Effects of Agents which Inhibit the Slow Channel on Sinus Node Automaticity and Atrioventricular Conduction in the Dog

By Douglas P. Zipes and John C. Fischer

ABSTRACT
A slow ionic current carried by calcium, sodium, or both constitutes transmembrane ionic flow through the slow channel; such a current may be involved in normal action potentials of sinus and atrioventricular (AV) nodal cells. In this study, we investigated the effects of the slow-channel inhibiting agents verapamil, D600, manganous chloride, and lanthanum chloride on sinus node automaticity and AV nodal conduction in open-chest dogs treated with atropine (0.5 mg/kg) and propranolol (1.0 mg/kg). The arteries to the sinus node and the AV node were cannulated and perfused with agents that inhibit the slow current. These agents slowed sinus node discharge rate, depressed AV nodal conduction, and lengthened the effective and the functional AV nodal refractory period. Effects were dose related and reversed with time. His-Purkinje conduction remained normal. Isoproterenol and epinephrine reversed the effects of slow-channel inhibiting agents, but calcium, sodium, glucagon, and phenylephrine did not. Concentrations of propranolol which produced beta-receptor blockade prevented isoproterenol-induced reversal of the effects of slow-channel inhibitors. We concluded that (1) agents which inhibit the slow channel directly depress sinus node discharge rate and AV nodal conduction, (2) effects of slow-channel inhibiting agents are not mediated through the activation of cholinergic discharge or inhibition of adrenergic discharge, and (3) beta-receptor stimulation reverses these effects.

KEY WORDS
manganese     verapamil     sodium     slow ionic current     D600     calcium

Paes de Carvalho et al. (1, 2) have suggested that the cardiac action potential might be composed of both a fast and a slow component. Recent experimental evidence supports the view that a fast initial inward sodium (Na+) current (3) is responsible for the upstroke and the spike of the action potential in atrium and ventricle and a second inward current, which may be carried by Na+ ions, calcium (Ca2+) ions, or both, is responsible for the plateau (4-9). Both currents are voltage and time dependent, but the fast current is (1) rapidly activated and inactivated, (2) dependent on extracellular sodium concentration, (3) abolished by the application of tetrodotoxin (TTX), and (4) inactivated by sustained depolarization. The second inward current, designated the slow current, involves transmembrane ionic flow through the slow channel (6-8) and is (1) activated and inactivated more slowly than the fast inward current, (2) relatively insensitive to TTX but is abolished by manganese (Mn2+) (6-8), verapamil (10), D600 (10), and lanthanum (LaCl3) (9, 11, 12), (3) sensitive to the external calcium concentration (13), and (4) activated at a threshold about 20 mv positive to that of the fast inward current (14-16). Additional separate channels regulating ionic movement through the cell membrane have been identified in cardiac action potentials (17), and analogous separate channels have also been demonstrated in nervous tissue (18).

The distinctive characteristic of action potentials recorded from cells in the sinus node or the midportion of the atrioventricular (AV) node is their slowly rising depolarization phase. Paes de Carvalho et al. (1, 2) have postulated that the ionic mechanisms responsible for the regenerative responses in AV nodal tissue differ from the ionic mechanisms responsible for regenerative responses in atrial and ventricular muscle and in the specialized ventricular conducting system in which the voltage change (dV/dt) is rapid.

Action potentials obtained in sodium-free, calcium-rich solutions (19) and after various conditions...
which produce slow conduction (20) possess electrophysiological features that are found in the action potentials of the normal sinus node and the AV node. In addition to strikingly similar contours, summation and prolonged recovery of excitability beyond full repolarization have been shown to occur in these depressed fibers (20) and in the normal AV node (21, 22). Lenfant et al. (23) have demonstrated in the isolated rabbit atrium that MnCl₂ suppresses action potentials in the sinus node but not in the atrial muscle and that TTX suppresses action potentials in the atrial muscle but not in the sinus node. Recently, Zipes and Mendez (24) have shown in the isolated rabbit AV node preparation that MnCl₂ suppresses AV nodal conduction without blocking conduction in the atrium or the His bundle and that TTX suppresses activity in the atrium and the His bundle but not in the AV node.

