Effects of Adrenergic Amines on Electrophysiological Properties and Automaticity of Neonatal and Adult Canine Purkinje Fibers

EVIDENCE FOR \(\alpha\) - AND \(\beta\)-ADRENERGIC ACTIONS

MICHAEL R. ROSEN, ALLAN J. HORDOF, JOSEPH P. ILVENTO, AND PETER DANILLO, JR.

With the assistance of Kathy J. Slavin

SUMMARY We determined age-related differences in automaticity and responsiveness of cardiac Purkinje fibers from adult and neonatal dogs to graded concentrations of epinephrine, isoproterenol, and phenylephrine. Purkinje fibers were studied with standard microelectrode techniques during superfusion with Tyrode's solution at 37°C. Control spontaneous rates for adults and neonates did not differ significantly. There was a biphasic response to all agonists such that rate decreased at low and increased at high concentrations. The decrease was greater with phenylephrine and epinephrine than with isoproterenol. The increase in rate was greater with isoproterenol and epinephrine than with phenylephrine. Propranolol shifted the dose-response curves downward and to the right for all agonists; phentolamine, shifted the curves upward and to the left. Epinephrine and isoproterenol dose-response curves for the neonates were upward and to the left of those for adults. Phenylephrine curves were identical for adults and neonates. Thus there are \(\alpha\) - and \(\beta\)-adrenergic effects on Purkinje fiber automaticity; the former decrease and the latter increase rate. Furthermore, the effects on automaticity of \(\alpha\)-adrenergic amines are greater in the neonates than in the adult.

IN THE present study we determined the effects of adrenergic amines on automaticity and action potential (AP) characteristics of Purkinje fibers from neonatal and adult dogs. A number of recent studies have indicated that adrenergic amines have \(\alpha\) and \(\beta\) agonistic effects on cardiac fibers. Ledda et al.¹ showed that phenylephrine, which has a high ratio of \(\alpha\) to \(\beta\) agonistic activity, increases AP duration of sheep Purkinje fibers. Giotta et al.² observed that isoproterenol decreases the duration of the Purkinje fiber AP, an action blocked by propranolol but not by phentolamine. They also found that norepinephrine decreases AP duration in the presence of phentolamine and increases AP duration in the presence of propranolol. These results led the authors to suggest that prolongation of the AP duration is an \(\alpha\)-adrenergic effect and that its shortening is a \(\beta\)-adrenergic effect.²

From the Departments of Pharmacology and Pediatrics, Columbia University College of Physicians and Surgeons, New York, New York.

Supported in part by Grants HL-12738 and HL-17766 from the National Heart and Lung Institute, U.S. Public Health Service, and a grant from the New York Heart Association. Dr. Rosen is a Career Scientist of the Irma T. Hirschl Foundation; Dr. Danilo is a Postdoctoral Fellow of the New York Heart Association.

Received July 12, 1976; accepted for publication October 15, 1976.

In 1971 Posner and Vassalle³ and Vassalle and Barnabei⁴ reported that low concentrations of norepinephrine decrease \(^{42}\)K uptake by canine Purkinje fibers but higher concentrations increase \(^{42}\)K uptake. They concluded that the reduction of the \(K^+\) uptake at low norepinephrine concentrations is an \(\alpha\)-adrenergic function mediated through depression of the \(Na^+\)-\(K^+\) exchange pump. Stanford,² in studies of rabbit atria, found epinephrine to increase \(^{42}\)K uptake; this action was blocked by dichloroisoproterenol but not by phenoxybenzamine. This study suggested that the epinephrine-induced increase in \(K^+\) flux is mediated by the activation of the \(\beta\)-receptor.

Blinks⁶,⁷ showed that epinephrine, isoproterenol, and norepinephrine induce a concentration-dependent increase in automaticity of feline atria, and Hoffman and Singer⁸ reported that epinephrine increases the slope of phase 4 depolarization of canine Purkinje fibers and results in enhanced automaticity. Posner et al.⁹ reported that low concentrations of epinephrine decrease Purkinje fiber automaticity and \(K^+\) uptake, and that higher concentrations increase automaticity and \(K^+\) uptake. The former effect, which was blocked by phentolamine, was interpreted as an \(\alpha\)-adrenergic and the latter as a \(\beta\)-adrenergic
action. Crandefield et al. in experiments using methoxamine, also demonstrated an α-adrenergic effect on Purkinje fibers. Hauswirth et al. and Tsien showed that epinephrine-induced increases in automaticity are associated with its action on the pacemaker potassium current, and further showed that its effects on automaticity and its concentration in the biophase would be the same for neonatal and adult tissue. Friedman14 showed that the contractile response of myocardial strips from fetal and newborn lambs to norepinephrine was greater than that of adult myocardium. However, for both age groups, the response to isoproterenol was equal. Friedman attributed the difference in responsiveness to norepinephrine to the fact that sympathetic terminals are not completely developed in the neonate, hence, norepinephrine—which normally is subject to uptake by sympathetic terminals—is taken up less by neonatal than by adult tissue. As a result, more norepinephrine would be available to act as a β agonist for the neonatal than for the adult tissue. On the other hand, because isoproterenol is not taken up by adrenergic terminals its concentration in the biophase would be the same for fetal and adult fibers.

We designed the present study to evaluate the extent to which ventricular automaticity and its response to adrenergic agonists change as a function of age. We found that although the spontaneous rates of Purkinje fibers from dogs 0–7 days old (neonates) and adult dogs do not differ, the younger dogs develop a far greater increase in rate on superfusion with adrenergic agonists. In addition, the responsiveness to norepinephrine to the fact that sympathetic terminals are not completely developed in the neonate, hence, norepinephrine—which normally is subject to uptake by sympathetic terminals—is taken up less by neonatal than by adult tissue. As a result, more norepinephrine would be available to act as a β agonist for the neonatal than for the adult tissue. On the other hand, because isoproterenol is not taken up by adrenergic terminals its concentration in the biophase would be the same for fetal and adult fibers.

