Adrenergic Modulation of the Transient Outward Current in Isolated Canine Purkinje Cells

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Single canine Purkinje cells were voltage clamped under Ca\(^{2+}\)-free conditions using the patch pipette. Depolarizing pulses from a holding potential of \(-42\) mV induced a time-dependent rapidly activating-slowly inactivating outward current, which was identified as the transient outward current. The current showed two exponential time constants of inactivation (48, 352 msec at \(+58\) mV and 53, 325 msec at \(+78\) mV). Norepinephrine in concentrations exceeding \(10^{-6}\) M modified the inactivation kinetics of this current without affecting the activation kinetics. The half-maximum dose for norepinephrine effect was \(1.9 \times 10^{-8}\) M, and the effect was saturated at \(10^{-7}\) M. Norepinephrine reduced the amplitude of the fast time constant component of inactivation, while increasing the amplitude of the slow component, without changing their time constants. Norepinephrine also increased the amplitude of a time-independent current component. The \(\beta\)-antagonist sotalol blocked the norepinephrine effect on the transient outward current. On the other hand, both activation of adenyl cyclase by forskolin and increase of intracellular cAMP concentration produced the same effect as exposure to norepinephrine. These results suggest a role for neurotransmitter regulation of the transient outward current in cardiac cells, perhaps by channel phosphorylation. (Circulation Research 1988;62:162-172)

Norepinephrine is known as an important transmitter of neural control in the heart.\(^1\) One of the most important actions of norepinephrine is the modulation of ion channels, but its effects on the action potential are complex. Norepinephrine shortens the action potential in various mammalian ventricular cells,\(^2\) in sheep Purkinje fibers,\(^3,4\) and in calf Purkinje fibers,\(^5,6\) while increasing the action potential duration in guinea pig ventricular cells\(^7\) and in isolated sheep Purkinje cells.\(^8,9\) It has been clearly shown that norepinephrine affects the slow inward current (\(I_{\text{s}}\)) and the delayed rectifier potassium current (\(I_{\text{K}}\)).\(^3,5,8,10,11\) However, these two actions are not sufficient to explain norepinephrine's complex effects on the cardiac action potential.

Another current of importance for the action potential plateau is the transient outward current (\(I_{\text{o}}\)), which has been identified in several types of heart cells: in calf,\(^12\) sheep,\(^13,14\) and dog Purkinje fibers;\(^15,16\) in rat\(^17\) and rabbit ventricular cells;\(^18\) in rabbit atrial cells;\(^19\) and in rabbit atrioventricular cells.\(^20\) In some cardiac tissues \(I_{\text{o}}\) may be regulated by intracellular calcium and therefore associated with \(I_{\text{s}}\).\(^21\) A similar current in neural cells, the A-current, has also been reported to be modulated by Ca\(^{2+}\) or cAMP-dependent processes.\(^22-24\)

Recently, we have studied \(I_{\text{o}}\) in isolated canine Purkinje cells. This current is modified by norepinephrine (NE) but not by intracellular Ca\(^{2+}\). Our studies suggest that the transient outward current channel can be phosphorylated by a cAMP-dependent process, altering the kinetics of the current.

