Effects of Acetylcholine on the Ventricular Specialized Conducting System of Neonatal and Adult Dogs

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SUMMARY We determined the effects of acetylcholine on automaticity of isolated cardiac Purkinje fibers from neonatal and adult dogs and on the idioventricular rhythm of adult dogs with complete atrioventricular block. Isolated Purkinje fibers were studied with standard microelectrode techniques during superfusion with Tyrode's solution at 37°C. For both age groups, spontaneous rate was decreased by acetylcholine, an effect which was reduced by atropine. The magnitude of the effect is equal in both neonatal and adult dogs. The negative chronotropic effect of acetylcholine was not prevented by phentolamine, indicating that an α-adrenergic mechanism was not involved. The idioventricular rate of conscious dogs with formalin-induced heart block was decreased by administration of acetylcholine. The effect was augmented by propranolol and attenuated by atropine. Thus, for both the in vitro and in situ ventricular specialized conducting system, acetylcholine decreases automaticity presumably through combination with a muscarinic receptor.

THE effects of vagal stimulation on heart rate of the intact animal and of acetylcholine (ACh) on the electrophysiology and automaticity of isolated sinus node and atrial fibers have been described in great detail.1-4 For mammals, it generally is agreed that the vagus, through the action of its neurotransmitter, ACh, slows sinus rate and decreases automaticity of other specialized atrial fibers by increasing maximum diastolic potential and decreasing the slope of phase 4 depolarization.3,4 In addition, the voltage-time course of repolarization is accelerated3,4 and resting membrane potential may be increased.4 These effects of ACh have been attributed to an increase in potassium conductance.4 The ACh-induced changes in sinus node and atrial resting and action potential and automaticity are blocked by atropine.

There is far less agreement on the effects of ACh on the electrical activity of the ventricular specialized conduction system. Studies of isolated tissues indicated that ACh has no significant effect on the normal Purkinje fiber action potential or automaticity.3 However, more recent studies have suggested that ACh does in fact decrease automaticity in the proximal5 and distal6 ventricular conducting system. Bailey et al.5 found that His-Purkinje preparations were hyperpolarized by ACh and that action potential amplitude, maximum rate of rise of phase 0, and conduction velocity were concomitantly increased (presumably as a result of increased membrane potential). The rate of spontaneous impulse initiation of these preparations was decreased by ACh. Tse et al.6 similarly demonstrated a negative chronotropic effect of ACh on canine Purkinje fibers resulting from a decrease in the slope of phase 4 depolarization and an increase in maximum diastolic potential. In neither study were dose-response relationships presented.

In a study of the effects of vagal stimulation on idioventricular rate in intact dogs, Eliakim et al.7 demonstrated a negative chronotropic effect of ACh as well as vagal stimulation. ACh also has been shown to decrease idioventricular rate in isolated rabbit hearts.8 Complete data on dose-response relationships are also lacking for these studies, however.

It was our purpose in this study to determine the extent to which ACh can modify automaticity and transmembrane potential characteristics of normal Purkinje fibers from adult and neonatal dogs. The study of adult animals was undertaken to attempt to settle the controversy between the older and more recent literature concerning the presence and magnitude of effect of ACh on Purkinje fibers. This was done by determining the dose-response relationships for ACh on automaticity of Purkinje fibers and on ventricular automaticity in the intact ventricle in dogs with heart block. Whereas earlier studies using one or two concentrations of ACh indicated either the presence or the absence of an effect, the dose-response determination permits a more careful description of that effect. The study of neonatal animals represents a continuation of our work on age-related changes in autonomic responsiveness. In a prior study, we found marked
differences in adrenergic responsiveness of Purkinje fibers from neonatal and adult dogs. In the present study, we determined the effects of ACh on Purkinje fibers from neonates as well as adult dogs to ascertain whether or not there were age-related differences in responsiveness to ACh.

Finally, to determine dose-response relationships for ACh in the intact animal and the effects of ACh on automaticity of Purkinje fibers in situ, we studied effects of ACh on the idioventricular rate of conscious adult dogs with complete atrioventricular block resulting from formalin injection into the His bundle.