Methods

Fifty-two mongrel dogs 1–9 years of age were anesthetized with pentobarbital sodium, 30 mg/kg, administered intravenously. Forty-three neonates, 0–7 days old, from 11 litters, were anesthetized with pentobarbital, 30 mg/kg, intraperitoneally. The chests were opened through a right lateral thoracotomy (adults) or midline sternotomy (neonates) and the hearts were removed rapidly and immobilized with pentobarbital sodium, 30 mg/kg, intraperitoneally. The preparations were stimulated, impaled with microelectrodes, and allowed to equilibrate for 1 hour before control measurements were made. The following control measurements then were recorded: activation voltage (AV), measured from the “0” reference potential to the level of membrane potential at which the AP was initiated; maximum diastolic potential (MDP), measured from “0” potential to the point of maximum membrane potential occurring at the end of phase 3 or the beginning of phase 4; AP amplitude, measured from the MDP to the peak of the overshoot; APD 90 (AP duration to 90% repolarization); and Vmax. These measurements were made by previously described methods17 and with the Nicolet 1090 digital processing oscilloscope.

For each preparation the drive stimulus then was discontinued and escape time (measured from the upstroke of the last driven AP to the upstroke of the first spontaneously occurring AP) and ensuing spontaneous rate and rhythm were recorded on the Brush recorder. A period of 3–10 minutes was required for the spontaneous rhythm to commence and stabilize. Preparations in which the spontaneous rhythm was irregular were not included in the study. The spontaneous cycle lengths for the remaining preparations were recorded and converted to beats per minute.

In certain of the preparations the fiber impaled had characteristics of a “pacemaker cell”; that is, during the spontaneous rhythm phase 4 depolarization merged smoothly with the phase 0 upstroke of the AP. When this occurred the spontaneous rhythm also was recorded on the Nicolet 1090 oscilloscope and the mean slope of phase 4 depolarization was measured. This was done by recording the MDP and AV for the spontaneously firing fiber, subtracting AV from MDP, and dividing the difference (Δ, in mV) by the time, in seconds, between the points at which these two voltages were measured. The AV for pacemaker fibers was defined by the point of inflection between phase 4 and phase 0 as recorded on the Nicolet oscilloscope.

After the control period the drive stimulus was resumed. The Purkinje fibers then were superfused with adrenergic amines by one of the following protocols: epi-
neprine (L-epinephrine bitartrate, Sigma), 1 x 10^-9 to 1 x 10^-4 M; isoproterenol (L-isoproterenol d-bitartrate, Sigma), 1 x 10^-11 to 1 x 10^-4 M; or phenylephrine-HCl (Sigma), 1 x 10^-9 to 1 x 10^-4 M. In four additional experiments on fibers from adults, the epinephrine concentrations studied were 1 x 10^-11 to 1 x 10^-4 M. After 20 minutes of superfusion with each concentration of drug, AP characteristics were recorded and measured, the stimulus was discontinued, and escape time, spontaneous rate and rhythm, and the other abovementioned characteristics of pacemaker fibers were recorded. In preliminary studies we found that a steady state effect of the adrenergic amines was reached within 5-10 minutes after the onset of superfusion.

To ascertain the reproducibility of spontaneous rate over a long period of time when studied by the aforementioned protocols, we performed five experiments in which the protocol for epinephrine was followed but epinephrine was not added to the superfusate. Although variations in rate were seen from one test period to the next these were statistically insignificant (paired t-test), small in magnitude, and inconsistent in direction.

To study the effects of α- and β-adrenergic blockade, phentolamine (Regitine-HCl, Ciba-Geigy), 1 x 10^-6 M, or propranolol, (dl-propranolol, Ayerst), 2 x 10^-7 M, was added to the superfusate after the 60-minute control period. The fiber was superfused with the antagonist, alone, for 40 minutes (time to steady state was 20-30 minutes) after which a second set of control readings was taken. Prior studies by others18 and by us19 have shown that these concentrations of the antagonists have no significant effect on the transmembrane resting and action potentials. Moreover, in the present study we found that phentolamine, 1 x 10^-6 M, had no effect on automaticity whereas propranolol, 2 x 10^-7 M, decreased automaticity consistently but not significantly (P > 0.05). The fibers then were superfused with adrenergic amines as described above, in the continued presence of phentolamine or propranolol.

DATA PROCESSING

Data concerning AP characteristics were retained only for those studies in which the microelectrode impalements were maintained throughout the duration of the experiment. Data concerning automaticity were retained for all studies in which the changes in rate were determined by a regular rhythm. When there were premature depolarizations or bursts of ectopic activity, or both, the data for the entire study were discarded because we could not rule out reentry as a contributory mechanism. When an impalement of a pacemaker cell was lost during a study, the data on automaticity still were recorded (using the electrogram and/or the microelectrode impalements at other sites). No effort was made to reimpale the pacemaker cell because of the possibility that the mechanical stimulus might reset or modify the basic rhythm. Hence, all data on pacemaker cells reported are from impalements that were maintained throughout the study.

The effects of each adrenergic amine and blocker on automaticity and on resting and action potentials of pacemaker and driven cells were analyzed by paired t-test. To compare data from one group with those from another a t-test for grouped data was used. In the studies of automaticity there were no significant differences in the control rates for the various groups of fibers. To facilitate presentation of the data and the comparison of one group of responses to another, we deemed it appropriate in our figures to express all control rates as 0 and the changes in rate that occurred as the percent increase or percent decrease. It is to be stressed, however, that the statistical significances reported were calculated from the absolute numbers and not from the percent changes. For values reported as statistically significant in all figures, P was <0.05.

