Effects of $\alpha$-Adrenergic Stimulation on Intracellular Sodium Activity and Automaticity in Canine Purkinje Fibers

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Approximately 60% of adult canine Purkinje fibers respond to $\alpha$-adrenergic stimulation with a decrease in automaticity. Recent studies of disaggregated Purkinje myocytes have suggested that this negative chronotropic effect results from $\alpha_1$-adrenergic activation of the Na-K pump. In this study we evaluated 1) whether Na-K pump activation is associated with the negative chronotropic effect of $\alpha_1$-adrenergic stimulation in adult canine Purkinje fibers and 2) if the effect of $\alpha$-agonists on the pump is direct or mediated by an increase in intracellular sodium activity ($aNa_k$). We used sodium selective microelectrodes to determine the effects of $5 \times 10^{-9}$ and $5 \times 10^{-8}$ M phenylephrine on $aNa_k$. Phenylephrine decreased automaticity in five of eight Purkinje fibers while an increase occurred in the other three. The rate decrease was always accompanied by a decrease in $aNa_k$ ($-3.9 \text{ mM; } p<0.05$), whereas in fibers showing an increase in rate, $aNa_k$ was unchanged. To evaluate the effect of phenylephrine in the absence of changes in automaticity, 10 Purkinje fibers were studied during pacing. A clear-cut reduction in $aNa_k$ ($-2.8 \text{ mM}$) was present in six fibers; no change was seen in the other four. The effect of phenylephrine was blocked by prazosin but not by propranolol. We conclude that the effect of $\alpha_1$-adrenergic stimulation to reduce $aNa_k$ is consistent with activation of the Na-K pump. Moreover, this action of $\alpha_1$-adrenergic stimulation is closely linked to its negative chronotropic effect. (Circulation Research 1990;66:416–426)

Supports of adult canine Purkinje fibers in vitro have shown that $\alpha_1$-adrenergic stimulation may either increase or decrease automaticity.\textsuperscript{1,2} Concentrations of $\alpha$-agonist less than $10^{-8}$ M induced a negative chronotropic response in approximately two thirds of preparations and an increase in automaticity in the remaining one third.\textsuperscript{3} Both the enhancement and the depression of automaticity result from postsynaptic $\alpha_1$-receptor stimulation\textsuperscript{4} but may operate via different receptor-effector pathways. To illustrate, our previous studies of canine Purkinje fibers in vitro and rat myocardium in tissue culture have shown that the negative chronotropic effect is mediated by a 41 kD GTP binding protein. However, conversion to a positive chronotropic effect is seen when this protein is ADP-ribosylated by pertussis toxin.\textsuperscript{4,5} We also found that in fibers not treated with pertussis toxin the population that increased in rate after $\alpha_1$-adrenergic stimulation tended to have a lower concentration of the 41 kD GTP binding protein than the population that decreased in rate.\textsuperscript{3} This finding and the additional fact that the depressant effect of $\alpha$-agonists on automaticity is abolished when the membrane is depolarized\textsuperscript{6} suggest that in any tissue preparation an equilibrium may exist between different mechanisms that exert opposing effects but are modulated by $\alpha_1$-receptors. The prevalence of one particular mechanism would determine the direction of the change in chronotropy.

A recent study\textsuperscript{7} has suggested that activation of the Na-K pump, generating an outward current, could well explain the negative chronotropic effect of $\alpha_1$-adrenergic stimulation. Furthermore, Na-K pump activation could result primarily from receptor stimulation or could be an epiphenomenon of the activation of pathways leading to an increase in intracellular sodium activity ($aNa_k$). One possible candidate for the latter could be the phosphatidylinositol cascade.\textsuperscript{8,9} A chance to discriminate between primary and secondary mechanisms for Na-K pump activation during $\alpha_1$-adrenergic stimulation is afforded by the fact that $aNa_k$ would be decreased by primary activation of the pump and would be increased in the second case. With this in mind, we designed the present study to evaluate the effect of $\alpha_1$-adrenergic stimulation on...
aNa, and to investigate whether the effect on aNa could be related to the chronotropic effect.

**Materials and Methods**

Mongrel dogs of either sex were anesthetized with 30 mg/kg i.v. sodium pentobarbital. The heart was quickly removed through a left thoracotomy and immersed in cold, oxygenated Tyrode’s solution. Free-running Purkinje fibers were dissected from both ventricles and pinned to the Sylgard bottom of a 12-ml Lucite chamber. The preparations were superfused at a rate of 12 ml/min with Tyrode’s solution prewarmed to 37±0.5°C and gassed with 95% O2-5% CO2. Temperature was monitored during the entire experiment by means of a thermistor probe (Yellow Springs Instrument, Yellow Springs, Ohio) placed near the fiber. The composition of the Tyrode’s solution (mM/l) was: NaCl 131, NaHCO3 18, KCl 4, dextrose 5.5, NaH2PO4 1.8, MgCl2 0.5, and CaCl2 2.7.

Single barrel sodium-selective microelectrodes were pulled from borosilicate glass (World Precision Instruments, New Haven, Connecticut), made hydrophobic by silanization (N-trimethylsilyldimethylamine, Serva Feinbiochemica, New York, New York), and back-filled with 160 mM NaCl solution containing 0.1 mM EGTA and buffered to pH 7.4 with 1 mM HEPES. The tip of the electrode was then immersed for 30 seconds in a resin cocktail containing the neutral carrier ETH 227 (Fluka Chemical, Hauppauge, New York). Stable filling of the electrode tip with a column of resin less than 500 μm high was obtained by this procedure.