**Methods**

**Cellular Electrophysiological Studies**

Adult (1-9 years) and neonatal (0-10 day) mongrel dogs were anesthetized with sodium pentobarbital, 30 mg/kg, iv, and the heart was removed through a lateral thoracotomy (adults) or midline sternotomy (neonates). Purkinje fibers were excised rapidly from both ventricles and immersed in Tyrode's solution containing (mmol/liter) NaCl, 137; NaHCO3, 12; CaCl2, 2.7; MaHPO4, 1.8; KCl, 4.0; MgCl2, 0.5; and dextrose, 5.5. Purkinje fiber bundles were trimmed of ventricular muscle, mounted in an acrylic tissue chamber, and superfused with Tyrode's solution which was equilibrated constantly with 5% CO2 in oxygen. Flow rate of the superfusing solution was 10-12 ml/min; temperature = 37.5 ± 0.5°C. Purkinje fiber bundles were stimulated at a cycle length of 500 msec through Teflon-coated bipolar silver wire electrodes using techniques previously described. As described below, the stimulus was discontinued intermittently for determination of automaticity. Fibers were impaled with 3 M KCl-filled glass capillary microelectrodes having tip diameters < 1 μm and resistances of 8-22 MΩ. The sites at which microelectrode impalements were made and maintained were the areas of least vigorous contraction. A silver-silver chloride junction was used to couple the microelectrodes to a preamplifier with high input impedance and input capacity neutralization; the signal was displayed on a cathode ray oscilloscope (Tektronix 565) and on a Gould Brush 220 chart recorder. The tissue chamber was continuous with ground through a 3 M KCl-Ag-AgCl junction. The methods used to calibrate the recording equipment have been described previously.

All Purkinje fiber preparations were stimulated, impaled with microelectrodes, and allowed to equilibrate for 1 hour before control measurements were made. These measurements were: maximum diastolic potential (MDP), measured from the level of zero potential to the point of maximum electro-negativity; action potential amplitude, measured from the MDP to the peak of the overshoot; action potential duration measured to 50% (APD50) and 100% (APD100) repolarization; the maximum rate of rise of phase 0 of the action potential (Vmax).

The method for the study of automaticity has been described in detail. For each preparation, the drive stimulus was discontinued and escape time (measured from the upstroke of the last driven action potential to that of the first spontaneous one) and the ensuing spontaneous rate and rhythm were recorded on the Brush recorder. Periods of 2-7 minutes were required for the spontaneous rhythm to begin and become regular. Only preparations in which regular spontaneous activity developed were used for the study. For these we measured MDP and activation voltage. This was measured from the level of zero potential to the level of membrane potential at which spontaneously occurring action potential was initiated. Spontaneous cycle lengths were measured and converted to rate, per minute.

Determination of the effect of ACh on phase 4 depolarization of pacemaker-like cells required that the cell impaled display pacemaker characteristics throughout the experiment. For all experiments in which automaticity was studied, the action potential during the spontaneous rhythm was recorded at high gain on a Nicolet 1090 oscilloscope. A pacemaker cell was defined as one in which there was a smooth transition between phase 4 depolarization and the upstroke of the action potential. As ACh exerted its effect on the fibers and altered spontaneous rate, there often was a shift in the pacemaker, and cells which initially exhibited a smooth transition from phase 4 to phase 0 lost this characteristic. In four of the preparations from adult dogs, however, the pacemaker characteristics persisted throughout the experiment; these were used to determine ACh effects on the slope of phase 4.

The mean slope of phase 4 was determined, for pacemaker fibers only, by measuring the difference between MDP and activation voltage and dividing this by the time interval between them.

For all preparations studied, two or three control determinations of automaticity were made, following which each preparation was superfused with Tyrode's solution containing graded concentrations of ACh Cl (Sigma) 1 × 10⁻¹¹ to 1 × 10⁻⁷ M. At the end of each 20-minute superfusion period, measurements were made of action potential characteristics. The stimulus then was discontinued and escape time and spontaneous rate were determined.