Results

TRANSMEMBRANE RESTING AND ACTION POTENTIAL CHARACTERISTICS AND AUTOMATICITY OF NEONATAL AND ADULT FIBERS

The control values for transmembrane potentials of all adult fibers studied by using adrenergic agonists and antagonists and in which impalements were maintained throughout the experiment are shown in Table 1 (column A). Columns B and C of Table 1 show, respectively, the control data for the adult and neonatal fibers which subsequently were superfused with either epinephrine, isoproterenol, or phenylephrine. The values for MDP, AP amplitude, and Vmax of neonatal fibers are lower than those of fibers from adults and their AP duration is shorter. Table 1 also shows the control escape time for all adult fibers as well as for adult and neonatal fibers subsequently superfused with epinephrine, isoproterenol, or phenylephrine. Although the fibers from the neonatal hearts tended to escape more rapidly than did those from the adults, the difference was not significant (P > 0.05). There also was no significant difference between the spontaneous rates of the fibers taken from neonatal and adult hearts (Table 1).

RESPONSE OF FIBERS FROM ADULT DOGS TO ADRENERGIC AGONISTS

Effects on the Action Potential

The effects of the three agonists on AP characteristics of electrically driven Purkinje fibers are shown in Tables 2 and 3. Isoproterenol, 10^-11 through 10^-5 M, significantly increased the MDP. This was associated with some increase in AP amplitude as well. Epinephrine, 10^-9 through 10^-6 M, had a similar effect on MDP and AP amplitude. Phenylephrine had no significant effect on these variables. Isoproterenol, 10^-6 through 10^-4 M, decreased APD50 significantly. It should be noted that the decreases that occurred in APD were associated with an increase in the height of the plateau. Epinephrine induced a small but significant increase in APD50 at 10^-4 and 10^-3 M and increased ADP100 at 10^-2 and 10^-1 M. Phenylephrine increased AP duration, although not significantly.

Effects on Automaticity

Thirty-seven Purkinje fiber bundles were studied: 15 were superfused with epinephrine, 11 with phenylephrine, and 11 with isoproterenol. Results of these studies are
shown in Figure 1. For all three drugs one of two types of response occurred. In the majority of fibers (10/15 epinephrine, 8/11 phenylephrine, 10/11 isoproterenol) the spontaneous rate slowed significantly at low drug concentrations and increased to a plateau value at high concentrations. We will refer to this type of response as a monophasic response. In the remainder of the fibers, rate was unchanged or increased on superfusion with the lowest concentration of agonist and continued to increase until a plateau was reached. This will be referred to as a biphasic response.

In the number of experiments is given in parentheses.

The following abbreviations are used in Tables 1-6. AP = action potential; MDP = maximum diastolic potential; $V_{\text{max}}$ = maximum upstroke velocity of phase 0 depolarization; APD90 = action potential duration to 50% repolarization; APD100 = action potential duration to full repolarization. Results for all tables are expressed as mean ± SE. The number of experiments is given in parentheses.

The following abbreviations are used in Tables 1-6. AP = action potential; MDP = maximum diastolic potential; $V_{\text{max}}$ = maximum upstroke velocity of phase 0 depolarization; APD90 = action potential duration to 50% repolarization; APD100 = action potential duration to full repolarization. Results for all tables are expressed as mean ± SE. The number of experiments is given in parentheses.

### Table 1

**Control Transmembrane Potential Characteristics and Automaticity**

<table>
<thead>
<tr>
<th>Variable and agonist</th>
<th>A: Adult</th>
<th>B: Adult</th>
<th>C: Neonatal</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP amplitude (mV)</td>
<td>125.7 ± 0.6</td>
<td>124.0 ± 0.8</td>
<td>120.7 ± 0.8*</td>
</tr>
<tr>
<td>(66)</td>
<td>(37)</td>
<td>(16)</td>
<td></td>
</tr>
<tr>
<td>MDP (mV)</td>
<td>90.5 ± 0.5</td>
<td>89.7 ± 0.7</td>
<td>86.7 ± 0.8†</td>
</tr>
<tr>
<td>(66)</td>
<td>(37)</td>
<td>(16)</td>
<td></td>
</tr>
<tr>
<td>$V_{\text{max}}$ (V/sec)</td>
<td>514.0 ± 12</td>
<td>511.0 ± 17</td>
<td>419.0 ± 23‡</td>
</tr>
<tr>
<td>(66)</td>
<td>(37)</td>
<td>(16)</td>
<td></td>
</tr>
<tr>
<td>APD90 (msec)</td>
<td>192.0 ± 3</td>
<td>194.0 ± 4</td>
<td>175.0 ± 4§</td>
</tr>
<tr>
<td>(66)</td>
<td>(37)</td>
<td>(16)</td>
<td></td>
</tr>
<tr>
<td>APD100 (msec)</td>
<td>306.0 ± 4</td>
<td>316.0 ± 5</td>
<td>275.0 ± 5‡</td>
</tr>
<tr>
<td>(66)</td>
<td>(37)</td>
<td>(16)</td>
<td></td>
</tr>
<tr>
<td>Escape time (sec)</td>
<td>48.7 ± 5.6</td>
<td>52.2 ± 7.9</td>
<td>34.5 ± 4.7</td>
</tr>
<tr>
<td>(66)</td>
<td>(37)</td>
<td>(16)</td>
<td></td>
</tr>
<tr>
<td>Spontaneous rate (beats/min)</td>
<td>12.2 ± 1.3</td>
<td>12.4 ± 1.6</td>
<td>9.7 ± 1.1</td>
</tr>
<tr>
<td>(66)</td>
<td>(37)</td>
<td>(16)</td>
<td></td>
</tr>
</tbody>
</table>

The following abbreviations are used in Tables 1-6. AP = action potential; MDP = maximum diastolic potential; $V_{\text{max}}$ = maximum upstroke velocity of phase 0 depolarization; APD90 = action potential duration to 50% repolarization; APD100 = action potential duration to full repolarization. Results for all tables are expressed as mean ± SE. The number of experiments is given in parentheses.

The following abbreviations are used in Tables 1-6. AP = action potential; MDP = maximum diastolic potential; $V_{\text{max}}$ = maximum upstroke velocity of phase 0 depolarization; APD90 = action potential duration to 50% repolarization; APD100 = action potential duration to full repolarization. Results for all tables are expressed as mean ± SE. The number of experiments is given in parentheses.