Materials and Methods
Preparation of Single Purkinje Cells

Single Purkinje cells were isolated according to procedures previously described.\(^25\) Briefly, canine free-running Purkinje fibers were obtained from adult dogs anesthetized with sodium pentobarbital (30 mg/kg). The Purkinje fibers were cut into 2–3 mm lengths and placed in bicarbonate Tyrode's solution gassed with 5% CO\(_2\) and 95% O\(_2\). The cut fibers were then placed for a period of 4 hours in the bottom of a plastic culture dish, which contained Eagles minimal essential medium (Gibco, Grand Island, N.Y.) modified to contain 0.1 mM free Ca\(^{2+}\), 5.6 mM Mg\(^{2+}\), 5 mg/ml collagenase (type I, Worthington, Freehold, N.J.), and 1 mg/ml albumin (Sigma, St. Louis, Mo.). The pH was adjusted to 6.2 by addition of 5.0 mM HEPES-NaOH buffer, and the temperature was kept at 37° C. Then, the digested fibers were washed three times and incubated for 15–20 minutes at 37° C in 130 mM K-glutamate, 5.7 mM MgCl\(_2\), 0.1 mM EGTA(ethylene glycol bis-(\(\beta\)-aminoethyl ether) N,N',N'-tetraacetic acid; Sigma), 5.5 mM dextrose, and 5.0 mM HEPES-KOH buffer (pH 6.2). The fibers were subjected to low-speed disruption (2.4 seconds) with a homogenizer (model 45, Virtis, Gardiner, N.Y.). The solution was centrifuged at 22g, and the resulting single Purkinje cells
were taken from the bottom of the centrifuged tube and maintained in the standard Eagles minimal essential medium buffered with 5.0 mM HEPES-NaOH (pH 7.2) containing 1.8 mM Ca\(^{2+}\), 5.4 mM K\(^+\), and 1 mg/ml albumin. We selected the smaller cells for these experiments. Their width and length while lying quiescent on the bottom of the chamber were 27.9 ± 5.9 x 120.6 ± 25.5 \(\mu\)m (n = 32).

**Solutions**

The composition of the Tyrode's solution with bicarbonate buffer was (in mM): NaCl 125, KCl 5.4, MgCl\(_2\) 1.05, NaHCO\(_3\) 22, NaH\(_2\)PO\(_4\) 2.4, CaCl\(_2\) 1.8, and glucose 11. The composition of the Tyrode's solution with HEPES buffer was (in mM): NaCl 142, KCl 5.4, CaCl\(_2\) 1.8, MgCl\(_2\) 0.5, glucose 5.5, and HEPES-NaOH buffer (pH 7.4) 5.0. The "Ca\(^{2+}\)-free" Tyrode's solution was prepared by omitting CaCl\(_2\) from the Tyrode's solution and adding 100 \(\mu\)M EGTA (pH 7.4). The internal solution in the recording pipette contained (in mM): K-aspartate 110, KCl 20, KH\(_2\)PO\(_4\) 2, glucose 10, ATP (adenosine 5'-triphosphate, magnesium salt; Sigma) 5, phosphocreatine (disodium salt, Sigma) 5, BAPTA (1,2-bis (O-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid, tetra potassium salt, cell impermeant type; Molecular Probes Inc., Eugene, Ore.) 5, HEPES 10, and the pH was adjusted to 7.2 with KOH.

Adenosine-3',5'-cyclic monophosphate (cAMP, free acid or sodium salt; Sigma) was directly dissolved in the internal solution. Forskolin (Calbiochem, San Diego, Calif.) was dissolved in ethanol as 10\(^{-2}\) M stock solution and was stored at 4° C. 8-Br-adenosine 3',5'-cyclic monophosphate (Br-cAMP, monosodium salt; Sigma) was also freshly dissolved in the bath solution. (-)-Norepinephrine (NE, Sigma) was dissolved in distilled water as 10\(^{-2}\) M stock solution and stored below -18° C, Sotalol (Mead Johnson and Co., Evansville, Ind.) was also dissolved in distilled water and stored in the refrigerator.