In some experiments we determined the effects of muscarinic blockade by adding atropine, 2 × 10⁻⁶ M, to the Tyrode's solution superfusing the fiber bundles. For these studies, each fiber bundle was superfused with atropine, alone, for 40 minutes at which time action potential characteristics were measured and automaticity determined. The concentration of atropine used has been reported to have no significant effect on automaticity of isolated Purkinje fibers. Our own preliminary experiments...
verified that this concentration of atropine had no statistically significant effect on the action potential or on automaticity.

As shall be apparent in the Results, certain of the effects of ACh might have been explained by catecholamine release. For this reason, in some experiments we determined the effects of the α-adrenergic blocker phentolamine or the β-adrenergic blocker propranolol on the ACh-induced changes. For these experiments, fibers were superfused with Tyrode’s solution containing phentolamine (Ciba-Geigy) 1 × 10⁻⁶ M, or propranolol (Ayerst), 2 × 10⁻⁷ M (final concentration). These concentrations of adrenergic blocking agents previously have been shown to exert no significant effect on Purkinje fiber action potential characteristics or automaticity.¹¹

Data on action potential characteristics reported here were included only if an impalement was maintained continuously during the course of an experiment. Data on automaticity are reported so long as the preparation under study displayed stable automatic rhythm. All data were analyzed using analysis of variance and the paired t-test (where applicable).¹² Results were accepted as statistically significant if P < 0.05.

Studies of Conscious Dogs

Complete atrioventricular block was produced using the method described by Steiner and Kovalik.¹³ Under sterile conditions, pentobarbital-anesthetized (30 mg/kg, intravenously) adult mongrel dogs were subjected to a right lateral thoracotomy. The heart was suspended in a pericardial cradle and acrylic plaque electrodes were sutured to the epicardial surface of the right atrial appendage and to the right ventricle. These were used for recording bipolar electrograms. Silver-silver chloride button electrodes (Beckman) were sutured in place subcutaneously to record an electrocardiogram. An indwelling polyethylene cannula was sutured into the left atrial appendage. The cannula and all leads subsequently were exteriorized through a skin incision between the scapulae and held in place with a Teflon button. The right atrial appendage was elevated and approximately 0.2 ml of 40% formalin was injected through a 26-gauge needle at the groove between the right atrium and the aorta. A lead II electrocardiogram was monitored continuously during the procedure. Complete heart block occurred within 1 minute following formalin injection and was manifested by a markedly widened QRS complex and a mean idioventricular rate of 48 ± 5 beats/min. For the studies reported here, all experiments were done 5–8 days after surgery. ACh, 0.1–500 μg/kg, was dissolved in sterile saline and a total volume of 5 ml was injected over 5 seconds into the left atrial cannula. Standard hematoxylin and eosin staining was used to demonstrate complete destruction of the His bundle in two of the dogs studied.

Effects of control injections of 5 ml of saline were determined at the beginning and end of all drug protocols. In no instance did a sham injection have any effect on ventricular rate.

Results

Action Potential Characteristics

The control action potential characteristics of adult and neonatal canine Purkinje fibers stimulated at CL = 500 msec are shown in Table 1. As reported in prior studies,¹¹¹² the transmembrane potentials recorded from the adult animals had significantly higher MDP, overshoot, Vₘₐₓ, and longer AP durations than those from the neonates (P < 0.05).

For both adults and neonates, ACh, 1 × 10⁻¹¹ to 1 × 10⁻⁴ M, had no significant effect on action potential amplitude, activation voltage, maximum diastolic potential, or Vₘₐₓ (Table 1). For both adults and neonates there was a tendency for APD to decrease, which was statistically significant for adults only at ACh, 1 × 10⁻⁴ M.

Automaticity

For neonates, ACh ≥ 1 × 10⁻⁶ M, induced a concentration dependent increase in escape time which was maximal at ACh, 1 × 10⁻⁵ M. Control escape time was 34.9 ± 6.1 sec and, at ACh, 1 × 10⁻⁵ M was 103.0 ± 20.0 sec (P < 0.02). For adults, the control escape time, 39.9 ± 8.7 sec did not differ significantly for that of the neonates. ACh, ≥ 1 × 10⁻⁷ M, increased escape time in adults to a maximum of 128.1 ± 31.4 sec at 1 × 10⁻⁵ M (P < 0.02). The maximum attained in neonates and adults did not differ significantly from one another. Atropine did not significantly alter control values for escape time in either age group but did completely prevent the ACh-induced increase in escape time for each group.