All of these observations suggest the possibility that the slow current may be responsible for the normal depolarization process in the sinus node and the AV node. Such a conclusion would help to explain the apparent resistance of these cells to the depolarizing effects of elevated potassium (25).

If the slow inward current is responsible for the normal depolarization process in cells of the sinus node and the AV node, then agents which suppress this slow tonic current might depress sinus node automaticity and AV nodal conduction. Agents which increase the slow current should reverse the effects of the slow-channel inhibitors. The purpose of this study was to test this hypothesis in vivo by selectively perfusing regions of the sinus node and the AV node with drugs that inhibit or enhance the slow inward current.

Methods

Healthy mongrel dogs of either sex weighing 15–30 kg were anesthetized with sodium pentobarbital (30 mg/kg, iv). Following intubation and ventilation with a Harvard respirator at a rate and volume predicted by a nomogram, the chest was opened along the midline, and the heart was suspended in a pericardial sling. The arterial blood pressure was monitored through an indwelling catheter placed in the femoral or the carotid artery. Tracings were displayed on a switched-beam oscilloscope (DB8 Electronics for Medicine) using filter settings between 40 Hz and 500 Hz and were recorded on photographic paper at speeds of 100 mm/sec. Measurements were made from the tracings on the photographic paper.

The branch of the right coronary artery that supplied the region of the sinus node under study (26) and the posterior septal artery that perfused the region of the AV node under study (27) were dissected free from the epicardium and cannulated with polyethylene tubing (Deseret, 22 gauge). Proper cannula placement was determined by injecting acetycholine (2 ml, 0.1 μg/ml) into the artery. Arrest of sinus node discharge or supra-His AV block resulted. To prevent clotting in the cannula, systemic anticoagulation was achieved with heparin (3 mg/kg) in about 50% of the dogs; but in the remaining dogs, the cannula was kept patent by periodically flushing between testing with freshly drawn arterial blood and a small amount of heparin.

Agents that inhibited the slow current produced an effect whether or not the autonomic nervous system was blocked. However, in the experiments reported, immediately prior to testing, dogs were vagotomized and treated with atropine (0.5 mg/kg, iv, and 20 μg into the artery supplying the node under study) and propranolol (1–1.5 mg/kg, iv, and 20 μg into the artery supplying the node under study). Adequate cholinergic and adrenergic blockade was demonstrated by the failure of maximal vagosympathetic trunk and stellate ganglion stimulation or of acetycholine (2 ml, 0.1–1 μg/ml) and isoproterenol (2 ml, 0.1 μg/ml) administered in the artery supplying the node to alter the rate of sinus node discharge or AV nodal conduction.

All drugs given intra-arterially were administered in the following fashion. The final concentration was made by mixing 1 ml of the drug dissolved in normal saline with 9 ml of freshly aspirated arterial blood; 2 ml of this final test solution was injected into the nodal artery over 30 seconds, followed by a 2-ml flush injection of fresh arterial blood without the drug over 30 seconds. The slow-channel inhibiting drugs verapamil, D600 (a methoxy derivative of verapamil), lanthanum chloride (LaCl₃) and manganese chloride (MnCl₂) were administered at various concentrations (see Results). Isoproterenol (2 ml) was administered at a concentration of 0.1 μg/ml or 1.0 μg/ml. The concentrations of other drugs tested are indicated in the Results.