Sodium-selective electrodes were calibrated before and after each experiment in standard Tyrode’s solution and in a set of five calibrating solutions containing NaCl and KCl in different proportions, summing to a constant total ionic strength of 160 mM ([Na+] /[K+]: 160/0, 130/30, 100/60, 10/150, and 5/155 mM). The calibrating solutions contained 0.1 mM EGTA and were buffered to pH 7.4 with 1 mM HEPES. The sodium electrode output was offset to zero in the Tyrode’s calibrating solution and then, after calibration, in the tissue bath Tyrode’s solution. Both Tyrode’s were identical. Vm output was also offset to zero in the tissue bath.

The calibration procedure was as follows: An approximated Nernstian slope (Bapp) was obtained by log-linear fitting of the potentials measured in the first three calibrating solutions ([Na]/[K]: 160/0, 130/30, and 100/60). With an expected maximum K/Na permeability ratio of 1/40,10 the change of [K] among these three solutions (60 mM) would reduce the total potential difference by less than 3% and was therefore considered negligible. The K/Na selectivity coefficient was computed by entering the potential difference recorded between the first ([Na]/[K]: 160/0) and fifth ([Na]/[K]: 5/155) calibrating solution in the Nicolsky-Eisenmann equation11 with the Nernstian slope set equal to Bapp. The Ca/Na selectivity coefficient was similarly calculated from the potential difference measured between Tyrode’s solution ([Na]/[Ca]: 137/2.7) and the second calibrating solution ([Na]/[Ca]: 130/0). The potential values recorded in all the six solutions were then corrected according to the K/Na and Ca/Na selectivity coefficients and fitted by log-linear regression. The regression coefficient, corrected for the temperature difference between the calibrating solutions and the tissue bath, was assumed as the electrode Nernstian slope. The calibration method was published previously.12 Intracellular free potassium and calcium concentrations were assumed to be 151 mM and 300 nM, respectively,12 for computation of the measured aNa. Free ion concentrations of sodium were calculated using the Nicolsky-Eisenmann equation and inserting the known ion concentration values, the calculated selectivity coefficients, and the measured sodium-selective microelectrode differential output. The free ion concentrations were then converted to the respective activities assuming a constant activity coefficient value of 0.74.13 Only electrodes with a Nernstian slope of 48 mV or more per decade were used. Average values for selectivity coefficients: KNaK= 0.023±0.003; KNaCa= 1.27±0.4.

Transmembrane potentials (reference potentials) were recorded by a microelectrode pulled from the same glass and with the same settings as the sodium-selective one (but filled with 3 M KCl) and inserted into the fiber bundle within 1 mm of the ion-sensitive microelectrode. Maximal capacity compensation was applied to the ion-sensitive electrode, while the electrode response of the reference electrode was delayed with a low-pass filter. The frequency response of the low-pass filter (adjustable between 1 Hz and 1 kHz) was set to make the potential time course of the reference barrel match that of the slowest components of the ion-sensitive barrel. Sodium-selective and reference potentials were fed into a high-input impedance (1014 Ω), low-bias current (<75 F) amplifier (Bloom Associates, Reading, Pennsylvania), and an analog subtraction was performed to obtain the net sodium potential (ENa). The reference potential was also separately recorded as the unfiltered output of a standard microelectrode amplifier with capacity compensation for evaluation of action potential parameters at a higher frequency response. The general features of the recording system have been described previously.14

The amplified signals from the reference and sodium-selective electrodes were fed into a digital programmable oscilloscope (Data 6000, Analogic Data Precision, Danvers, Massachusetts), allowing simultaneous sampling from up to four separate input signals at two different sampling rates (A/D converter resolution, 14 bit; input range, 0.5 V; maximum sampling rate, 100 kHz). The action potential recorded from the reference electrode was sampled at 4 kHz, and the action potential parameters were automatically measured for every beat using a program triggered by the end of the waveform acquisition process. The values of the parameters com-
puted from each action potential were then stored as consecutive data points in a separate buffer, called "trend," and transferred to disk at the end of the experiment. The action potential waveforms, the delimiters assisting the parameter computation, and the forming trends buffer were directly visualized during the entire experiment. Action potential parameters at steady state and before and during each intervention were determined off-line by placing a cursor over the appropriate region of the trend buffer display and computing the mean value of the points included in the cursor (at least 10). Therefore, parameter computation was totally automatic, with an accuracy of ±30 μV and ±125 μsec (determined from the characteristics of the A/D converter) and obtained as the mean of at least 10 measurements.