ACh induced a concentration-dependent decrease in spontaneous rate in fibers from both neonates and adults. The magnitude of the effect was the same for both age groups, and the decrease (compared to control) was statistically significant at ACh ≥ 1 × 10⁻⁵ M (Fig. 1). For both age groups, this negative chronotropic effect of ACh was reversible on washout with drug-free Tyrode’s solution. Records of action potentials from adult fibers made at a high gain (Fig. 2) show that the negative chronotropic effect of ACh results from a decrease in the slope of phase 4 depolarization rather than from alterations in activation voltage or maximum diastolic potential. Table 2 lists mean data from four preparations in which the fibers impaled exhibited pacemaker characteristics throughout the experiment.

To determine whether ACh exerted its effects on automaticity through combination with a cardiac muscarinic receptor, we superfused six adult and
To test whether this paradoxical effect of ACh in fibers from both age groups occurred. To test whether this paradoxical effect of ACh in fibers from both age groups was not prevented by propranolol and presumably might be due to release of endogenous catecholamines (Fig. 1). Atropine effectively blocked the negative chronotropic effect which was reduced by the pathomimetic amines of Purkinje fiber automaticity. Recently, we reported a biphasic effect of sym patheticomimetic amines of Purkinje fiber automaticity. Low concentrations of amines induced a negative chronotropic effect which was reduced by the pathomimetic amines of Purkinje fiber automaticity.

Recently, we reported a biphasic effect of sympthe omimetic amines of Purkinje fiber automaticity. Low concentrations of amines induced a negative chronotropic effect which was reduced by the α-adrenergic blocking agent, phentolamine. It has been reported that ACh is not a result of liberation of endogenous catecholamines (Fig. 3).