During studies on sinus node automaticity, the cycle length of the spontaneous sinus rate was measured at
various intervals beginning 30 seconds after the flush injection had been completed to avoid the pressure effects (28) of the injection. The first measurement is indicated as time zero in Figures 1 and 2. The reversing effects of isoproterenol were evaluated by administering the drug during the peak lengthening of the cycle length caused by the slow-channel inhibiting agent. Atrial activity was considered to originate in the region of the sinus node and thus represent sinus node cycle length as a measure of sinus node automaticity when the recording electrode situated in the sinus node area registers the earliest recordable atrial activity. Conceivably, diverse mechanisms such as sinus node exit block could account for the changes in atrial cycle length and, without an intracellular recording of sinus node potentials, we can only be absolutely certain that the spontaneous atrial rate decreased.

During evaluation of AV nodal conduction, the right atrium was paced at a fixed cycle length (A1) with a bipolar electrode placed in the region of the sinus node or the atrial appendage. A premature atrial stimulus (A2) was delivered at progressively earlier intervals following each last stimulus of the basic train of eight or ten regularly delivered stimuli. Appropriate curves relating the response of His bundle activity (H1-H2) to the A1-A2 interval were plotted. The effective refractory period of the AV node was defined as the longest A1-A2 interval at which A2 failed to conduct to the His bundle. The functional refractory period of the AV node was defined as the minimum H1-H2 interval that conducted from the atrium. The A-H interval was measured from the onset of the first rapid atrial deflection (A) to the first rapid His bundle deflection (H) recorded in the catheter electrode monitoring His bundle activity. The V-H interval was measured from the onset of the His deflection to the beginning of ventricular depolarization recorded in either the His bundle lead or the standard lead II electrocardiogram.

AV nodal conduction was tested 2 minutes after the completion of the flush injection and then periodically until normal AV nodal conduction returned or conduction remained stable. Isoproterenol was injected at times of peak depression in AV nodal conduction, and conduction through the node was tested periodically.

At the conclusion of each experiment, the heart was removed from the chest, and crystal violet was injected into the artery supplying the node under study. In each acceptable experiment, a deep blue color stained that region of the node, thus verifying the anatomic site that was perfused.

**Results**

In all dogs tested, verapamil, D600, MnCl2, and LaCl3 impaired AV nodal conduction and, in sufficient concentrations, resulted in AV nodal conduction and, in sufficient concentrations, resulting in a two-to-one or Wenckebach supra-His AV block during the basic driving cycles (Table 2). The values reported in Table 2 were averaged from data recorded 2 minutes after the flush injection. A single representative example illustrating the time course for each agent is shown (Figs. 3–6). AV nodal conduction improved toward normal control values with time, but a degree of impaired conduction sometimes remained (Fig. 5). Isoproterenol reversed the effects of the slow-channel inhibiting agents (Figs. 3 and 4).

None of the slow-channel inhibitors prolonged the H-V interval in any dog. Distal His block was never demonstrated, and the His electrogram and the QRS complex did not alter significantly.

**REVERSAL OF THE EFFECTS OF SLOW-CHANNEL INHIBITING AGENTS**

Isoproterenol (2 ml, 0.1 \( \mu g/ml \), ia) reversed the effects of slow-channel inhibiting agents in the absence of beta-receptor blockade. If propranolol prevented isoproterenol (0.2 \( \mu g \)) from exerting an effect in the control state, this dose of isoproterenol also failed to reverse the effects of the slow-channel inhibitors. However, propranolol (1.0–1.5 mg/kg) failed to prevent isoproterenol (2 ml, 1.0 \( \mu g/ml \), ia) from exerting an effect in the control state, and this concentration of isoproterenol reversed the effects of slow-channel inhibiting agents by in-
Effects of Slow-Channel Inhibiting Agents on Sinus Node Cycle Length