To demonstrate system performance, in Figure 1 we include data from a well-studied phenomenon, the fall in aNa, after an abrupt increase in cycle length. This illustrates how changes in E\textsubscript{Na} of only 1 mV and of aNa of 0.2–1.0 mM can readily be measured and tabulated. In Figure 1, values of E\textsubscript{Na} were obtained from the digitized signal during every cycle. Panels A and B represent the actual E\textsubscript{Na} signal sampled at 250 Hz, and the horizontal bars in panels A and B correspond to the position of a cursor 200 msec in duration. The position of the cursor was chosen at the beginning of each experiment by moving it along the flat portion of the signal until the voltage difference between the extremes of the cursor (directly displayed on the screen) was negligible. Flatness of the portion of the tracing on which the cursor was positioned was also controlled by taking the mean time derivative of the points included in the cursor and verifying that its absolute value was less than 50 μV/sec. Within each experiment the cursor, once positioned, was kept at a constant interval after the preceding action potential, irrespective of changes in the duration of the diastolic interval. Figure 1 also shows that the portion of the tracing on which the cursor was positioned did not drift, even when the cycle length was increased by 1,000 msec.

The mean values of the points included in the cursor at each cycle were stored as consecutive data points in a trend buffer file (Figure 1C).

Figure 1 shows that an increase in cycle length from 2,500 to 3,500 msec results in a slow 1.1 mV negative shift of E\textsubscript{Na}, corresponding in this experiment to a 0.25 mM change in aNa. The relative scatter of points in the trend is due to low-frequency random noise. The error introduced by this type of noise was minimized by averaging the largest possible number of E\textsubscript{Na} data points (at least 10) included in a cursor placed on the steady-state portions of the trend before and after the experimental intervention.

This procedure resolves E\textsubscript{Na} changes as small as 1 mV. Therefore, a 1 mV change in E\textsubscript{Na} was assumed as the resolution of our system throughout the study. Since each electrode has a different sensitivity and the relation between E\textsubscript{Na} and aNa, is logarithmic, a 1 mV change in E\textsubscript{Na} corresponded to different changes in aNa, according to the slope coefficient of the electrode and the value of aNa, from which the change took place. In our study, the resolution, expressed in change of aNa, ranged from 0.2 to 1 mM in different experiments. Thus, the sodium electrode used in Figure 1 had better than average

![Figure 1](http://circres.ahajournals.org/content/66/2/418/F1)

**Figure 1.** Example of on-line determination of net sodium potential (E\textsubscript{Na}) from digitized signals (for details see text). Panels A and B: Digitized E\textsubscript{Na} signal, as displayed on the oscilloscope during the experiment, at drive cycle lengths of 2,500 msec (panel A) and 3,500 msec (panel B). The spikes are artifacts due to the different frequency responses of the reference and ion-selective electrodes during an action potential. The horizontal bar marks the position of a cursor 200 msec in duration; the cursor was set at a fixed time after the upstroke of the action potential artifact on the differential output. For each cycle, the points included in the cursor were averaged, and the mean value was stored as a data point in a separate buffer called "trend," shown in panel C. Panel C: Plot of the trend of E\textsubscript{Na} values during the change in rate. Since data points are plotted with equal spacing irrespective of the cycle length and there is only one data point per cycle, the horizontal axis is not a linear time scale except when the cycle length is constant. Arrows mark the points computed from the signals shown in panels A and B. The period during which stimulation cycle length increased from 2,500 to 3,500 msec is marked by the crosshatched bar. At basic cycle length of 3,500 msec, after a lag of several cycles, there was a negative shift of E\textsubscript{Na}, corresponding to a change in intracellular sodium activity of 0.25 mM. Close to the end of panel C, stimulation at the faster rate was resumed (end of crosshatched bar), and E\textsubscript{Na} began to rise again. The values of E\textsubscript{Na}, before and after the change in rate, were computed off line by averaging the points of the trend included in a cursor placed in the positions marked by the solid bars. E\textsubscript{Na} at basic cycle length of 2,500 msec was \(-81.475±0.056\) mV (mean±SEM), and at basic cycle length of 3,500 msec, it was \(-82.614±0.038\) mV.
selectivity and sampled a cell with lower than average aNa$_i$. The recording technique described above was developed to allow resolution of small changes in aNa$_i$ in beating fibers, without filtering the output of the ion selective and reference electrodes as suggested by Dagostino and Lee.\textsuperscript{11} We did this because of concern that the use of the filter might obscure changes in aNa$_i$ that occur in experiments such as ours in which rate and action potential shape vary.

Finally, the frequency response characteristics of the sodium-selective electrode limited the maximal rate of action potential firing at which aNa$_i$ could be measured reliably. At best, the frequency response was such that aNa$_i$ was measured reliably at rates of 30 beats/min. For this reason, all experiments were performed in a setting in which both the spontaneous and paced rates selected were sufficiently low to permit full equilibration of the aNa$_i$ measurement. Paced fibers were stimulated by standard techniques and a bipolar Teflon-coated silver electrode.\textsuperscript{13}

In the first group of experiments, the effect of $\alpha_1$-adrenergic stimulation on aNa$_i$ was correlated with its effect on automatic rate. In these experiments, drug superfusion was begun after the rate had attained a stable value for at least 20 minutes (overall equilibration period of about 1 hour). The rate was then averaged over 5-minute intervals before drug superfusion and at each steady-state drug effect. In a second group of experiments, we analyzed the net effect of $\alpha_1$-adrenergic stimulation on aNa$_i$, in the absence of changes in automaticity, in fibers stimulated at a constant rate. For both experimental protocols, E$_{na}$ was required to be constant within 0.5 mV before the experiment could begin; fibers were then superfused with 5×10$^{-9}$ and 5×10$^{-8}$ M phenylephrine for two consecutive 20-minute periods.