**Electrophysiological Experiments**

Single Purkinje cells were added to the recording chamber (0.5–1 ml volume) filled with Tyrode's solution. After the cells had settled to the glass bottom of the chamber, superfusion was started at a rate of 1–2 ml/min. The single-pipette whole cell clamp technique was similar to that described by Hamill et al. The electrodes had tip diameters of 3–8 \(\mu\)m and resistances of 0.5–3.0 M\(\Omega\) when filled with the internal solution. The sealing resistance of 5–50 G\(\Omega\) was established by applying a negative hydrostatic pressure less than 50 cm inside of the pipette. After seal, the membrane patch was disrupted by more suction. The patch clamp amplifier (model 8900, Dagan Corp., Minneapolis, Minn.) was modified by changing the feedback resistor from 100 M\(\Omega\) to 20M\(\Omega\) and disconnecting the capacitive compensation circuit. A liquid junction potential from 100 \(\Omega\) to 20MD and disconnecting the capacitive compensation circuit. A liquid junction potential from 100 \(\Omega\) to 20MD and disconnecting the capacitive compensation circuit.

**Results**

**Isolation of the Transient Outward Current**

Cardiac Purkinje fibers demonstrate six separate ionic currents: sodium current (I\(_{Na}\)), slow inward calcium current (I\(_{L}\), or I\(_{Ca}\)), inward rectifier potassium current (I\(_{K,\text{in}}\), or I\(_{K1}\)), delayed rectifier potassium current (I\(_{K,\text{out}}\), or I\(_{K1}\)), pacemaker current (I\(_{p}\), or I\(_{p}\)) and transient outward current (early outward current, I\(_{\text{out}}\), or I\(_{\text{out}}\)). In these studies, we separated the transient outward current (I\(_{\text{out}}\)) from other currents by the following procedures. First, the holding potential was held at -42 mV to inactivate the sodium current. Second, Ca\(^{2+}\) was reduced to very low levels on both sides of the membrane by its omission from the bathing solution and the addition of EGTA and BAPTA. This condition should lower free Ca\(^{2+}\) to levels below 10\(^{-4}\) M, and it will be identified by the term "Ca\(^{2+}\)-free." In low divalent ion solutions, Na\(^{+}\) can pass through the Ca\(^{2+}\) channel in cardiac cells. However, these experiments were done with 0.5 mM Mg\(^{2+}\) added to the outside bath solution, and inward current going through the Ca\(^{2+}\) channel was not seen. Furthermore, we analyzed the potential near the reversal potential of Na\(^{+}\) (+70 mV) at which the amplitude of any Na\(^{+}\) component must be small. In two experiments with 20 \(\mu\)M ouabain added to the bath solution under Ca\(^{2+}\)-free conditions, the currents induced by various depolarizing pulses were not changed (not shown), indicating that the Na-K pump current was negligible in these experiments, possibly due to the low temperature. The Na\(^{+}\)-Ca\(^{2+}\) exchange current was eliminated by the low Ca\(^{2+}\) level.

Exposure of the cell to the low Ca\(^{2+}\) outside solution was followed by an increase in the peak amplitude of I\(_{\text{L}}\) at every depolarizing voltage (Figure 1A and 1B), either because of reduced I\(_{\text{Na}}\) channel block by Ca\(^{2+}\) and/or because of reduction of an overlapping slow inward Ca\(^{2+}\) current. Figure 1B shows the family of current traces under the Ca\(^{2+}\)-free conditions and Figure 1C shows the current-voltage (I-V) relation of the peak and the late current at 700 msec after the onset of the command pulses from the recordings in Figure 1B. Upon depolarization from the holding potential of
-42 mV to more positive potentials than -22 mV, an outward current activated rapidly, then slowly inactivated. The peak amplitude of the outward current became larger with increasing depolarization. Seven hundred milliseconds after onset of the command pulse, the time-dependent current was complete, leaving a large outward steady current. The I-V relation of the late current was almost linear at voltages more positive than 0 mV, with a conductance of 21 nS. Upon stepping back to the holding potential of -42 mV, we observed no tail current. The absence of tail current is shown more clearly in Figure 1D. The membrane was stepped from -42 mV to +78 mV for 1 second and stepped back to +18 mV or -2 mV. If IK or other increasing, time-dependent K+ currents existed, we would expect to see a tail current. Under these conditions, the time-dependent Ik was negligibly small. The inward rectifier current Ik, and the pacemaker current Ir or Ih also could be neglected because of the voltage range of these studies.6,14,34-36

The Ik current system has the unusual property of quite slow recovery from inactivation. To provide additional evidence as to the Ik component of the recorded current, we studied recovery, as illustrated in Figure 2. The recovery time constants were 73 and 922 msec at -42 mV. The steady-state current showed no recovery because it was not inactivated, suggesting that the steady-state current component was not a part of Ik.