### Table 2

**Effects of Adrenergic Amines on the Transmembrane Potential**

<table>
<thead>
<tr>
<th>Variable and agonist</th>
<th>Control</th>
<th>$10^{-11}$ M</th>
<th>$10^{-10}$ M</th>
<th>$10^{-9}$ M</th>
<th>$10^{-8}$ M</th>
<th>$10^{-7}$ M</th>
<th>$10^{-6}$ M</th>
<th>$10^{-5}$ M</th>
<th>$10^{-4}$ M</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP amplitude (mV)</td>
<td>123.0</td>
<td>120.5</td>
<td>120.5</td>
<td>120.5</td>
<td>120.5</td>
<td>120.5</td>
<td>120.5</td>
<td>120.5</td>
<td>120.5</td>
</tr>
<tr>
<td>Epi (17)</td>
<td>±4.0</td>
<td>±2.0</td>
<td>±2.0</td>
<td>±2.0</td>
<td>±2.0</td>
<td>±2.0</td>
<td>±2.0</td>
<td>±2.0</td>
<td>±2.0</td>
</tr>
<tr>
<td>Iso (9)</td>
<td>±2.9</td>
<td>±1.5</td>
<td>±1.5</td>
<td>±1.5</td>
<td>±1.5</td>
<td>±1.5</td>
<td>±1.5</td>
<td>±1.5</td>
<td>±1.5</td>
</tr>
<tr>
<td>Phenyl (8)</td>
<td>±1.4</td>
<td>±1.4</td>
<td>±1.4</td>
<td>±1.4</td>
<td>±1.4</td>
<td>±1.4</td>
<td>±1.4</td>
<td>±1.4</td>
<td>±1.4</td>
</tr>
<tr>
<td>MDP (mV)</td>
<td>87.4</td>
<td>87.4</td>
<td>87.4</td>
<td>87.4</td>
<td>87.4</td>
<td>87.4</td>
<td>87.4</td>
<td>87.4</td>
<td>87.4</td>
</tr>
<tr>
<td>Epi (17)</td>
<td>±3.0</td>
<td>±2.0</td>
<td>±2.0</td>
<td>±2.0</td>
<td>±2.0</td>
<td>±2.0</td>
<td>±2.0</td>
<td>±2.0</td>
<td>±2.0</td>
</tr>
<tr>
<td>Iso (9)</td>
<td>±5.7</td>
<td>±5.7</td>
<td>±5.7</td>
<td>±5.7</td>
<td>±5.7</td>
<td>±5.7</td>
<td>±5.7</td>
<td>±5.7</td>
<td>±5.7</td>
</tr>
<tr>
<td>Phenyl (8)</td>
<td>±1.1‡</td>
<td>±1.1‡</td>
<td>±1.1‡</td>
<td>±1.1‡</td>
<td>±1.1‡</td>
<td>±1.1‡</td>
<td>±1.1‡</td>
<td>±1.1‡</td>
<td>±1.1‡</td>
</tr>
<tr>
<td>$V_{\text{max}}$ (V/sec)</td>
<td>525.0</td>
<td>525.0</td>
<td>525.0</td>
<td>525.0</td>
<td>525.0</td>
<td>525.0</td>
<td>525.0</td>
<td>525.0</td>
<td>525.0</td>
</tr>
<tr>
<td>Epi (17)</td>
<td>±24.0</td>
<td>±24.0</td>
<td>±24.0</td>
<td>±24.0</td>
<td>±24.0</td>
<td>±24.0</td>
<td>±24.0</td>
<td>±24.0</td>
<td>±24.0</td>
</tr>
<tr>
<td>Iso (9)</td>
<td>±24.0</td>
<td>±24.0</td>
<td>±24.0</td>
<td>±24.0</td>
<td>±24.0</td>
<td>±24.0</td>
<td>±24.0</td>
<td>±24.0</td>
<td>±24.0</td>
</tr>
<tr>
<td>Phenyl (8)</td>
<td>±32.0</td>
<td>±32.0</td>
<td>±32.0</td>
<td>±32.0</td>
<td>±32.0</td>
<td>±32.0</td>
<td>±32.0</td>
<td>±32.0</td>
<td>±32.0</td>
</tr>
</tbody>
</table>

Agonists are epinephrine (epi), isoproterenol (iso), and phenylephrine (phenyl); other abbreviations as in Table 1.

* $P < 0.025$.
† $P < 0.02$.
‡ $P < 0.05$.
§ $P < 0.005$.
¶ $P < 0.01$. 

Downloaded from http://circres.ahajournals.org/ on June 20, 2017.
rately for the remainder of the paper, as there were differences between the points on the biphasic and monophasic curves which were statistically significant in all studies.

Figure 2 shows the biphasic dose-response curves for comparable concentrations of epinephrine, isoproterenol, and phenylephrine. Note that the threshold concentration for a rate increase was lowest for isoproterenol (between $10^{-9}$ and $10^{-8}$ M) and highest for phenylephrine (between $10^{-6}$ and $10^{-5}$ M). Similarly, the maximum rate was attained at the lowest concentration ($10^{-7}$ M) by fibers exposed to isoproterenol and at the highest concentration ($10^{-5}$ M) by fibers exposed to phenylephrine. The minimum and maximum rates attained did not differ significantly for all three agonists.

**RESPONSES OF FIBERS FROM ADULT DOGS TO ADRENERGIC ANTAGONISTS AND AGONISTS**

**Effects on the Action Potential**

The effects of epinephrine on the transmembrane potential characteristics of phentolamine and propranolol superfused fibers are shown in Tables 4 and 5. The antagonists alone had no significant effects on AP characteristics. Note that in the presence of either propranolol or phentolamine epinephrine had no effect on MDP and AP.
TABLE 4  Effect of Phenolamine (Ph) (1 × 10⁻⁶ M) and Propranolol (Pr) (2 × 10⁻⁶ M) on Epinephrine-Induced Changes in Transmembrane Potential

<table>
<thead>
<tr>
<th>Variable Control</th>
<th>Antagonist</th>
<th>Epinephrine conc. (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10⁻¹</td>
</tr>
<tr>
<td>AP amplitude (mV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi (17)</td>
<td>123.0</td>
<td>±4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pr-e (10)</td>
<td>125.0</td>
<td>±4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDP (mV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi (17)</td>
<td>87.4</td>
<td>±3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pr-e (10)</td>
<td>126.0</td>
<td>±4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vmax (V/sec)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi (17)</td>
<td>482.0</td>
<td>±2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pr-e (10)</td>
<td>482.0</td>
<td>±2.0</td>
</tr>
</tbody>
</table>

Values for epinephrine are the changes that occurred as compared to values for the antagonist. Ph-e = phenolamine + epinephrine; Pr-e = propranolol + epinephrine; other abbreviations and explanations are given in preceding tables.