In experiments studying effects of $\alpha$- or $\beta$-adrenergic blockade, 1×10$^{-6}$ M prazosin or 2×10$^{-7}$ propranolol was first superfused alone, and then phenylephrine was added. We selected these concentrations because our previous studies indicated these are the highest ones at which no significant direct effects on the transmembrane potential or automaticity occur.\textsuperscript{3} The source of phenylephrine and propranolol was Sigma Chemical, St. Louis, Missouri. Prazosin was a generous gift from Pfizer, Groton, Connecticut.

**Statistical Analysis**

Within each experiment, a change in aNa$_i$ is defined as a positive or negative shift of E$_{na}$ of 1 mV or more (electrode resolution). In addition to aNa$_i$ and automatic rate, the variables measured were maximum diastolic potential (E$_{max}$) and action potential duration at 50% repolarization (APD$_{50}$). All results are expressed as mean±SEM. Changes in the mean values of measured variables at different drug concentrations were analyzed by analysis of variance for repeated measurements with repetitions for a single factor. Post hoc comparisons were performed by means of Tukey's test. Differences among proportions were evaluated by $\chi^2$ analysis. Correlations were determined by least-squares linear regression. All analyses were performed using the SYSTAT statistical software package.

**Results**

**Effects of Phenylephrine on Automatic Fibers**

Eight fibers were studied. Control values for aNa$_i$, automatic rate, and action potential parameters are shown in Table 1, and the changes in aNa$_i$ and rate induced by phenylephrine are in Figure 2. Phenylephrine induced a decrease in rate (~29% from 12.6±3.2 beats/min) in five fibers (group A), whereas an increase occurred in the remaining three fibers (group B) (~14% from 25.0±12.1 beats/min). Control values for rate, aNa$_i$, and action potential parameters did not differ significantly between the two groups (Table 1). In both groups E$_{max}$ was not significantly changed by phenylephrine whereas APD$_{50}$ was prolonged (from 287±40 to 359±52 msec; $p<0.05$) only in the group showing a decrease in automaticity.

**TABLE 1. Control Values for Spontaneously Beating Fibers**

<table>
<thead>
<tr>
<th></th>
<th>Rate (beats/min)</th>
<th>aNa$_i$ (mM)</th>
<th>E$_{max}$ (mV)</th>
<th>APD$_{50}$ (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A, n=5</td>
<td>12.6±3.2</td>
<td>9.9±1.5</td>
<td>-87.9±6.6</td>
<td>307±41</td>
</tr>
<tr>
<td>Group B, n=3</td>
<td>25.0±7.0</td>
<td>8.2±1.4</td>
<td>-89.1±1.6</td>
<td>297±58</td>
</tr>
<tr>
<td>Overall</td>
<td>17.3±3.7</td>
<td>9.3±1.2</td>
<td>-88.3±1.8</td>
<td>304±31</td>
</tr>
</tbody>
</table>

aNa$_i$, intracellular sodium activity; E$_{max}$, maximal diastolic potential; APD$_{50}$, action potential duration at 50% repolarization; $\alpha_1$-adrenergic fibers in which phenylephrine induced a rate decrease; Group B, fibers in which an increase in rate was observed; Overall, pooled values from Groups A and B. None of the differences among the values listed here are significant ($p>0.05$).

![Figure 2. Plots showing effects of phenylephrine on intracellular sodium activity (aNa$_i$) (panel A) and on spontaneous rate (panel B). Results are expressed as changes from control (C). The white circles represent five fibers whose rate decreased on superfusion with phenylephrine. The black circles represent three fibers whose rate increased. See Table 1 for control values. *p<0.05 vs. control.](http://circres.ahajournals.org/abstracts/download/320143522.png)
In the fibers showing a decrease in rate, aNa, decreased from 9.9±1.5 to 5.8±1.7 mM at 5×10⁻⁹ M phenylephrine and to 6.1±1.3 mM at 5×10⁻⁸ M phenylephrine (p<0.05). The decreases in E₉ at the two phenylephrine concentrations were by 9.5±3.1 and 8.1±2.9 mV, respectively. The decrease in aNa, was observed in all fibers from this group and ranged from 9% to 83% of the control value. Figure 3 depicts a representative experiment. In the three fibers showing an increase in rate, aNa, was unchanged (8.2±1.4 mM during control, 8.5±1.2 mM at 5×10⁻⁹ M phenylephrine, and 8.7±1.4 mM in 5×10⁻⁸ M phenylephrine).

Figure 4 demonstrates the relation between the changes in aNa, and in rate during phenylephrine superfusion. The points derived from the experiments in which rate decreased could be fitted by a line (r=0.99; p<0.05) with a regression coefficient of 0.57 mM/beats/min. The experiments in which rate increased could not be fitted to the same line.