From these results, we may assume that when depolarizing pulses of +58 mV or +78 mV were applied from the holding potential of -42 mV in Ca2+-free conditions, the time-dependent outward current component was the Ik current, and the time-independent current component was a combination of leak current and a time-independent Ik.

Effect of Norepinephrine

After application of 10^-6 M NE, the current rapidly changed as shown in Figure 3A. The single Purkinje cell was held at -42 mV and depolarizing pulses were applied to +58 mV and +78 mV. The overall inactivation time course of the Ik component was remarkably slowed in all experiments (n = 24), with little change in time-to-peak current. Figure 3B also demonstrates that this phenomenon was not due to an NE-induced increase in Ik.10,17 The protocol of the experiment was almost the same as that of Figure 1D. After the Ik outward current was inactivated, the membrane potential was clamped back from +98 mV to +18 mV or -2 mV. No tail current was detected, which would have been expected if the effect of NE was to increase a developing time-dependent K+ current.
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FIGURE 2. Recovery from inactivation. The cell potential was clamped at -42 mV, and twin depolarizing pulses of + 78 mV were applied with various interpulse durations (A). Interpulse times are shown on the bottom with a log scale. The main interval between the twin pulses was set to 1 minute. When the interpulse duration was set shorter, the time-dependent component of \( I_a \) was smaller, but the time-independent component was almost the same. B, Time course of the recovery from inactivation. The time constants of recovery from inactivation are separated into two components (73 and 922 msec) at the holding potential of -42 mV. In this experiment and those in the figures to follow, the external solution was "Ca\(^{2+}\)-free."

The time course of onset and washout of the NE effect on \( I_a \) kinetics is shown in Figure 3C and 3D, respectively. NE affected the \( I_a \) within 1 minute, and after 4 minutes, steady-state effects of NE were achieved in all four experiments of this sort. However, washout of the NE effect on \( I_a \) was much slower. In 4 of 6 cases, almost complete recovery occurred after 15 to 25 minutes of washout. In 2 of 6 cases, the current records recovered only about 50 to 80% after 25 minutes of washout. In the following experiments, the recordings were started 5 minutes after adding NE to the bath, to be sure that full effect had occurred.

Effects of Norepinephrine on \( I_a \) Inactivation

The decay of \( I_a \) inactivation was fitted with 3 or less exponential components. In the Ca\(^{2+}\)-free control condition, only 2 current decays out of 22 were fitted to 3 exponential components as the best solution. The fastest exponential components in these two cells were 10.9 and 18.0 msec. These fast time constants are similar to values that were reported by Coraboeuf and Carmeliet\(^{38}\) to represent a component that was suppressed by caffeine. Since NE affects mainly the slower components of \( I_a \) decay (Figure 3A), this very fast time constant was not considered in this analysis. In the following analysis, the decay of inactivation was fitted to one or two time constants.