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (m)</th>
<th>No. of exp.</th>
<th>Average sinus slowing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verapamil</td>
<td>(1 \times 10^{-7})</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>(1 \times 10^{-6})</td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>(1 \times 10^{-5})</td>
<td>7</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>D600</td>
<td>(1 \times 10^{-7})</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>(1 \times 10^{-6})</td>
<td>8</td>
<td>&gt; 68</td>
</tr>
<tr>
<td></td>
<td>(1 \times 10^{-5})</td>
<td>1</td>
<td>200</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>(1 \times 10^{-3})</td>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>(5 \times 10^{-3})</td>
<td>1</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>(1 \times 10^{-2})</td>
<td>3</td>
<td>&gt; 105 in 2 experiments; total arrest for 31 minutes in 1 experiment</td>
</tr>
<tr>
<td>LaCl₃</td>
<td>(1 \times 10^{-4})</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>(1 \times 10^{-3})</td>
<td>4</td>
<td>&gt; 71</td>
</tr>
<tr>
<td></td>
<td>(1 \times 10^{-2})</td>
<td>1</td>
<td>83</td>
</tr>
</tbody>
</table>

Increasing the spontaneous discharge rate of the sinus node or by improving AV nodal conduction. Intra-arterial infusion of calcium gluconate or calcium chloride (2 ml, 1 mg/ml) and normal saline failed to reverse the effects of the slow-channel inhibiting agents. Higher doses of Ca²⁺ depressed sinus node automaticity or AV nodal conduction or produced atrial or ventricular fibrillation, depending on whether the drug was infused into the sinus or the posterior septal artery. In a few of the dogs that were tested, epinephrine (2 ml, 0.1 mg/ml, ia) reversed the effects of the slow-channel inhibiting agents; less concentrated amounts of epinephrine were not used. Phenylephrine (2 ml, 0.1 μg/ml, ia) and glucagon (2 ml, 0.1 mg/ml, ia) failed to reverse the effects of these agents. Phentolamine (5 ml, 1 mg/ml, iv) failed to block the reversal produced by isoproterenol.

Although we did not systematically evaluate the alterations in contractility that the slow-channel in-
Effects on the spontaneous sinus node cycle length of three infusions into the sinus node artery of manganous chloride (MnCl₂) at concentrations of 1 × 10⁻⁵ M, 1 × 10⁻⁴ M, and 1 × 10⁻³ M.

Habiting agents produced, it was clear that electrophysiological effects of these agents did not necessarily parallel effects on contractility. The systemic arterial blood pressure could be normal at a time when AV block was produced; conversely, AV conduction could have returned to normal when the systolic pressure was only 90 mm Hg.

TABLE 2
Effects of Slow-Channel Inhibiting Agents on Atrioventricular Conduction

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (M)</th>
<th>No. of expt.</th>
<th>Average increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Effective refractory period</td>
</tr>
<tr>
<td>Verapamil</td>
<td>10⁻⁷</td>
<td>3</td>
<td>&gt; 24</td>
</tr>
<tr>
<td></td>
<td>10⁻⁶</td>
<td>3</td>
<td>&gt; 48</td>
</tr>
<tr>
<td></td>
<td>10⁻⁵</td>
<td>3</td>
<td>&gt; 102</td>
</tr>
<tr>
<td></td>
<td>10⁻⁴</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>10⁻³</td>
<td>5</td>
<td>&gt; 41</td>
</tr>
<tr>
<td></td>
<td>10⁻²</td>
<td>1</td>
<td>&gt; 48</td>
</tr>
<tr>
<td>D600</td>
<td>10⁻⁷</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>10⁻³</td>
<td>5</td>
<td>&gt; 29</td>
</tr>
<tr>
<td></td>
<td>10⁻²</td>
<td>3</td>
<td>&gt; 69</td>
</tr>
<tr>
<td>LaCl₃</td>
<td>10⁻⁶</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>10⁻⁴</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>5 × 10⁻³</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>10⁻²</td>
<td>1</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

When the test drug produced second degree AV block in the basic cycles, the percent increase in the effective refractory period and the functional refractory period was arbitrarily assigned a value of 100%. If the control effective refractory period and functional refractory period were less than the refractory period of atrial muscle, the percent increase was calculated using the control value obtained for atrial muscle refractory period. This method of calculation reduced the percent increase and those values are indicated by >.
Discussion