* P < 0.05.
† P < 0.02.
‡ P < 0.01.
§ P < 0.005.

amplitude. Further, in the presence of phenolamine, epinephrine, ≥10⁻⁶ M, decreased APD and in the presence of propranolol, epinephrine increased APD.

Effects on Automaticity

The effects of phenolamine, 1 × 10⁻⁶ M, on the responses of adult Purkinje fibers to epinephrine, isoproterenol, and phenylephrine were studied in 10, six, and seven experiments, respectively (Fig. 3). In seven of the 10 epinephrine experiments and five of the seven phenylephrine experiments there was a biphasic response. There was no biphasic response in any of the six isoproterenol experiments. Hence, a biphasic response occurred in 12 of 23 fibers treated with a combination of phenolamine, 1 × 10⁻⁶ M, and agonist, as compared to 28 of 37 fibers treated with agonist alone.

TABLE 5  Effect of Phenolamine (Ph) (1 × 10⁻⁶ M) and Propranolol (Pr) (2 × 10⁻⁶ M) on Epinephrine-Induced Changes in Action Potential Duration

<table>
<thead>
<tr>
<th>Variable Control</th>
<th>Antagonist</th>
<th>Epinephrine conc. (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10⁻¹</td>
</tr>
<tr>
<td>APDₘₐ (msec)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi (17)</td>
<td>195.0</td>
<td>±5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pr-e (10)</td>
<td>197.0</td>
<td>±5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APDₘₐ (msec)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi (17)</td>
<td>310.0</td>
<td>±8.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pr-e (10)</td>
<td>294.0</td>
<td>±8.0</td>
</tr>
</tbody>
</table>

Values for epinephrine are the changes that occurred as compared to values for the antagonist. Explanations are given in preceding tables.

* P < 0.001.
† P < 0.05.
‡ P < 0.025.
§ P < 0.02.
¶ P < 0.005.
‖ P < 0.01.
The changes in rate that occurred when phenolamine-treated fibers were superfused with isoproterenol are shown in Figure 3, as are curves obtained for those fibers showing a biphasic response to epinephrine and phenylephrine. Although not shown in this figure, the biphasic and monophasic curves for epinephrine did not differ significantly from one another, nor did the biphasic and monophasic curves for phenylephrine. As shown previously (Fig. 2) the isoproterenol curve was to the left of that for epinephrine. However, the peak rate attained was not significantly different for the isoproterenol- and epinephrine-superfused fibers. For the fibers superfused with epinephrine or phenylephrine in the presence of phenolamine there was less slowing than occurred during superfusion with agonist alone. For all the agonists the maximum change in rate attained in the presence of phenolamine was greater than for the experiment in which agonist alone was studied (compare Figs. 3 and 1).

The effects of propranolol, 2 × 10^{-7} M, were studied in 19 experiments, 11 on fibers superfused with epinephrine and eight with phenylephrine. In 10 of 11 epinephrine-superfused fibers and eight of eight phenylephrine-superfused fibers, the response to agonist was biphasic. The effects of propranolol and phenolamine on the dose-response curves for epinephrine and for phenylephrine are shown in Figure 4. For both epinephrine (A) and phenylephrine (B), propranolol shifted the dose-response curve downward and to the right. However, the peak increase in rate did not differ significantly from that with agonist alone. Phenolamine shifted the curve upward and to the left.

**FIGURE 3** Response of adult Purkinje fibers to epinephrine, isoproterenol, and phenylephrine in the presence of phenolamine, 1 × 10^{-4} M. The biphasic epinephrine curve (seven of 10 fibers), the biphasic phenyllephrine curve (five of seven fibers), and the isoproterenol curve (six of six fibers) are shown. For the epinephrine curve values at 10^{-6} and 10^{-4} through 10^{-4} M differ significantly from control. For the phenyllephrine curve values obtained at 10^{-3} and 10^{-4} to 10^{-4} M differ significantly from control. For the isoproterenol curve values obtained at concentrations ≥10^{-6} M differ significantly from control. Comparing the isoproterenol and epinephrine curves, values at 10^{-9} and 10^{-9} M differ significantly. Comparing the phenyllephrine and epinephrine curves, values at 10^{-4} and 10^{-8} M differ significantly.