The time course of decay of the \( I_a \) current both before (control) and after application of NE at a test potential of + 78 mV are plotted in Figures 4A and 4B using log current versus time. In both A and B, the decay of the current was fitted to 2 exponential components in the control condition, with fast time constants (\( \tau_f \)) of 46 and 53 msec and slow time constants (\( \tau_s \)) of 429 and 412 msec, respectively. In 20 cells, \( \tau_f \) was 48.2 ± 16.7 msec and \( \tau_s \) was 352.3 ± 125.8 msec at + 58 mV, and \( \tau_s \) was 52.7 ± 11.4 msec and \( \tau_f \) was 325.0 ± 110.4 msec at + 78 mV. These values are similar to those reported in intact sheep Purkinje fibers\(^{39-39}\) and in rabbit atrioventricular node cells.\(^{30}\) After application of NE, the inactivation time constants also showed two components (\( \tau_f = 56 \) msec, \( \tau_s = 545 \) msec) in Figure 4A, but the component decaying with the fast time constant almost disappeared, as shown in Figure 4B (\( \tau_f = 470 \) msec). The cumulative results for 7 cells are shown in Figure 5D and 5E. In 2 of 7 cells, the fast component could not be identified after exposure to NE. In the other five cells, there was no consistent change. The slow time constants tended to be slower in all cases except two (dotted lines in Figure 5, p < 0.05 with \( t \) test). The slow time constants were 364 ± 107 and 551 ± 99 msec at + 58 mV and 333 ± 115 and 533 ± 68 msec at + 78 mV before and after application of NE, respectively.

The amplitudes of both exponential decay components of \( I_a \) are taken from the equation

\[
I_a = K_f e^{-t/\tau_f} + K_s e^{-t/\tau_s} + I_w
\]

where \( K_f \) and \( K_s \) represent the extrapolated initial amplitudes of the fast and slow components and \( I_w \) is the amplitude of the steady-state current. \( I_a \) is the current at any time \( t \), and \( \tau_f \) and \( \tau_s \) are the respective fast and slow time constants. Figures 5A and 5B show the amplitude of each component (\( K_f \) and \( K_s \)) in 7 cells. The amplitude of the fast component was decreased after NE at both potentials, while the amplitude of the slow component was increased after NE. The total amplitude (fast, slow, and steady-state components) was increased by NE compared with the control (the ratio was 1.27 ± 0.34 at + 58 mV and 1.54 ± 0.83 at + 78 mV, \( n = 7 \)), and the peak amplitude was also increased (the ratio of the peak amplitude after application of NE to those in the control condition was 1.47 ± 0.28 at + 58 mV and 1.49 ± 0.32 at + 78 mV, \( n = 7 \)). However, when the total amplitude of the two time-dependent components was compared, there was no significant change after NE (the ratio of the time-dependent component after NE to that in the control was 0.86 ± 0.32 at + 58 mV and 1.24 ± 1.04 at + 78 mV). From this comparison it would appear that the sum of the time-dependent components was not changed significantly on average, but the time-independent component was slightly increased (less than 2 times) by NE.
Because the cells differed in size, the currents were of different magnitude. An alternative way to compare currents between cells is to calculate the ratio of the amplitude of the fast time component to the total time-dependent component amplitude. Under control Ca\(^{2+}\)-free conditions, the amplitude ratios of the fast time component to the total time-dependent component were 0.62 ± 0.11 and 0.64 ± 0.17, and the ratios of the time-dependent amplitude to the total current amplitude were 0.57 ± 0.09 and 0.60 ± 0.09 at +58 mV (n = 20) and +78 mV (n = 19), respectively. The ratio did not differ much between cells. Figure 5C shows the ratio of fast current component to the total transient current before and after application of NE. The fast current decay was fitted with two exponential components (solid curves) of 46 and 429 msec in the absence of NE and of 56 and 545 msec in the presence of NE. After applying NE the amplitude of the fast component decreased from 1,327 pA to 624 pA and the amplitude of the slow component increased from 416 pA to 916 pA. In Panel B, the current decay was also fitted with two exponential components (time constants of 53 and 412 msec) in the absence of NE, but in the presence of NE, the decay was fitted with only one exponential with a time constant of 470 msec. The amplitude of slow component was almost the same (801 pA in the absence of NE and 753 pA in the presence of NE). See text for more details of curve fitting.
component was dramatically reduced from 0.64 ± 0.05 and 0.69 ± 0.09 to 0.19 ± 0.14 and 0.14 ± 0.13 at +58 and +78 mV, respectively. These mean values are significantly different (p<0.001, paired t test).