The results of this study clearly establish that agents which inhibit the slow channel in tissue bath preparations depress sinus node automaticity and impair AV nodal conduction of hearts in situ. The ionic mechanisms responsible for these effects can only be conjectured from this investigation. However, the fact that four different drugs, which inhibit transmembrane ionic flow through the slow channel without affecting the rapid Na\(^+\) channel, produced similar results supports the possibility that the suppression of the slow current accounted for these effects and that the generation of normal action potentials in the sinus node and the AV node depends on the slow ionic current carried by Na\(^+\), Ca\(^{2+}\), or both (24). A recent in vivo study (29) provides support for this conclusion by demonstrating the failure of TTX to directly affect the sinus node or the AV node.

An increase in the maximum diastolic potential, a decrease in the level of the threshold potential, a decrease in the rate of diastolic depolarization, or a combination of these factors may depress automaticity of the sinus node. Lenfant et al. (23) found that, after exposure of the rabbit atrium to Mn\(^{2+}\), the rhythm slowed markedly and, in 20–25 minutes, all spontaneous electrical activity disappeared. Aronson and Cranefield (19) observed spontaneous diastolic depolarization in the absence of Na\(^+\), suggesting that, under certain circumstances, Ca\(^{2+}\) can carry the current necessary for pacemaker activity. Blocking the slow channel in sinus node cells should slow or suppress spontaneous diastolic depolarization if the slow channel is important for pacemaker activity in these cells.
Zipes and Mendez (24) demonstrated that Mn²⁺ initially diminished the dV/dt of cells located within the AV node until only local subthreshold responses occurred; resting membrane potential remained unaltered. These changes explain the AV nodal conduction delay and the eventual AV nodal block observed in vivo after the administration of slow-channel inhibitors, but they may be insufficient to explain the prolongation of refractoriness. Refractoriness outlasts repolarization in normal AV nodal cells (22) and in cells manifesting very slow conduction (20). This feature may be a characteristic of cells dependent on a slow current that is activated and inactivated more slowly than the fast current. Conceivably, slow-channel inhibitors might lengthen refractoriness by further delaying reactivation of the slow current. Under these circumstances, refractoriness might considerably exceed repolarization. Other mechanisms may be operative, but additional speculation on the basis of the present study is unwarranted.

Without histological study, we cannot completely eliminate the possibility that slow-channel inhibiting agents directly injured nodal tissue, because, following repeated injections, the node often became discolored. Therefore, the reported data were derived from initial injections performed immediately after cannulation of the artery. A return to normal values with time in many experiments or after isoproterenol administration in virtually all experiments refutes the presence of irreversible cellular damage. In vitro studies support the reversibility of these agents.

Zipes and Mendez (24) demonstrated that epinephrine restored activity in rabbit atria that had been rendered inexcitable by TTX. Because of the difficulty of maintaining an impediment in the midportion (N region) of the rabbit AV node after epinephrine administration, these authors (24) were unable to conclusively show the effects of epinephrine on N region action potentials. Epinephrine increased the dV/dt of N cells and reversed Mn²⁺-induced AV block (unpublished observations). The present study supports the observation that catecholamines (isoproterenol and epinephrine) reverse the effects of the slow-channel inhibiting agents in vitro (10).

In the present in vivo study, calcium chloride or calcium gluconate (2 ml, 1 mg/ml) failed to reverse the effects of the slow-channel inhibiting agents and, in some instances, appeared to increase sinus slowing or AV block. Larger amounts of Ca²⁺ usually produced atrial or ventricular fibrillation.