**FIGURE 4** Effects of phenolamine, 1 × 10^{-4} M and of propranolol 2 × 10^{-7} M on the response of adult Purkinje fibers to epinephrine (A) and to phenylephrine (B). In panel A, the epinephrine and phenyllephrine-epinephrine curves are reproduced from Figures 1 and 3, respectively. For propranolol and epinephrine, the following curves differ significantly from control: values obtained at epinephrine, 10^{-9} to 10^{-1} M and at 10^{-5} and 10^{-4} M. Comparing the three curves, the control-epinephrine and propranolol-epinephrine curves differ significantly at 10^{-3} and 10^{-4} M. The epinephrine and phenyllephrine-epinephrine curves differ significantly at 10^{-6} through 10^{-4} M. The propranolol-epinephrine and phenyllephrine-epinephrine curves are reproduced from Figures 1 and 3, respectively. For propranolol and phenylephrine, results differ significantly from control at phenylephrine, 10^{-4}, 10^{-5}, and 10^{-6} M. Comparing the three curves, the control phenylephrine and propranolol-phenylephrine curves differ significantly only at phenylephrine, 10^{-4} M. The control phenylephrine and phenyllephrine-phenylephrine curves differ significantly at phenylephrine, 10^{-4} through 10^{-5} M.
The data for the epinephrine-superfused fibers are shown in Table 6. Six fibers developed a biphasic response and four, a monophasic response. The control values for rate, MDP, AV, and slope of phase 4 for both groups of fibers did not differ significantly (Table 6, columns A, and B2). For fibers in which the biphasic response occurred there was no significant change in the MDP and AV at the concentration at which greatest slowing occurred (column A5). There was, however, a significant \( P < 0.01 \) decrease in the slope of phase 4 depolarization (column A3). At the concentrations at which rate was increased maximally for both groups of fibers MDP and AV did not differ significantly from control, and the only significant change was an increase in the slope of phase 4 (columns A3 and B3). Column B2 shows that, in contrast to the biphasic group, the slope of phase 4 begins to increase in the monophasic group when agonist concentrations equal to those in column A2 (biphasic) are administered. Hence it appears that for some fibers (biphasic response, Table 6A) low concentrations of the agonist induce a marked decrease in the slope of phase 4 depolarization and higher concentrations increase the slope. For other fibers (monophasic response, Table 6B) the only change induced by the agonist is an increase in the slope of phase 4.

Pacemaker characteristics were also studied in six fibers superfused with isoproterenol and five with phenylephrine. The small number of experiments prevented a mean-

**FIGURE 5** Effects of epinephrine, isoproterenol, and phenylephrine on spontaneous rates of fibers from neonates. Epinephrine: For the monophasic curve (O), results at \( 10^{-9} \) and \( 10^{-8} \) to \( 10^{-4} \) \( M \) differ significantly from control. For the biphasic curve ( ), results at \( 10^{-8} \) and \( 10^{-7} \) to \( 10^{-4} \) \( M \) differ significantly from control. Isoproterenol: The monophasic curve differs significantly from control at \( 10^{-11} \) through \( 10^{-3} \) \( M \) concentrations. The biphasic curve differs significantly from control at \( 10^{-4}, 10^{-3}, \) and \( 10^{-2} \) to \( 10^{-1} \) \( M \) concentrations. The two curves differ from one another significantly at all concentrations. Phenylephrine: The monophasic curve differs significantly from control at \( 10^{-2} \) and \( 10^{-4} \) \( M \). The biphasic curve differs significantly from control at \( 10^{-4}, 10^{-3}, \) and \( 10^{-2} \) \( M \) concentrations. The two curves differ significantly from one another at all concentrations.

**DIFFERENCES IN AUTOMATICITY BETWEEN NEONATAL AND ADULT FIBERS**

There was no significant difference in the magnitude of the response to phenylephrine of neonatal (Fig. 5) and adult (Fig. 1) fibers. However, there were significant differences in the responses of neonatal and adult fibers to epinephrine and to isoproterenol. For the fibers that generated a biphasic rate response to epinephrine (Fig. 6A), the change in rate at any concentration was greater for the neonate than for the adult. For the fibers showing the monophasic response (Fig. 6B), there was no significant difference between neonates and adults.

**FIGURE 6** Effects of epinephrine on the biphasic and monophasic dose-response curves for neonatal and adult fibers. For differences between neonatal curves and control see Figure 5; for adult curves and control, see Figure 1. The biphasic neonatal and adult curves differ significantly at epinephrine, \( 10^{-2} \) through \( 10^{-4} \) \( M \). There is no significant difference between the monophasic curves.
A, rate increased in B; columns A, and B, = concentration at which maximum increase in rate occurred.

 Nigel and paired Mest.
 concentrations of epinephrine and phenylephrine was di-
 effect on the pacemaker current and phase 4 depolariza-
 response curves such that spontaneous rate increased at
 the presence of phentolamine there was a shift in the dose-
 antagonist. The slowing induced by isoproterenol was
 due to changes in the slope of phase 4 depolarization
 cause both slowing (at low concentrations) and accelera-
 (Table 6, epinephrine). This is consistent with prior stud-
 been shown that a-adrenergic antagonists such as phenoxy-
 has been shown to result in an increase in the response of
 tolamine data should be viewed with some caution because
 this agent may have other effects. For example, it has
 pret the enhancement of the β response to epinephrine
 is suggested by the finding that α-adrenergic blockade
 significantly enhances the peak β response. The β effect of
 the agonists was inhibited by propranolol. The concentra-
 propranolol used was shown not to have significant effects
 on the Purkinje fiber action potential by Davis and Temte (18)
 and by our own study (Tables 4 and 5).

 We have interpreted the slowing of rate as an α-adre-
 nergic effect because it was more prominent and sustained
 (i.e., maintained over a wider range of agonist concentra-
 tions) with epinephrine and phenylephrine (see dose-re-
 sponse curves), two agonists with known α-adrenergic
 capabilities, and less sustained with isoproterenol—the β-
 agonist. The data obtained with phentolamine and pro-
 pranolol support this interpretation. However, the phentol-
 amine data should be viewed with some caution because
 this agent may have other effects. For example, it has
 shown that α-adrenergic antagonists such as phenox-y-
 benzamine may inhibit access of catecholamines to cate-
 choline orthomethyltransferase (COMT). This effect
 has been shown to result in an increase in the response of
 isolated cat papillary muscles to catecholamines. Phen-
 tolamine, too, inhibits COMT. Here, one might interpret
 the enhancement of the β response to epinephrine and
 isoproterenol in the presence of phentolamine as res-
 ulting from an inhibition of COMT or block of access of
 agonist to COMT by phentolamine. This interpretation

 Discussion

 EFFECTS OF ADRENERGIC AMINES ON PURKINJE FIBER AUTOMATICITY

 Isoproterenol, epinephrine, and phenylephrine can cause both slowing (at low concentrations) and acceleration (at high concentrations) of the spontaneous rate of Purkinje fibers. These effects of the agonists appear to be due to changes in the slope of phase 4 depolarization (Table 6, epinephrine). This is consistent with prior studies that have shown catecholamines to exert their major effect on the pacemaker current and phase 4 depolarization.