In summary, the main NE effect on the inactivation process of I_o was to reduce the amplitude of the fast component and increase the amplitude of the slow component by an approximately equivalent amount and to increase the amplitude of the time-independent component. The effect of NE on the decay rate constants themselves was minimal.

**Effect of Norepinephrine on I_o Activation.**

The activation phase of I_o is shown in Figure 6A at the temperature of 11 ± 1° C. The membrane potential was held at −42 mV and depolarizing pulses of various voltages were applied. The times-to-peak current were not changed by NE. Note also that the peak current was somewhat increased by NE. Figure 6B shows the voltage dependence of time-to-peak in three different cells before and after application of NE. When the current was activated by steps to more depolarized potentials, the time-to-peak was faster. NE does not appear to influence the activation kinetics of I_o, while the activation kinetics are themselves quite voltage-dependent.

**Dose Dependence of the Norepinephrine Effect**

Figure 7 shows the current traces at +78 mV from the holding potential of −42 mV before and after

![Image of Figure 5](https://example.com/image5.png)

**Figure 5. The effect of norepinephrine (NE) on time constants and amplitudes of the I_o inactivation components.** A, Amplitudes of the fast components from seven different cells at +58 and +78 mV in the absence (C) and in the presence (H) of 10^-7 M NE. In all cases the amplitude was decreased after application of NE. The triangles represent data from the cells in which the two-exponential decay changed to one exponential after NE. B, Amplitude of the slow component of inactivation. In all cases except one, the amplitude increased. C, Ratio of the fast component amplitude to the total amplitude of the time-dependent current. In all cases, the amplitude ratio was 0.6–0.8 in the absence of NE and 0–0.4 in the presence of NE. D and E, Time constants of fast and slow inactivation in the absence (C) and presence (H) of NE at +58 and +78 mV. The time constants were not significantly changed. See the text for further description.

![Image of Figure 6](https://example.com/image6.png)

**Figure 6. Norepinephrine (NE) and activation of I_o.** A, Currents of the I_o activation phase for different potentials at the low temperature of 10° C. , current in the presence of NE; o, current in the absence of NE. The time-to-peak (time from the onset of the command pulse to the peak point of the current amplitude) and the amplitudes of the time-dependent component of the I_o were almost the same in the absence or presence of NE for these voltages. B, Times-to-peak plotted against the membrane voltage in three cells at temperatures of 10–12° C. The time-to-peak is quite voltage-dependent, but it was not changed by NE.
application of various concentrations of NE. The drug action was allowed to reach its full effect before recording.

There was little or no effect of the different concentrations of NE on the fast or slow time constants (not shown). The dose-response relation can be more clearly seen by examining NE effects on amplitudes of the two components of current decay. Figure 8 shows each component's current amplitude extracted by exponential fitting of current decay at the various NE concentrations. The amplitude of the fast component was decreased at more than $10^{-9}$ M NE concentration and the effect saturated at $10^{-6}$ M (Figure 8A). The amplitude of the slow component was increased at more than $10^{-9}$ M NE concentration and saturated at $10^{-6}$ M NE concentration (Figure 8B). Higher concentrations of NE were not used since in preliminary experiments repeated exposure to a high concentration of NE resulted in a large current leak and, finally, in cell death. Figure 8C shows the ratio of the fast component to the total time-dependent current at the various NE concentrations. The ratio began to decrease at a concentration of $10^{-10}$ M NE, was half-maximal with $1.9 \times 10^{-8}$ M NE, and saturated with $10^{-6}$ M NE. These concentrations are similar to those measured by an affinity labeling method for the $\beta$-adrenergic receptor, with $K_d = (5 \times 10^{-10}) - (2 \times 10^{-7})$ M, and the isoproterenol increase of the slow inward $Ca^{2+}$ current with a threshold concentration of $2 \times 10^{-9}$ M, a half-maximum effect concentration of $3.7 \times 10^{-4}$ M, and a maximal concentration of $10^{-6}$ M.

