Potassium channel blockade: A mechanism for suppressing ventricular fibrillation

ABSTRACT

The suppression of ventricular fibrillation by antidysrhythmic drugs is well correlated with their ability to block potassium channels in nerve and cardiac membranes. Blockade of potassium channels reduces electrical inhomogeneities in action potential and conduction parameters that lead to ventricular fibrillation. These actions tend to effectively decrease the electrical size of the heart, which suggests a mechanism for antifibrillatory drug action. The receptor sites for antifibrillatory drug action (I_K blockade) appear to be on the outside of the cardiac membrane whereas receptors for antiarrhythmic drug action (I_Na blockade) appear to be on the inside of the cardiac membrane.

Ventricular fibrillation, the major cause of sudden cardiac death, has long been regarded as an extension of less lethal ventricular arrhythmias that could be prevented by class I antidysrhythmic drugs (1). However, almost every controlled study has failed to show a reduction in the incidence of ventricular fibrillation by prophylactic administration of lidocaine even when arrhythmias per se were suppressed (2–11). In contrast, ventricular fibrillation can be suppressed by bretylium tosylate and its pharmacologic analog, bethanidine sulphate (2, 12–31). Both drugs (termed class 3) increase ventricular fibrillation threshold manyfold, facilitate electrical defibrillation, and induce episodes of pharmacologic (spontaneous) defibrillation under conditions where it does not normally occur (refs. 2, 12, 21–31 and Fig. 1a). Class 1 antidysrhythmic drugs such as lidocaine and procainamide do not have comparable antifibrillatory effects (12, 30, 31). These drugs also differ from bretylium and bethanidine in their effects on cardiac action potential parameters (33–37), which further indicates that cardiac and antiarrhythmic drug actions are not directly related. This report demonstrates that these differing drug actions are correlated with differences in the blockade of membrane ionic currents. Preliminary reports of these experiments have been published (38, 39, 44).

METHODS

Experiments were performed on internally perfused squid giant axons, chicken embryonic heart cell aggregates, and anesthetized mongrel dog hearts by using standard techniques for each preparation (12, 40–43).

Squid Axon. Membrane currents were measured by axial wire voltage-clamp technique in squid axon before and after addition of test drugs to either the internal or the external bathing medium. The voltage-clamp protocol consisted of serial depolarizations of membrane potential lasting several milliseconds from a holding potential of −80 mV (inside negative) to −50, −40, −30, ... +30 mV with a rest interval of 5 sec between each step. Drugs tested were bretylium, bethanidine, lidocaine, procainamide, meobentine, guanethidine, and cevimeline.

Chicken Heart Cells. Voltage-clamp measurements were performed on embryonic chicken heart cell aggregates before and after addition of bretylium to the external medium. Chick hearts were dissected from 7- to 12-day-old embryos and dissociated into their component cells with trypsin (0.05%). An inoculum of cells was placed into a flask containing tissue culture medium, which had been gassed with an atmosphere of 5% CO_2, 10% O_2, and 85% N_2. The flask was placed on a gyratory shaker for 48 to 72 hr. The temperature was maintained constant at 37°C. Aggregation of cells into spherical clusters occurs during this process. The clusters were transferred to a plastic culture dish to which they adhered within 1 hr. Mineral oil was layered over the medium to prevent evaporation and maintain an unclouded view of the cells. Temperature was maintained at 37°C, and pH was maintained at 7.3. Membrane currents were measured with a standard two-microelectrode voltage-clamp technique (43). Electrode resistance was typically 50 MΩ. Tetrodotoxin (1 μM) was used to block the fast sodium current component (I_Na). External potassium ion concentration was 1.3 mM. After control measurements were made, bretylium was added to the tissue culture dish at a final concentration of 1 mM.

Dog Heart. Electrical ventricular fibrillation threshold (VFT) was measured in the hearts of 68 open-chested dogs before and after intravenous drug infusion in each animal. A single constant current shock 10 msec in duration was applied during the vulnerable period of the cardiac cycle by means of fishhook electrodes embedded within the ventricle. The lowest current amplitude that induced sustained fibrillation lasting 30 sec, or longer, was taken as the VFT (12, 23, 29). Drugs used were bretylium, bethanidine, lidocaine, procainamide, meobentine, guanethidine, tetraethylammonium, tetramethylammonium, 4-aminopyridine, and cesium. The doses are given in Fig. 1 and Table 1.

RESULTS

In the dog heart bretylium and bethanidine each caused a significant sustained increase in VFT. In contrast, lidocaine typically induced a small transient increase in VFT and procainamide had virtually no effect (Fig. 1a).