**Effects of 5×10⁻⁹ and 5×10⁻⁸ M Phenylephrine During Drive at a Constant Rate**

To estimate the effect of α₁-adrenergic stimulation on aNa, in the absence of rate changes, 10 fibers were

**Figure 3.** A representative experiment on a spontaneously beating fiber. Top panel: Intracellular sodium activity (aNa) is plotted for every second action potential, before and during phenylephrine (phe) superfusion. aNa values were obtained by entering net sodium potential (E₉ₐ) data points and the electrode calibration parameters in a computer program based on procedures described in “Materials and Methods.” When aNa reached a plateau during exposure to the first concentration, the second concentration was started (arrows) and was maintained during the remainder of the recording. An overall time reference is given on the horizontal scale. The tracing is interrupted to permit the visualization of a longer recording time. Bottom panel: Analog recordings of the transmembrane potential (E₉ₐ) and of the difference between the sodium-sensitive electrode potential and E₉ₐ, representing E₉ₐ. The recordings were made during steady state in control and during phe superfusion. During phe superfusion, a decrease in aNa was accompanied by a decrease in automatic rate.

**Figure 4.** Graph showing relation between changes in rate and intracellular sodium activity (aNa) induced by phenylephrine in spontaneously beating fibers. Only the points representing the experiments in which rate decreased during α₁-adrenergic stimulation (white circles) were fitted by the regression line. Black circles are those fibers in which rate increased. The regression function and its correlation coefficient (R) are also shown.
studied during constant pacing. A representative experiment is shown in Figure 5A. To effect a compromise between the requirements imposed by each electrode’s frequency response and the need to overdrive the spontaneous rhythms of individual fibers, we varied the pacing rates among experiments (range, 12-20 beats/min). However, within each experiment the stimulation rate was kept constant. Control values of \( aNa_v \), \( E_{\text{max}} \), and \( \text{APD}_{50} \) are shown in Table 2. Overall, phenylephrine induced a decrease in \( E_{\text{Na}} \) of 3.3±1.1 mV and in \( aNa_v \) from 9.1±1.4 to 7.4±0.9 mM at \( 5 \times 10^{-8} \) M \((p<0.05)\); \( \text{APD}_{50} \) was prolonged from 308±24 to 408±41 msec \((p<0.05)\), and \( E_{\text{max}} \) increased from \(-83.5±2.4 \) to \(-85.6±2.0 \) mV \((p<0.05)\).

Even in these driven fibers, the response to phenylephrine superfusion was not homogeneous; in some

Figure 5. Effects of phenylephrine alone and in the presence of \( \alpha \)- or \( \beta \)-blockade. Top left panel: A representative experiment in a driven fiber. Intracellular sodium activity (aNa) is plotted for every second action potential, before and during phenylephrine (phe) superfusion. aNa values were obtained by entering net sodium potential (E\(_{\text{Na}}\)) data points and the electrode calibration parameters in a computer program based on procedures described in “Materials and Methods.” When aNa reached a plateau during exposure to the first concentration, the second concentration was started (arrow) and was maintained during the remainder of the recording. An overall time reference is given on the horizontal scale. Only the effect of phe is shown. Lower section of this panel shows analog recordings of the transmembrane potential (E\(_{\text{m}}\)) and of the difference between the sodium-sensitive electrode potential and E\(_{\text{m}}\), representing E\(_{\text{Na}}\). The recordings were made during steady state in control and during phe superfusion. During phe superfusion, a decrease in aNa was accompanied by a decrease in automatic rate. Top right panel: Effect of phe in the presence of \( \alpha \)-blockade with prazosin (praz). Praz was started before and was continued during phe superfusion. Left panel: Effect of phe in the presence of \( \beta \)-blockade. Propranolol (prop) superfusion was started before the beginning of the digital record displayed and continued during phe superfusion. Only the effect of the first dose of phe is shown. The last action potential of the chart relative to the control panel is shown at a rapid paper speed (5 mm/sec).
there was a clear-cut decrease in $aNa_i$ (range, $-12\%$ to $-39\%$), and in others, no change. This was not unexpected, because two different types of response to phenylephrine had been recognized in the previous set of experiments in unpaced fibers, in which the rate was allowed to vary (Figure 2). Therefore, we also analyzed the data by dividing the paced fibers into two groups: those showing a decrease in $aNa_i$ of more than $8\%$ and those showing a lesser decrease. The argument for arbitrarily selecting a cutoff is as follows: In previous studies,2,3,15 we have shown that the automatic response to $\alpha_1$-adrenergic stimulation is such that fibers fall into one of two significantly different subgroups, one that increases in automaticity and one that decreases. We also have shown that the inhibitory $\alpha_1$-adrenergic response depends on the presence of a 41 kD GTP regulatory protein that is a pertussis toxin substrate; fibers decreasing in automaticity have this substrate in larger amounts than those that do not.5 The presence of this protein is independent of heart rate and, in voltage clamp experiments, is essential if $\alpha_1$-adrenergic stimulation is to stimulate Na-K pump current.7 Finally, in the present study of automatic fibers, we found that about two thirds showed a decrease in automaticity and in $aNa_i$ on phenylephrine superfusion (Figure 2). The information just reviewed demonstrates that two statistically different groups exist using two different descriptors relating to $\alpha_1$-adrenergic response (automaticity and G protein). Therefore, we considered it likely that even in paced fibers, the change in $aNa_i$ that occurs as a result of pump current stimulation by $\alpha_1$-agonist should be distributed into two groups with a proportion similar to that in our other experiments (i.e., 2/3:1/3). A specific cutoff of $8\%$ was selected because the automatic fibers manifesting decreased automaticity showed decreases in $aNa_i$ of $9\%$ or more. The analysis (Figure 6) demonstrated that $5 \times 10^{-6}$ M phenylephrine reduced $E_{Na}$ by $3.5 \pm 1.2$ mV and $aNa_i$ from $10.9 \pm 1.9$ mM to $9.4 \pm 1.4$ mM in six fibers (group A). At $5 \times 10^{-6}$ M phenylephrine, $E_{Na}$ was reduced by $5.5 \pm 1.2$ mV, and $aNa_i$ was $8.1 \pm 1.4$ mM ($p<0.05$). Phenylephrine had no effect in the remaining four fibers (group B) ($6.3 \pm 0.6$ mM control and $6.3 \pm 0.6$ mM in $5 \times 10^{-6}$ M phenylephrine). The group showing the decrease in $aNa_i$ had a higher control $aNa_i$ ($p<0.05$) but had similar $E_{max}$ and $APD_{50}$ to the other group (Table 2). Hence, the use of the cutoff provided a proportion of $aNa_i$ change in driven fibers entirely consistent with that in automatic fibers. Moreover, the absolute values of $aNa_i$ attained in the experiments on driven fibers were such that no changes in the range of $-4\%$ to $-11\%$ occurred (i.e., the change was $\geq -12\%$ or $\leq -3\%$). This indicates that despite potential concern over the accuracy of discriminating small changes in the $-8\%$ range, this was not a problem in the experiments performed.