Similar effects on $I_{n}$ could be produced by exposure to isoproterenol. Figure 9 illustrates the isoproterenol effect on decay of $I_n$ and on the magnitude of the steady-state outward current, which were qualitatively similar to those seen with NE. The dose-response curve was also similar to that of NE.

**Study of Mechanisms**

Sotalol, a pure $\beta$-antagonist, was applied to the bath solution after the NE effect had developed (Figure 10A). Under control conditions, the inactivation of $I_n$ was fitted to the two exponential components of 42, 205 msec at +58 mV and 56,401 msec at +78 mV. After application of $10^{-8}$ M NE, the current amplitude was increased and overall inactivation was slower. The current decay was fitted to one exponential with time constants of 313 msec at +58 mV and 343 msec at +78 mV. Immediately after recording the current, $10^{-4}$ M sotalol was added to the bath solution containing $10^{-8}$ M NE. After 10-15 minutes, the currents were almost back to control, with two inactivation time constants of 45,234 msec at +58 mV and 61,458 msec at +78 mV. The same results of the sotalol were obtained from 3 cells. In two of the three cases, NE abolished the fast inactivation component, which reappeared after exposure to sotalol.

Forskolin, a potent activator of adenylate cyclase, was added to the bath solution to increase the intracellular cAMP concentration. Figure 10B shows the forskolin effect on $I_n$. First, sotalol was added without...
change in the current. Then $2 \times 10^{-3}$ M forskolin was added to the bath solution in addition to sotalol, producing the same effects on $I_m$ as NE (3 cells). In only one of the three cells was the fast component of the inactivation decay detected at +78 mV after application of forskolin. The forskolin effect of shifting current inactivation to the slowly decaying component was identical to the NE effect on $I_m$. In addition, forskolin increased the time-independent current, perhaps to a greater extent than NE. Sotalol had no effect on its own, and it did not alter the forskolin effect.

If the NE effect is achieved by activation of adenylyl cyclase and increasing cAMP inside of the cell, then it should be possible to imitate the effect of NE by addition of cAMP to the pipette solution. Figure 10C shows the NE effect when cAMP was introduced to the cytoplasm through the pipette. Two hundred micromolar cAMP was added to the pipette solution, the control current inactivation time courses appeared slower, with fast and slow time constants of 43,550 at +58 mV and 54,644 msec at +78 mV, respectively. The ratios of the amplitude of the fast component to the total time-dependent current was 0.21 at +58 mV and 0.36 at +78 mV. These ratios are slightly higher compared with those after NE of 0.19 and 0.14, but small compared with control values of 0.64 at +58 mV and 0.69 at +78 mV. The same results were seen in three cells after addition of 100–200 μM cAMP to the pipette solution. Therefore, under these conditions, the increase of intracellular cAMP imitated the effects of NE.

When NE was applied in the presence of cAMP, the current decay was only slightly changed (compare with Figure 3A). The same results were obtained from the three cells. The small additional effect of NE suggested that the 200 μM cAMP in the pipette did not produce maximal activation or that there was some nonhomogeneous distribution of cAMP.

Extracellular application of Br-cAMP was also used to increase the intracellular cAMP concentration. Figure 10D shows the result of the effects of the Br-cAMP. The control inactivation decay was fitted to two exponential components of 42,268 msec at +58 mV and 48,288 msec at +78 mV. After application of 500 μM Br-cAMP to the bath solution, the fast component was abolished and the inactivation decay was fitted to one exponential component of 349 msec at +58 mV and 459 msec at +78 mV. In one other similarly treated cell, the control inactivation decay was also fitted to two exponential components of 82,558 msec and fitted to one component of 402 msec after 500 μM Br-cAMP at +58 mV. The fast component disappeared in both cases, but the absolute amplitudes of the slow component also increased after Br-cAMP was applied. The ratio of the amplitude of the slow component after application of Br-cAMP to control was 1.96 at +58 mV, 1.82 at +78 mV from...
the cell shown in Figure 10D, and 1.86 at +58 mV in the other cell. These results were the same as the effect of NE.