Squid Axon. The effects of these drugs on voltage-clamped squid axons are shown in Fig. 2. In each case depolarizing voltage steps elicited an inward sodium current (I_Na) and an outward potassium current (I_K), similar to the original results of Hodgkin and Huxley (45). Bretylium, bethanidine,

Abbreviations: I, current; VFT, ventricular fibrillation threshold; APD, action potential duration.
Fibrillation Threshold. To further relate our observations in squid axon to dog heart, we tested the effects of tetraethylammonium (3 dogs), 4-aminopyridine (3 dogs), cesium (2 dogs), and tetramethylammonium (2 dogs) on fibrillation threshold. These diverse chemical agents, all of which are known blockers of potassium current in nerve and cardiac membranes (46–52), significantly increased VFT in dog hearts (Fig. 1b).

Adrenergic Blockade. Bretylium inhibits transmitter release from sympathetic neurons, which may suggest that adrenergic blockade is the mechanism for its antifibrillatory effect (34). Our results with guanethidine and meobentine argue against this view. Guanethidine, which has the same antiaadrenergic effect as bretylium, has little antifibrillatory effect (12, 53). Furthermore, meobentine, the O-methyl analog of bethanidine, has no antiaadrenergic action but it increases VFT significantly (ref. 54 and Table 1) and also induces pharmacologic defibrillation (unpublished observation, M.B.B.). The results with both drugs are well correlated with their effects on \( I_K \) in squid axons (Table 1). These experiments further indicate that blockade of \( I_K \) underlies antifibrillatory drug action.

Cardiac Cells. To test our prediction that bretylium would also block \( I_K \) in cardiac cells, voltage-clamp studies on chicken embryonic heart cell aggregates were carried out. When bretylium (1 mM) was placed in the external medium, outward current was significantly reduced as shown by the reduction in \( I_K \) at the end of the voltage steps (Fig. 4). In seven preparations bretylium consistently blocked both a time-dependent outward current and a time-independent (background) component. Blockade of the time-dependent outward current is especially evident by the reduction of the tail current amplitude (Fig. 4, Inset) upon return to the holding potential (−55 mV). Blockade of the background component is apparent in the reduction of the initial current jump immediately following the voltage step (Fig. 4, Inset). The time-dependent component has been demonstrated to be a potassium ion-selective current (A.S. and J.R.C., unpublished observations). The time-independent, or background component, is also carried at least partly by potassium ions (A.S. and J.R.C., unpublished observations).

Site of Action. Our data provide some insight into which side of the membrane the receptors are located for drugs that block sodium and potassium channels. Lidocaine and procainamide partially blocked \( I_{Na} \) and \( I_K \) current components when the drugs were added to the internal perfusing solution (Fig. 2 Table 1). The effects of these drugs on \( I_K \) are further illustrated in Fig. 3 along with the effects of tetraethylammonium, which is a standard potassium channel blocker. Tetrodotoxin was added to the external solution in these experiments to block the \( I_{Na} \) component. These results demonstrate that equimolar concentrations (5 mM) of bretylium and bethanidine produce a significantly greater blockade of \( I_K \) than either lidocaine or procainamide.

Table 1. Drug effects on membrane currents and on fibrillation threshold

<table>
<thead>
<tr>
<th>Drug</th>
<th>Block of ( I_K ) in squid axons, %</th>
<th>Increase of VFT in infarcted dog hearts, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>bretylium</td>
<td>87 88 89</td>
<td>504 (18)</td>
</tr>
<tr>
<td>bethanidine</td>
<td>70 75 72</td>
<td>327 (12)</td>
</tr>
<tr>
<td>meobentine</td>
<td>68 64 70</td>
<td>278 (3)‡</td>
</tr>
<tr>
<td>lidocaine</td>
<td>51 49 47</td>
<td>53 (12)</td>
</tr>
<tr>
<td>procainamide</td>
<td>41 36 33</td>
<td>17 (7)</td>
</tr>
<tr>
<td>guanethidine</td>
<td>32 40 46</td>
<td>14 (6)‡</td>
</tr>
</tbody>
</table>

*Drug concentration in the internal perfusate equal to 5 mM. Data taken from a total of 3 axons (a, b, and c) for each drug.

‡Average percentage increase. Parentheses, number of dogs tested. Drug concentration equal to 20 mg/kg (therapeutic dose) for bretylium, bethanidine, and meobentine. The doses for the other drugs are equivalent to the maximum doses used clinically (lidocaine, 7 mg/kg; procainamide, 9.4 mg/kg; and guanethidine, 15 mg/kg).