A previous study (32) reported the effects obtained after infusing verapamil (18 μg/kg min⁻¹) into the canine right coronary artery after ligating “all branches except those supplying the sinus node and interventricular septum.” Sinus slowing resulted after a 4–8-minute infusion of about 1,000 times the amount of verapamil we found effective. Impairment of AV nodal conduction was found “in some experiments,” but the author (32) felt that the AV node was less sensitive to the effects of verapamil than was the sinoatrial node probably because of the failure to directly perfuse the AV node. In the dog, the arterial supply to the AV node is provided jointly by two branches of the left, but not the right, coronary artery (27). Without a His bundle recording, the site of block could not be determined in that study (32). No mention was made of the possible slow-channel inhibiting role played by verapamil.

The site of AV block produced by Mn²⁺ in vitro (24) and by verapamil, D600, MnCl₂, and LaCl₃ in vivo is at the AV node. Although Mn²⁺ reduced the rate of rise and produced some distortion of the intracellular His bundle action potential in vitro (24), the H–V interval in vivo remained constant after perfusion of the AV nodal artery with all of the slow-channel inhibiting agents. Certainly, the distal His-Purkinje system did not receive high concentrations of the slow-channel inhibiting agents with this method of arterial perfusion, which may partly account for the unchanged H–V interval. Even after spillover of the slow-channel inhibitors into the systemic circulation when repeated doses were injected into the nodal arteries, the H–V interval remained normal. Direct perfusion of the His-Purkinje system is required to conclusively eliminate a significant effect of slow-channel inhibiting agents on this portion of the conduction system.

Verapamil has received both animal and clinical trial as an antiarrhythmic agent and as a coronary vasodilator for patients with angina pectoris (33–40). Although verapamil seems to be fairly well tolerated intravenously and orally, the slow-channel inhibitors do possess a negative inotropic effect which may produce detrimental
hemodynamic effects. Verapamil slows the sinus rate, decreases the ventricular response to atrial fibrillation, and is effective against various arrhythmias (41). It is possible that verapamil and similarly acting drugs will provide an alternate therapeutic approach to some arrhythmias. These drugs may be beneficial in situations that require slowing of the sinus rate or additional AV nodal conduction delay. Also, if the normal mechanism for depolarization in some cells involves positive currents moving through the slow channel, it is reasonable that this mechanism may go awry and cause arrhythmias. Recent investigations involving ouabain-induced arrhythmias demonstrated transient depolarizations (42) (low-amplitude potentials [43]); conceivably these depolarizations could have been generated via the slow channel. Observations from our laboratory (44) and other studies (35, 37, 38) have shown the effectiveness of verapamil in eliminating some digitalis-induced arrhythmias. Similar results have recently been obtained with nanganese (45).

Another area of clinical concern in which more effective antiarrhythmic agents are needed involves patients with Wolff-Parkinson-White syndrome. However, if bypass tracts in these patients are composed of atrial muscle dependent on a rapid Na+ channel, verapamil probably will not significantly affect conduction over the anomalous pathway. Providing additional block at the AV node might be helpful in some instances.

The concentrations at which the slow-channel inhibiting agents produced sinus slowing or AV block in this study can only be regarded as estimates of the effective dose range. Although selective cannulation of one branch of the arterial supply does not produce gross changes in the physiological parameters of the sinus node and the AV node for some hours because of collateral circulation (26, 27), ischemia (46), trauma resulting from the repeated infusions, and effects of drug accumulation are probably unavoidable. The extent to which these factors alter sinus and AV node physiology varies from one preparation to another, particularly as the collateral blood supply to the node varies. Thus, a strict comparison of one experiment with another is not entirely valid. However, these handicaps do not negate the basic conclusions of these experiments. (1) A dose could be reached for all of the slow-channel inhibiting agents which would slow the spontaneous discharge rate of the sinus node; impair AV nodal conduction, and lengthen AV nodal refractory period; this dose was similar to concentrations which effectively and selectively block ionic flow in the slow channel in vitro. (2) The effects of the slow-channel inhibiting agents were not mediated through activation of the cholinergic nervous system or inhibition of the adrenergic nervous system. (3) Beta-receptor stimulation reversed the effects of the slow-channel inhibiting agents.

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