### Table 1: Effect of Acetylcholine on Transmembrane Action Potential Characteristics

<table>
<thead>
<tr>
<th>Acetylcholine (m)</th>
<th>Control</th>
<th>10^-11</th>
<th>10^-10</th>
<th>10^-9</th>
<th>10^-8</th>
<th>10^-7</th>
<th>10^-6</th>
<th>10^-5</th>
<th>10^-4</th>
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<td><strong>A. Adult</strong></td>
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<tr>
<td>Amplitude (mV)</td>
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<td>130.3</td>
<td>130.4</td>
<td>131.4</td>
<td>130.2</td>
<td>130.8</td>
<td>129.5</td>
<td>129.6</td>
<td>128.8</td>
</tr>
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<td>±1.5</td>
<td>±1.5</td>
<td>±1.5</td>
<td>±1.5</td>
<td>±1.4</td>
<td>±1.4</td>
<td>±1.3</td>
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<td>-94.0</td>
<td>-94.6</td>
<td>-95.0</td>
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<td>-94.3</td>
<td>-94.2</td>
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<tr>
<td>(mV)</td>
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<td>±1.2</td>
<td>±1.0</td>
<td>±0.9</td>
<td>±0.6</td>
<td>±0.8</td>
<td>±0.7</td>
<td>±0.6</td>
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<td>529</td>
<td>525</td>
<td>527</td>
<td>524</td>
<td>536</td>
<td>514</td>
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<td>(V/sec)</td>
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<td>±30</td>
<td>±31</td>
<td>±32</td>
<td>±38</td>
<td>±37</td>
<td>±33</td>
<td>±35</td>
<td>±42</td>
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<td>APD50 (msec)</td>
<td>186.4</td>
<td>185.0</td>
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<td>184.8</td>
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<td>(msec)</td>
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<td>±6.6</td>
<td>±7.3</td>
<td>±7.3</td>
<td>±6.4</td>
<td>±6.8</td>
<td>±6.6</td>
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<tr>
<td>APD100 (msec)</td>
<td>324.1</td>
<td>323.3</td>
<td>322.9</td>
<td>318.2</td>
<td>312.5</td>
<td>312.3</td>
<td>312.3</td>
<td>309.1</td>
<td>304.4*</td>
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<tr>
<td>(msec)</td>
<td>±10.6</td>
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<td>±10.5</td>
<td>±11.4</td>
<td>±12.3</td>
<td>±11.1</td>
<td>±10.9</td>
<td>±10.5</td>
<td>±9.6</td>
</tr>
<tr>
<td><strong>B. Neonates</strong></td>
<td></td>
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<tr>
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<td>125.3</td>
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<td>127.0</td>
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<td>±2.1</td>
<td>±1.6</td>
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<tr>
<td>(mV)</td>
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<td>±0.9</td>
<td>±1.5</td>
<td>±1.4</td>
<td>±1.5</td>
<td>±1.5</td>
<td>±1.5</td>
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<td>(V/sec)</td>
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<td>±14</td>
<td>±19</td>
<td>±22</td>
<td>±22</td>
<td>±18</td>
<td>±18</td>
<td>±29</td>
<td>±22</td>
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<tr>
<td>APD50 (msec)</td>
<td>166.4</td>
<td>165.3</td>
<td>163.0</td>
<td>160.5</td>
<td>161.0</td>
<td>159.7</td>
<td>153.4</td>
<td>159.5</td>
<td>165.0</td>
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<tr>
<td>(msec)</td>
<td>±7.1</td>
<td>±8.0</td>
<td>±7.8</td>
<td>±6.2</td>
<td>±7.2</td>
<td>±6.9</td>
<td>±7.7</td>
<td>±6.0</td>
<td>±7.7</td>
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<tr>
<td>APD100 (msec)</td>
<td>273.6</td>
<td>274.1</td>
<td>270.8</td>
<td>267.9</td>
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<td>(msec)</td>
<td>±12.5</td>
<td>±11.1</td>
<td>±10.5</td>
<td>±10.1</td>
<td>±10.2</td>
<td>±9.5</td>
<td>±6.1</td>
<td>±5.8</td>
<td>±6.0</td>
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</table>

Results (mean ± SE) were obtained from 8-11 maintained impalements of adult Purkinje fibers and from 7 maintained impalements of neonatal Purkinje fibers. All were stimulated at a cycle length of 500 msec. Abbreviations used: Amplitude = total action potential amplitude; MDP = maximum diastolic potential; Vmax = maximum rate of rise of phase 0 depolarization; APD50 and APD100 = action potential duration measured to 50% and 100% repolarization, respectively.

*B. Neonates*

### Table 2: Effect of Acetylcholine on Spontaneously Initiated Action Potentials

<table>
<thead>
<tr>
<th>Acetylcholine (m)</th>
<th>Control</th>
<th>10^-4</th>
<th>10^-7</th>
<th>10^-5</th>
</tr>
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<tr>
<td>AV (mV)</td>
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<td>-76.2 ± 1.2</td>
<td>-77.0 ± 1.6</td>
<td>-76.3 ± 1.2</td>
</tr>
<tr>
<td>MDP (mV)</td>
<td>-82.0 ± 1.3</td>
<td>-82.3 ± 1.7</td>
<td>-83.0 ± 1.5</td>
<td>-81.7 ± 1.4</td>
</tr>
<tr>
<td>Δt (sec)</td>
<td>5.53 ± 1.2</td>
<td>6.87 ± 1.6</td>
<td>7.01 ± 1.5</td>
<td>65.4 ± 9.9</td>
</tr>
<tr>
<td>MDP-AV/Δt</td>
<td>1.031 ± 0.05</td>
<td>0.885 ± 0.04</td>
<td>0.856 ± 0.06</td>
<td>0.083 ± 0.23</td>
</tr>
</tbody>
</table>

n = 4 (mean ± SE). Results were obtained from 4 impalements maintained during control superfusion and during superfusion with ACh, 1 × 10^-9, 1 × 10^-7, and 1 × 10^-5 M. The fibers were not subjected to external electrical stimulation. Abbreviations used: AV = activation voltage; MDP = maximum diastolic potential; Δt = time from MDP to the next action potential upstroke; MDP-AV/Δt = the slope of phase 4 depolarization, in mV/sec.
EFFECT OF ACETYLCHOLINE ON SPONTANEOUS RATE OF ISOLATED PURKINJE FIBERS