 The slowing of spontaneous rate induced by low concentrations of epinephrine and phenylephrine was diminished by addition of phentolamine, an α-adrenergic antagonist. The slowing induced by isoproterenol was abolished completely by the β-antagonist. As a result, in the presence of phentolamine there was a shift in the dose-response curves such that spontaneous rate increased at lower agonist concentrations, and the peak rate attained was greater than that which occurred in the presence of agonist alone. In the presence of propranolol, a β-adrenergic antagonist, the peak response to agonist was not decreased for epinephrine or phenylephrine; however, the curve was shifted to the right. Therefore, a higher concentration of agonist was needed to increase rate, and the slowing of rate that occurred was sustained through higher agonist concentrations than observed in the absence of the β-antagonist. Interestingly, the magnitude of the decrease in rate observed with low agonist concentrations was not altered by addition of the β-antagonist.

 These results are consistent with the presence of α- and β-adrenergic receptors in canine Purkinje fibers. The slowing, or α, response was inhibited by the α-receptor blocker phentolamine in a concentration that has been shown previously to have no effect on cardiac cellular resting and action potentials. That the α effect exerts a significant inhibitory action on the acceleratory or β effect is suggested by the finding that α-adrenergic blockade significantly enhances the peak β response. The β effect of the agonists was inhibited by propranolol. The concentration of propranolol used was shown not to have significant effects on the Purkinje fiber action potential by Davis and Temte (18) and by our own study (Tables 4 and 5).

 We have interpreted the slowing of rate as an α-adrenergic effect because it was more prominent and sustained (i.e., maintained over a wider range of agonist concentrations) with epinephrine and phenylephrine (see dose-response curves), two agonists with known α-adrenergic capabilities, and less sustained with isoproterenol—the β-agonist. The data obtained with phentolamine and propranolol support this interpretation. However, the phentolamine data should be viewed with some caution because this agent may have other effects. For example, it has shown that α-adrenergic antagonists such as phenoxbenzamine may inhibit access of catecholamines to catecholamine orthomethyltransferase (COMT). This effect has been shown to result in an increase in the response of isolated cat papillary muscles to catecholamines. Phenolamine, too, inhibits COMT. Here, one might interpret the enhancement of the β response to epinephrine and isoproterenol in the presence of phentolamine as resulting from an inhibition of COMT or block of access of agonist to COMT by phentolamine. This interpretation

 Table 6 Changes in Transmembrane Potentials of Spontaneously Beating Fibers

| Table 6 Changes in Transmembrane Potentials of Spontaneously Beating Fibers |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|
|                                | A,  | A,  | A,  | B,  | B,  | B,  |
| MDP (mV)                       | 83.7 ± 1.57 | 82.2 ± 1.44 | 83.2 ± 1.95 | 83.3 ± 2.08 | 83.7 ± 2.00 | 83.4 ± 0.92 |
| AV (mV)                        | 71.6 ± 0.72 | 70.6 ± 0.86 | 71.5 ± 2.37 | 72.1 ± 1.18 | 72.5 ± 1.45 | 72.0 ± 1.35 |
| Δ (mV)                         | 12.0 ± 1.27 | 11.3 ± 0.93 | 11.7 ± 1.41 | 10.2 ± 1.60 | 11.3 ± 1.63 | 11.4 ± 1.54 |
| t (sec)                        | 3.9 ± 0.78  | 9.3 ± 1.21 | 1.7 ± 0.36  | 6.0 ± 2.41  | 5.3 ± 1.86  | 2.9 ± 0.95  |
| Δ (mV)/t (sec)                 | 3.6 ± 0.62  | 1.4 ± 0.21 | 8.8 ± 1.55  | 2.9 ± 1.01  | 4.1 ± 1.53  | 6.8 ± 2.27  |

AV = activation voltage; t = time; Δ = MDP - AV (mV); Δt = slope of phase 4 (mV/sec); other abbreviations and explanations as in preceding tables.

* P < 0.001, cf. control.  
† P < 0.01, cf. control.  
‡ P < 0.005, cf. control.  
§ P < 0.05, cf. control.
might lead one to conclude that phentolamine was not acting primarily as an α-adrenergic blocker in our studies and, further, to state that slowing of rate is not an α-adrenergic effect. However, in our experiments with phenolamine and phenylephrine, the β response to phenylephrine was increased as compared to the experiments with agonist alone. It is unlikely that COMT plays a major role in the metabolism of phenylephrine because the hydroxyl group on the aromatic ring of this amine is in the meta, not the ortho, position. The slowing of rate achieved with phenylephrine alone and the partial blockade of this action by phentolamine are plausibly interpreted as resulting from an α-adrenergic effect on the Purkinje fiber. Hence, we consider our data to be consistent with α- and β-adrenergic effects of isoproterenol, epinephrine, and phenylephrine on Purkinje fibers, while recognizing that the experiments using α-antagonists may be open to question.

One might speculate that the fibers which develop the monophasic response constitute a population that is devoid of α-receptors; however, we believe this not to be the case. Evidence supporting the presence of α-receptors in Purkinje fibers showing a monophasic response was obtained in the studies of epinephrine and of phenylephrine in the presence of propranolol (Fig. 4). With epinephrine and propranolol 10 of 11 fibers generated a biphasic response (compared to 10 of 15 with epinephrine alone); with phenylephrine and propranolol eight of eight fibers generated a biphasic response (compared to eight of 11 with phenylephrine alone) (Fig. 1). These results suggest that in the presence of β blockade there is a greater tendency for the occurrence of a biphasic curve, consistent with the unmasking of an α-adrenergic effect.