The effects of phenylephrine on $APD_{50}$ did not differ between the two groups of paced fibers. A significant hyperpolarization (from $-81.1 \pm 1.4$ to $-84.0 \pm 1.7$ mV; $p<0.05$) was observed only in the group in which $aNa_i$ did not change. In the group showing a decrease in $aNa_i$, changes in $E_{max}$ were nonhomogeneous: hyperpolarization occurred in four fibers, and a slight depolarization occurred in two (overall change from $-85.2 \pm 3.8$ to $-86.7 \pm 3.1$ mV; $p>0.05$). No correlation was present between the changes in $aNa_i$ and $E_{max}$ in this group.

**Effects of Phenylephrine in the Presence of $\alpha$- and $\beta$-Adrenergic Blockade**

In another six fibers that were paced at a constant rate, $1 \times 10^{-6}$ M prazosin was administered before and during superfusion with $5 \times 10^{-6}$ M phenylephrine (the concentration found maximally effective in the previous experiments). A representative tracing is shown in Figure 5B. Prazosin alone did not alter $aNa_i$. In the presence of prazosin, phenylephrine had no effect on $aNa_i$ (Figure 7) and on $APD_{50}$, although a slight hyperpolarization still was present ($E_{max}$ changed from $-80.9 \pm 2.2$ mV to $-81.8 \pm 0.2$ mV; $p<0.05$). A phenylephrine-mediated decrease in $aNa_i$ occurred in one of the six fibers tested but was less

**TABLE 2. Control Values for Fibers Driven at a Constant Rate**

<table>
<thead>
<tr>
<th>Group</th>
<th>Rate (beats/min)</th>
<th>$aNa_i$ (mM)</th>
<th>$E_{max}$ (mV)</th>
<th>$APD_{50}$ (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>11.5±1.1</td>
<td>10.9±1.9*</td>
<td>-85.1±3.8</td>
<td>291±22</td>
</tr>
<tr>
<td>Group B</td>
<td>12.1±1.2</td>
<td>6.3±0.6*</td>
<td>-81.1±1.4</td>
<td>330±44</td>
</tr>
<tr>
<td>Overall</td>
<td>11.7±0.8</td>
<td>9.1±1.4</td>
<td>-83.5±2.4</td>
<td>308±24</td>
</tr>
</tbody>
</table>

$aNa_i$, intracellular sodium activity; $E_{max}$, maximal diastolic potential; $APD_{50}$, action potential duration at 50% repolarization; Group A, fibers showing a decrease in $aNa_i$ (>8% of the control value); Group B, fibers showing no change; Overall, pooled values from Groups A and B.

*p<0.05 when the two groups are compared.
than 8%. Overall aNa was 9.3±1.9 mM in prazosin alone and 9.4±1.9 mM in phenylephrine plus prazosin (p>0.05) (range of aNa changes, −7.7% [ΔE\textsubscript{Na}−1.65 mV] to +13.2% [ΔE\textsubscript{Na}+2.2 mV] of control). Based on the previous findings, the likelihood of six consecutive fibers failing to show a decrease in aNa of greater than 8% (in the absence of β-blockade) are given by (0.4)\textsuperscript{6} = 0.004. Therefore, we conclude that prazosin inhibited the effect of phenylephrine on aNa.

In the presence of 2×10\textsuperscript{-7} M propranolol, phenylephrine reduced E\textsubscript{Na} by 8.7±0.9 mV and aNa from 11.0±0.8 mM to 7.5±0.7 mM (p<0.05) in three of five fibers. Of the two remaining fibers, aNa did not change in one and increased in one (from 5.1 to 6.1 mM) (Figures 5C and 7). Thus, both the magnitude of aNa reduction and the proportion of fibers in which this effect was observed were similar in the presence or absence of β-adrenergic blockade.