**FIGURE 1** Effect of ACh (alone and in the presence of atropine) on spontaneous rate of adult (A) and neonatal (B) Purkinje fibers. Spontaneous rates of adult and neonatal Purkinje fibers were determined during control superfusions with normal Tyrode's solution or Tyrode's containing atropine, $2 \times 10^{-6}$ M. Each preparation was then superfused with Tyrode's solution containing graded concentrations of ACh. Results are expressed as mean ± SE of the change in rate (beats/min). For adults, $n = 7$ for ACh groups and $n = 6$ for ACh and atropine. For neonates, $n = 11$ for ACh and $n = 8$ for ACh and atropine. For both age groups, $^* = P < 0.05$ compared to control. Control values for spontaneous rate: (mean ± SE) adult = 16.5 ± 5.8; adult (atropine) = 12.8 ± 3.3; neonate = 10.2 ± 6.5; neonate (atropine) = 12.6 ± 3.9.

been suggested\(^ {\text{15}} \) that ACh may cause a release of norepinephrine from nerve terminals. Therefore, to determine whether ACh might release low concentrations of norepinephrine which combine with $\alpha$-receptors on Purkinje fibers to induce a negative chronotropic effect, we superfused five adult Purkinje fiber preparations with phentolamine, $1 \times 10^{-6}$ M, for 40 minutes. Following this control period, we continued superfusing with Tyrode’s solution containing, in addition to phentolamine, ACh (final concentrations, $1 \times 10^{-9}$, $1 \times 10^{-7}$, and $1 \times 10^{-5}$ M). This did not prevent the negative chronotropic effect of ACh (Fig. 4).

**Effects of ACh on Adult Dogs with Complete Heart Block**

Five adult dogs with stable idioventricular rhythms were studied 5-8 days after surgery. That the rhythm was ventricular in origin rather than atrioventricular junctional was evident from the following: (1) formalin was injected into the area of the His bundle rather than the atrioventricular node; (2) histological examination of this area revealed complete obliteration of the His bundle; (3) the QRS complex changed markedly in duration and configuration as a result of injection of formalin: (mean ± SE) control QRS duration = 48 ± 5 msec, vs. 103 ± 9 msec following induction of heart block.

Three preliminary experiments were performed to determine the effects of ACh on systemic arterial blood pressure and the relationship of these changes to those in idioventricular rate. Figure 5 shows the results of one experiment. Injection of ACh caused a rapid decrease in blood pressure and a simultaneous decrease in idioventricular rate. This was followed by an increase in idioventricular rate. We corrected the effect of ACh for rate by subtracting the cycle length of the last beat (before ACh) from the maximum cycle length observed following ACh.

**FIGURE 2** Records of transmembrane action potentials of adult Purkinje fiber were made at relatively high gain, so that effects on phase 4 depolarization were more apparent. In this experiment, following the control superfusion, the preparation was superfused sequentially with Tyrode’s solution containing ACh, $1 \times 10^{-6}$, $1 \times 10^{-7}$, and $1 \times 10^{-5}$ M. MDP was −84 mV, AV was −76 mV during control.
ACh, M

Figure 3 Comparison of effects of ACh, alone, and in the presence of propranolol, 2 × 10⁻⁷ M on spontaneous rate. The curve for ACh, alone, is that shown for neonates in Figure 1. The curve for ACh plus propranolol (n = 4) shows that β-adrenergic blockade did not prevent the increase in spontaneous rate at ACh, 10⁻⁹ M. No significant difference in response to ACh in the presence or absence of propranolol was found. Results expressed as mean ± SE. Control values for spontaneous rate: 102 ± 6.5 (in absence of propranolol); 9.0 ± 1.5 (in presence of propranolol). Difference is not statistically significant.

injection. A different response was seen in sinus rate. Rather than decreasing, sinus rate increased concomitantly with the ACh-induced hypotension.