One might, further, consider why in clinical situations or experiments in situ, a biphasic effect on automaticity has not been reported. The only situation in situ which is referable to the experimental situation in our study would be that in which there is atrioventricular block and an idioventricular pacemaker is driving the ventricle. Even in this situation it may be that with cardiac adrenergic input intact the basal catecholamine level available to the receptors that modulate ventricular pacemaker fiber function is greater than the concentration that induces a slowing of rate. By removing adrenergic input to our isolated preparation we simply may have been diminishing catecholamine availability to the receptor site and therefore unmasking the α, or slowing, effect. This interpretation is, of course, speculative and must await experimental verification.

DEVELOPMENTAL CHANGES IN AUTOMATICITY

There were two major differences between the adrenergic responses of the neonatal and adult fibers. First, the proportion of fibers that developed a biphasic response differed markedly between the two groups: for the adults, 28 of the 37 fibers (76%) had a biphasic response; for the neonates 26 of 52 fibers (50%) had a biphasic response. Second, the dose-response curves for epinephrine and isoproterenol in the neonates were significantly displaced upward and to the left of those for the adults. The dose-response curves for phenylephrine were comparable for both.

These results suggest the following: There is an α response capability present in the neonatal fiber, but this is counteracted by the β response to a greater extent than in the adult. When an agent with primary α agonistic effects was studied (phenylephrine) the responses for fibers in the two age groups did not differ. When the effects of epinephrine were studied, the results we obtained were comparable to those of Friedman; that is, the response was greater in the neonatal fibers. Viewing our data in light of Friedman’s interpretation, the greater neonatal response to epinephrine (which is taken up at adrenergic terminals) is consistent with incompletely developed adrenergic terminals in the neonate. In contrast to the results of Friedman, the neonatal fibers we superfused with isoproterenol developed a β response of greater magnitude than that of the adults. However, the difference between the neonatal and adult responses to epinephrine (Fig. 6) clearly was far greater than that to isoproterenol (Fig. 7).

Studies have indicated that both isoproterenol and epinephrine can be metabolized by COMT at nonneuronal sites. Reports of developmental changes in COMT in human liver indicate that this enzyme increases in concentration with age if one compares the fetus to the adult. We are aware of no comparable information for the human or canine heart although studies on the feline heart have shown COMT to be present in the kitten. The fact that the magnitude of the response of neonatal fibers to phenylephrine, an α-adrenergic agonist that probably is not metabolized by COMT, did not differ from that of the adult fibers suggests that differences in COMT may be responsible for the differences between neonates and adults in sensitivity to adrenergic amines. Certainly, immaturity of this enzyme system is a plausible explanation for the finding that responses to isoproterenol and epinephrine were of greater magnitude in the neonatal than the adult hearts, whereas responses to phenylephrine were equal. Another possibility is that the transport mechanism necessary to carry the catecholamines to the intracellular sites at which COMT acts may be less developed in the neonate than in the adult.

The response to epinephrine in the adult can be made to approximate that in the neonate by inducing α blockade. This can be seen by comparing the curves for adult-epinephrine and adult-phentolamine-epinephrine (Fig. 4A) to that for neonate-epinephrine (Fig. 5). Although it is tempting to view this as indicating that a greater sensitivity to α agonists determines the lesser acceleratory response in the adult, the fact that the neonatal and adult responses to phenylephrine are nearly identical makes such an interpretation unlikely.

THE TRANSMEMBRANE ACTION POTENTIAL

Referring to Table 1, it is apparent that for the neonatal fibers, MDP, AP amplitude, $V_{\text{max}}$, APD$_{50}$, and APD$_{90}$ all were significantly lower for the neonate than for the adult. This result is consistent with data from our previous study of ouabain effects on neonatal and adult fibers. Although some differences in values for AP characteristics are seen...
comparing the neonates and adults in this group to the same group in our previous study it must be understood that in the latter all fibers impaled were at the area of maximum AP duration, whereas in the present study we deliberately selected sites at which the least motion was apparent during contraction. The actions of the agonists and antagonists on AP duration are consistent with those described by other investigators, who have identified catecholamine-induced increases in AP duration as α-mediated and decreases in AP duration as β-mediated effects. In addition, epinephrine and isoproterenol induced a small but statistically significant increase in MDP and AP amplitude of adult Purkinje fibers. These results are consistent with earlier studies that have shown epinephrine either to have no effect on or to increase resting membrane potential and AP amplitude of electrically stimulated mammalian cardiac fibers. Phenylephrine had no effect on MDP and amplitude and none of the agonists studied significantly affected MDP, AP amplitude, and $V_{\text{max}}$ in neonatal fibers.

Both phenolamine and propranolol blocked the increases in MDP and AP amplitude induced in Purkinje fibers from adult dogs by epinephrine and isoproterenol. That both antagonists had this effect suggests that the hyperpolarization of normal Purkinje fibers induced by catecholamines may not be either α- or β-receptor-mediated but, rather, may be due to a nonspecific effect of these agonists. This suggestion is consistent with a view that not all catecholamine-induced effects on the cardiac cell are mediated by the α- and β-receptors. For example, Vassalle and Barnabei, have demonstrated that although norepinephrine stimulates the active transport of potassium this action can be dissociated from norepinephrine-induced increases in automaticity. Stimulation of active transport—if it results in an increase in the intracellular potassium concentration—might well induce an increase in membrane potential. Whether such a dissociation of catecholamine effects on automaticity (mediated through α- and β-receptors) and membrane potential (mediated, perhaps, through a direct effect on active transport) does in fact occur must await direct experimental verification.

Acknowledgments

We thank Margaret Alonso for her technical assistance in the performance of many of these studies, and Dinah Lowenstein for typing the manuscript. We also acknowledge, with profound thanks, the advice and encouragement of Drs. Robert M. Weiss and Brian F. Hoffman throughout the performance of these studies and in the preparation of the manuscript, and the comments on the manuscript provided by Dr. Shih-Hsuan Ngai.

References

12. Tsien R: Effects of epinephrine on the pacemaker potassium current of cardiac Purkinje fibers. J Gen Physiol 64: 293-319, 1974

M R Rosen, A J Hordof, J P Ilvento and P Danilo, Jr

Circ Res. 1977;40:390-400
doi: 10.1161/01.RES.40.4.390

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1977 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/40/4/390

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/