulfipyrazone eliminated the pulmonary response to complement-activated plasma without altering the leukopenic response. Pulmonary histology in rabbits and sheep confirmed the presence of intracapillary leukostasis after the plasma infusions, whether or not sulfipyrazone had been administered previously. The pulmonary response to complement activation is associated with pulmonary capillary leukostasis, but leukostasis alone is not an adequate explanation of the phenomenon. Circ Res 46:175-180, 1980
Electrophysiological effects of imipramine on ovine cardiac Purkinje and ventricular muscle fibers.

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