We assumed that the secondary increase in idioventricular rate was mediated through the cardiac sympathetic nerves due to catecholamine release resulting from hypotension. We therefore reasoned that pretreatment with propranolol should suppress the secondary increase in idioventricular rate and might enhance the ACh-induced slowing.

ACh, M

Figure 4 Comparison of effects of ACh, alone, and in the presence of phentolamine, 1 × 10⁻⁶ M. The curve for ACh alone is that of adults shown in Figure 1. The curve for ACh plus phentolamine (n = 4) indicates that the negative chronotropic effect of ACh is not due to an α-adrenergic effect occurring as a result of release of adrenergic amines.

Figure 5 Effects of ACh on idioventricular rhythm and blood pressure of conscious, adult dogs with complete heart block. For each panel are shown a body surface electrocardiogram (ECG) and the systemic arterial pressure (BP). The top panel ("C") shows the control with the ECG also shown in an expanded time scale. The middle panel shows the effect of ACh, 1 ng/kg, on idioventricular rate and blood pressure. Note that ACh slowed rate and decreased blood pressure simultaneously and that this effect was then followed by an increase in the idioventricular rate. The bottom panel illustrates the effect of ACh, 10 ng/kg. Note that idioventricular rate is decreased further (compared to ACh, 1 ng/kg) and that the decrease in blood pressure coincides with this effect. This is followed by an increase in rate.

To test this, we administered propranolol 0.5 mg/kg, iv, and again administered ACh. The rapid decrease in idioventricular rate and blood pressure occurred simultaneously, but the secondary increase in idioventricular rate was markedly attenuated.

Figure 6 shows results of five subsequent experiments in which ACh, 0.1–500 µg/kg, was injected in the presence of propranolol or propranolol + atropine, 0.1 mg/kg. ACh, alone, 1–50 µg/kg, induced a concentration-dependent decrease of the idioventricular rate. Doses of ACh > 75 µg/kg occasionally caused only a tachycardia. ACh alone, 200–500 µg/kg, caused marked side effects (tremors, seizures, respiratory distress). When ACh was administered in the presence of propranolol, the dose-response relationship for the idioventricular rate was shifted upward and to the left of that obtained with ACh alone. A maximum suppression of the idioventricular rhythm was observed at ACh, 75
Administration of atropine in the presence of propranolol significantly attenuated the effect of ACh on idioventricular rate. Differences are not statistically significant.

Discussion

We have demonstrated a consistent negative chronotropic effect of ACh on Purkinje fibers from adult dogs and a biphasic effect on spontaneous rate in fibers from neonatal dogs. In addition, we have confirmed that the idioventricular rate of adult dogs with chronic complete heart block is decreased by administration of exogenous ACh, and have provided the dose-response relationship for this effect.

In our studies of adult dogs, ACh significantly decreased APD_{100} only at the highest concentration studied, \(1 \times 10^{-4} \text{ M}\). APD_{100} was insignificantly decreased in neonatal dogs. No other consistent effects on action potential characteristics were observed. Studies by other investigators have shown that ACh can increase maximum diastolic potential, rate of rise of phase 0, action potential amplitude, and conduction velocity. In both of these studies, preparations were used which had lower membrane potentials than those in our study prior to superfusion with ACh. In reviewing the contents of Table 1, it is apparent that in our study small increases in MDP did occur with ACh; however, these are statistically insignificant. It is reasonable to assume that, in our preparations of adult fibers, which have higher levels of MDP (i.e., \( \geq -90 \text{ mV} \)) than either those of Bailey et al., or Tse et al., the potassium conductance may have been higher initially and therefore less amenable to change by ACh. Following this line of reasoning, one might question why a greater increase in MDP was not seen in the neonatal fibers, for which the control MDP was significantly lower than in the adult fibers. It has been shown recently that intracellular potassium activity (\( a_{\text{K}} \)) and the potassium equilibrium potential (\( E_{\text{K}} \)) are significantly lower for neonatal than for adult dogs, which, at least in part, the difference in resting membrane potentials for the two groups of animals. Whether, in addition, there are age-related differences in \( g_{\text{K}} \) remains to be seen, but this is plausible.