Effect of Rate Changes on aNa

To assess the role of automatic rate changes per se on aNa, two quiescent fibers were paced at a given rate during superfusion with 5×10\textsuperscript{-8} M phenylephrine, until aNa reached a plateau value. Pacing was then discontinued, allowing complete recovery. The difference between aNa immediately after discontinuation of pacing and after complete recovery was measured for various pacing rates. The mean aNa in the quiescent state was 6.5 mM; it increased by 1.27 mM at 60 beats/min, by 0.42 at 30 beats/min, and by 0.30 mM at 20 beats/min (Figure 8).

Discussion

We have shown that α\textsubscript{1}-adrenergic receptor stimulation decreased aNa in nearly two thirds (61%) of Purkinje fibers studied and had no effect on aNa in the remainder. We also have shown that the negative chronotropic effect of α\textsubscript{1}-adrenergic stimulation is closely related to the decrease induced in aNa. In this regard, it is worth noting that the proportion of fibers responding with a decrease in automaticity is very consistent in this and previous studies\textsuperscript{5} and that this same proportion describes the fibers responding with a decrease in aNa.

Changes in Purkinje fiber automaticity may result from effects on different ionic conductances: the Na-K electrogenic pump, the background potassium conductance, and the pacemaker current. The ionic mechanisms underlying the chronotropic effects of α\textsubscript{1}-adrenergic stimulation were addressed in a recent study by Shah et al\textsuperscript{7} on isolated canine Purkinje myocytes. Here, phenylephrine induced an increase in the outward current carried by the Na-K pump and a decrease in background potassium conductance (presumably iK\textsubscript{s}). Shah et al\textsuperscript{7} used a higher phenylephrine concentration (100 nM) than was the case in the present study. Their intent was to induce a maximal effect of α\textsubscript{1}-adrenergic stimulation. In our study, the effects of 5 and 50 nM phenylephrine on aNa, did not differ from one another. Moreover, in preliminary studies\textsuperscript{16} we used concentrations as high as 100 nM and found no additional effect. Hence, the overall concentration range in the present study and that of Shah et al is comparable, and the data may be viewed as reflecting similar pharmacological actions. Shah et al found that the effects of phenylephrine on Na-K pump current and background potassium conductance were blocked by prazosin and were mediated by a 41 kD GTP binding protein, inactivated by pertussis toxin. Both these findings fit with previous
observations showing that $\alpha_1$-adrenergic stimulation increases myocardial potassium uptake in the pig\textsuperscript{17} and prolongs the action potential in Purkinje fibers.\textsuperscript{18}

Our finding that the negative chronotropic effect of $\alpha_1$-adrenergic stimulation is closely associated with a decrease in aNa\textsubscript{1}, strongly supports the notion that activation of the Na-K pump plays a role in the $\alpha_1$-mediated decrease of automaticity. This view is strengthened by the earlier observation\textsuperscript{7} that the phenylephrine-induced outward current is attenuated in the presence of dihydrouabain. However, in pursuing this interpretation we must exclude other possible sources of a relation between aNa\textsubscript{1} and rate changes.

For example, a reduction in the rate of impulse initiation can, of itself, result in a decrease in aNa\textsubscript{1} due to less sodium influx per unit time\textsuperscript{17} (Figure 1). In our experiments, a phenylephrine-induced 1 beat/min reduction in rate was associated with a 1.75 mM decrease in aNa\textsubscript{1} (see Figure 4), that is, a change far beyond that expected due to a rate change alone, especially at the slow rates studied. According to Cohen et al.,\textsuperscript{19} for example, one might expect a change of approximately 0.03 mM/beat/min. As a further test, in two experiments (see Figure 8) we studied the effect on aNa\textsubscript{1} of pacing fibers to different rates in the presence of phenylephrine. Consistent with our hypothesis, rate changes between quiescence and 30 beats/min resulted in changes in aNa\textsubscript{1} smaller than 0.5 mM, implying that phenylephrine-induced aNa\textsubscript{1} decreases were not secondary to the slowing of rate. Moreover, from Figure 4 it is clear that the relation between rate and aNa\textsubscript{1} changes, found in the presence of a negative chronotropic response, did not hold true for the three fibers in which automaticity increased during phenylephrine superfusion. The most likely cause of the change in aNa\textsubscript{1} is thus Na-K pump stimulation.

The direct effects of $\alpha_1$-adrenergic stimulation on aNa\textsubscript{1} were more accurately studied in the experiments in which rate changes were eliminated by constant rate pacing. The finding, in these experiments, of a primary decrease in aNa\textsubscript{1} leads us to reject the hypothesis that $\alpha_1$-adrenergic stimulation might increase Na-K pump activity indirectly (i.e., by raising aNa\textsubscript{1}). A primary decrease in aNa\textsubscript{1} during norepinephrine superfusion has also been reported recently by Wang et al.\textsuperscript{20} Our results support the view of specific linkage of the receptor to the Na-K pump, analogous to what has been shown for other hormones.\textsuperscript{21}