Normal hearts without coronary ligation.
failure to decrease upstroke velocity ($V_{\text{max}}$) of the normal cardiac action potential (33–35), which suggests that bretylium is also unable to cross the cardiac membrane. The lack of effect of external bretylium and bethanidine on $I_K$ in squid axons is attributable to the lack of an external receptor for most potassium channel blockers in this preparation, in contrast to other nerve and cardiac preparations where it is present (46, 47). Our results on chicken heart cell aggregates (Fig. 4) indicate that bretylium is, in fact, an effective blocker of $I_K$ components in cardiac membrane when the drug is added to the external solution. Failure of bretylium to reduce $V_{\text{max}}$ in cardiac cells suggests that the site for primary antiarrhythmic drug action ($I_{Na}$ blockade) is on the internal side of the cardiac membrane, which bretylium does not reach. In contrast, the primary site for antifibrillatory drug action ($I_K$ blockade) appears to be on the external side of the cardiac membrane, although effects on $I_K$ by drugs that cross the cardiac membrane to internal receptors are not excluded.

**DISCUSSION**

Ischemic injury results in asynchronous excitability states between normal and injured cardiac cells causing decreased conduction velocity, local conduction blocks, conduction over aberrant pathways, and decreased space constant (2,
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The control current-voltage relation inwardly rectified at $V > -30$ mV. Bretylium in the external medium significantly reduced outward current at potentials positive to $-40$ mV with a region of negative slope conductance positive to $V = -30$ mV. (Inset) Membrane currents in response to 5-sec duration voltage-clamp steps in control (a) and following addition of bretylium to the external medium (b). Holding potential was $-55$ mV. Step potential was $-25$ mV in (a) and $-22$ mV in (b). Time-dependent and time-independent (background) currents were elicited by the voltage step. The time-dependent component is most accurately represented by the amplitude of tail current following return to the holding potential. The time-independent component is most accurately represented by the instantaneous current jump immediately following the voltage step. Both currents were reduced by bretylium. Similar results were observed in seven preparations. Control, solid squares; 1 mM bretylium, open diamonds.

55–59). These effects can cause ventricular fibrillation when the disruption of normal ion transport causes action potential and conduction inhomogeneities that critically desynchronize normal excitation and contraction coupling over a sufficient mass of myocardium (2).

It has long been known that sustained fibrillation is related to the absolute size of the heart (32). For example, ventricular fibrillation usually terminates spontaneously in the hearts of small animals (cat, rat, and rabbit). In large hearts (dog, beef, and man) ventricular fibrillation persists until death unless terminated by electrical countershock, because the large number of cells and long conduction paths make spontaneous electrical and contractile resynchronization improbable. Similarly, the ischemic or infarcted myocardium can become effectively "enlarged" electrophysiologically because of abnormal and aberrant conduction characteristics caused by action potential inhomogeneities. Bretylium has been shown to decrease such inhomogeneities in conduction times between normal and ischemic myocardium (58). Thus, the large heart or the ischemically injured heart may take on some characteristics of the small heart following administration of bretylium, bethanidine, or meobentine, as indicated by episodes of pharmacologic defibrillation and an increase in VFT to supernormal levels.

The blockade of $I_K$ explains the most consistent electrophysiologic action of bretylium, which is to increase the action potential duration (APD) (33–35). Bethanidine and meobentine have also been reported to have similar effects on APD in rat heart (37). Moreover, clofilium, another class 3 antiarrhythmic drug, has been reported to block $I_K$ in guinea pig heart cells (60). Blockade of $I_K$ could exert an important antiarrhythmic action by blocking an increase in potassium conductance (61) with shortening of APD that often immediately precedes the onset of fibrillation (61, 62). In ischemic or infarcted myocardium, bretylium prolongs APD in both the uninvolved normal cells and in injured cells with abnormally shortened APD so that duration is prolonged about equally in both groups (63). Since bretylium has little effect on APD in ischemic injured cells where it is already abnormally prolonged, the net effect is a reduction in the temporal and spatial disparity of APDs between normal and injured cells throughout the heart (33). Increased homogeneity of both APD and conduction parameters (58) would result in more synchronous and uniform coupling of excitation and contraction. These actions tend to effectively reduce the electrical size of the heart, which could explain the increased resistance to initiating and sustaining fibrillation induced by antiarrhythmic drugs. Our results suggest that one important mechanism underlying this effect is the blockade of one or more potassium ion currents.

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