We have shown a concentration-dependent negative chronotropic effect of ACh on Purkinje fibers from both adult and neonatal dogs. This effect is of equal magnitude for fibers from both age groups, is readily reversible on washout of ACh, and is prevented by atropine, a muscarinic blocking agent. Further, this effect of ACh is apparently due to a decrease in the slope of phase 4 depolarization without significant effects on either activation voltage or maximum diastolic potential. Such an effect is consistent with an increase in \( g_{\text{K}} \) during phase 4 of the transmembrane potential.

It is difficult to compare the effects of ACh on Purkinje fibers to those on sinus node because there are only two studies of which we are aware, in which a dose-response relationship for canine sinus node was determined. In one of these studies, isolated superfused rabbit sinus nodes showed a peak decrease of 43% in sinus rate at ACh, \( 5 \times 10^{-5} \text{ M} \). This is in contrast to our study in which only \( 1 \times 10^{-8} \text{ M} \) ACh was required to induce a 50% decrease in canine Purkinje fiber automaticity. Although this comparison might be interpreted as suggesting there is a relatively greater effect of ACh on Purkinje fibers than sinus node, it is likely that the species differences between the two studies account for this disparity. The results of the second study support our interpretation. Here, the canine sinus node, which was studied during perfusion of its artery, required ACh, \( 5.5 \times 10^{-8} \text{ M} \), to abolish spontaneous activity, whereas for superfused Purkinje fibers, spontaneous activity ceased at ACh, \( 1 \times 10^{-4} \text{ M} \). There are difficulties in interpretation of this study, too. Although it suggests a greater sensitivity.
of canine sinus node to ACh than occurs for Purkinje fibers, the sinus node was studied during perfusion of its artery, the Purkinje fiber during superfusion of the preparation. It is possible that these differences in ACh delivery might result in the apparent differences in the dose-response relationship.

That the magnitude of the negative chronotropic effect of ACh was approximately equivalent for both neonatal and adult dogs was not unexpected. The cardiac parasympathetics (and, by implication, the acetylcholine receptor) are well developed at birth, unlike the sympathetic neurons which are relatively immature and continue to develop postpartum. Given the relative maturity of the parasympathetic system at birth, an end organ response to ACh approximating that of the adult was anticipated. What was unexpected was the consistent, marked increase in automaticity of neonatal fibers at ACh, 10^-9 M. This effect was not blocked by propranolol and, therefore, is presumably not due to release of endogenous catecholamines. The mechanism responsible for this effect remains to be described.

Evidence that catecholamines are not responsible for the negative chronotropic effects of ACh is supplied by our experiments using phentolamine. It has been hypothesized that ACh can combine with receptors to cause a release of catecholamines from preganglionic (i.e., neuroeffector junction) sites. We and others previously have shown that low concentrations of catecholamines exert an alpha-adrenergic effect that decreases Purkinje fiber automaticity. However, our experiments showing the lack of effect of phentolamine, an alpha-blocking agent, on the negative chronotropic effect of ACh suggest that endogenous catecholamines are not responsible for the decrease in automaticity.

Our results with intact dogs with chronic heart block indicated that ACh decrease automaticity in situ. This is consistent with earlier reports, which showed that vagal stimulation as well as exogenous ACh could decrease ventricular automaticity. The negative chronotropic effect of ACh is dose dependent and atropine sensitive.

The question of whether the ventricular rhythm arose from the His bundle or from the more distal Purkinje system is important in bridging the in vitro and in vivo effects of ACh. In all intact dogs studied, the induction of heart block was accompanied by a marked widening of the QRS complex. Histological studies of two hearts showed destruction of the entire His bundle, suggesting that the rhythm arose from more distal segments of the ventricular conduction system. Thus, it seems likely that the neurotransmitter of the parasympathetic nervous system can exert a negative chronotropic effect on the distal ventricular specialized conducting system of the intact animal.

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