The decrease seen in aNa\textsubscript{1} might be attributed to Na-K pump activation and/or to a decreased sodium influx through channels or other ion exchangers. We believe that sodium influx is unlikely to be reduced for the following reasons: 1) The absence of effects of $\alpha_1$-adrenergic stimulation on the maximum velocity of the action potential upstroke\textsuperscript{22} suggests lack of a major influence on fast sodium channel conductance. 2) $\alpha$-Agonists stimulate intracellular metabolic pathways such as the phosphoinositide cascade\textsuperscript{22,23}; this stimulation leads to an increase in intracellular free calcium and, potentially, to a diacylglycerol-mediated activation of the Na-H exchanger.\textsuperscript{24,25} Both these effects should result in an increase in aNa\textsubscript{1}, which we did not see, rather than a decrease. Finally, it is possible that the lack of effect of phenylephrine on aNa\textsubscript{1} in one third of our experiments was due to a degree of pump activation insufficient to completely offset an increase in aNa\textsubscript{1} induced by other effects of $\alpha_1$-adrenergic stimulation. Nonetheless, it seems reasonable to conclude that the measured decrease in aNa\textsubscript{1} was the consequence of Na-K pump activation.

Our findings cannot rule out a role for a depressant effect on the pacemaker current in the negative chronotropic effect of $\alpha_1$-adrenergic stimulation. In studies by Hauswirth et al\textsuperscript{26} and by Tsien,\textsuperscript{27} $\alpha_1$-adrenergic stimulation had no effect on the pacemaker current in cardiac Purkinje fibers. In these studies, however, the pacemaker current was interpreted as a decaying outward current Purkinje fibers. These studies, however, the pacemaker current was reinterpreted as a slowly activating inward current ($i_t$ in Reference 28), and the relevance of cleft accumulation/depletion phenomena in distorting its measurement has been emphasized.\textsuperscript{29} Since $\alpha_1$-adrenergic stimulation is likely to influence cleft accumulation/depletion phenomena by reducing background potassium conductance, its net effect on the pacemaker current, $i_o$, could have been improperly analyzed based on the earlier studies.\textsuperscript{26,27} However, in these studies, the pacemaker current was examined at potentials positive to the reversal potential for potassium currents. In this case, a reduction of $i_K$, by reducing the (outward decreasing) accumulation current, should result in an artifactual decrease of the observed pacemaker current, more evident in the range of potentials where $i_K$ is maximal. Therefore, the findings reported by Hauswirth et al\textsuperscript{26} and by Tsien,\textsuperscript{27} although inadequate to rule out a stimulatory effect of $\alpha$-agonists on $i_o$, strongly support the lack of an inhibitory effect.

Overall, the maximal diastolic potential was slightly increased by phenylephrine. However, hyperpolarization was not a uniform finding among fibers showing a decrease in aNa\textsubscript{1}. $\alpha_1$-Receptor stimulation, besides increasing Na-K pump current, decreases background potassium conductance.\textsuperscript{7,26} These actions result in opposite changes in the maximal diastolic potential, and the overall effect of phenylephrine on this parameter is probably a balance between the two. Therefore, a nonhomogeneous effect of phenylephrine on the maximal diastolic potential is expected. On the other hand, hyperpolarization is not essential to explain the negative chronotropic effect of Na-K pump activation for the following reasons: 1) Hyperpolarization does not necessarily result in a decrease in rate because, besides displacing the membrane potential away
from threshold, it activates the pacemaker current to a greater extent. The net effect of these changes probably depends on many factors and is not easily predicted in different situations. 2) In previous studies, we have shown that the negative chronotropic effect of phenylephrine is associated with a reduction of the slope of phase 4 and not with an increase of the difference between maximum diastolic and take-off potentials. 3) The activation of a constant outward current (Na-K pump current) in the presence of a gradually increasing membrane input resistance during diastole can itself reduce the rate of diastolic depolarization resulting in negative chronotropy. Hence, there is more than one possible contribution to the decrease in rate; there is a) the activation of Na-K pump current in the presence of a gradually increasing membrane input resistance during diastole and b) the hyperpolarization of the membrane, which occurs in a subset of fibers during α-adrenergic stimulation. Either of these alone or the two together might decrease automaticity, with the response being further modulated by any effect of α-agonist to decrease background potassium conductance.

Another factor to be considered in our experiments is that the group of driven fibers responding to phenylephrine with a decrease in aNa had a higher aNa in the control state. The difference in baseline aNa could not be explained either by differences in the level of $E_{\text{max}}$ or rate, since these variables were similar in the two groups. Although the cause of a higher initial aNa in this subgroup of fibers is unclear, the finding that fibers having low aNa show no effect of phenylephrine on the variable might provide a basis for the absence of a negative chronotropic effect of α1-adrenergic stimulation in depolarized fibers such as those of sinus node. In fact, prolonged depolarization results in a decrease of aNa, and in this setting either no effect or an increase in automaticity as a result of α1-adrenergic stimulation might be anticipated.

In one third of the spontaneously beating fibers, phenylephrine did not change aNa, significantly but produced an increase in rate. The mechanism of this positive chronotropic effect is still unclear. One possible explanation relies on the reduction of background potassium conductance, which, in the absence of a “sufficient” enhancement of the Na-K pump current, would induce an increase in automaticity. Although this mechanism is quite plausible, it is probably not the only one in operation. In fact, both the Na-K pump activation and the reduction in potassium conductance are abolished by pertussis toxin pretreatment, whereas the positive chronotropic response persists in all the preparations after such intervention. Therefore, the existence of another effector, linked to the α1-receptor by a pertussis toxin insensitive pathway, must be postulated and is currently under investigation.

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