In summary, the effect of NE appears to be via binding to β-receptors, activation of adenyl cyclase, and production of cAMP. It remains to be determined how cAMP acts to alter inactivation kinetics of I_{w}.

**Discussion**

**Ca^{2+} Dependence of I_{w}**

The I_{w} current persisted or increased in these experiments after extracellular and intracellular Ca^{2+} were reduced to very low levels. Consequently, it is apparent that the I_{w} studied in these dog Purkinje cells is not a Ca^{2+}-activated current. DiFrancesco and McNaughton\(^4\) have shown a substantial I_{w} in sheep cardiac Purkinje fibers that was not affected by moderate reduction of extracellular Ca^{2+}. Coraboeuf and Carmeliet\(^5\) reported that the transient outward current in sheep Purkinje fibers consisted of two components, a large Ca^{2+}-insensitive current and a smaller, very rapidly inactivating Ca^{2+}-activated one. It seems likely that the I_{w} studied here corresponds to the large Ca^{2+}-insensitive component of Coraboeuf and Carmeliet.\(^5\) We saw the small, rapidly inactivating current only twice in these studies, possibly because of the Ca^{2+} buffer in the intracellular solution. In most experiments, presence of extracellular Ca^{2+} reduced the peak amplitude of the I_{w}. While this was partly because of the overlap of I_{0} with I_{w},\(^34\) this effect could be seen even in cells where the I_{w} had run down. Perhaps there was some change in the membrane field as a result of shielding of membrane surface charge by Ca^{2+} or, alternatively, some Ca^{2+} block of the I_{w} channel.

**Norepinephrine Effect on I_{w}**

It is well known that catecholamines increase I_{ca},\(^2\) but catecholamines also affect other cardiac currents. Kass and Tsien\(^1\) showed that catecholamines increase the delayed rectifier K+ current, and this effect has been studied further by Kass and Weigers\(^9\) and Bennett et al.\(^37\) Some of the effects of catecholamines on the sinoatrial pacemaker cells may be through a K+ current mechanism.\(^43\)\(^44\)

These experiments show the dramatic effect of catecholamines on I_{w}. This effect appears to be exerted through β-receptor stimulation since the effect was also produced by isoproterenol and sotalol blocked it. The presumed mechanism of intracellular action of β-receptor stimulation is the resulting production of cAMP, which is consistent with our finding that cAMP and forskolin mimicked the effect of NE. It has been shown that the action of cAMP on the Ca^{2+} channel occurs by phosphorylation of a membrane receptor by the catalytic subunit of the cAMP-dependent protein kinase,\(^45\) increasing the probability that the depolarized channel is in the open state.\(^46\) It is reasonable to think that a similar mechanism may explain the effect of NE on the I_{w} channel.

An increase in the steady-state outward current was also seen when the cells were exposed to NE or isoproterenol. This effect could also be produced by intracellular cAMP and by exposure to forskolin, and it was prevented by sotalol. We do not have direct evidence of the nature of this outward current component, but it is likely to be mostly a K+ current. This effect may reflect the same phenomenon reported by Gadsby,\(^49\) who showed an increase in steady-state outward K+ current at the plateau voltage range in canine Purkinje fibers produced by β-receptor stimulation. A similar effect of NE on steady-state K+ conductance in canine coronary sinus tissue has also been seen.\(^50\)

**Effect of Norepinephrine on the Action Potential**

As already indicated, a slowing of the decay of K+ current would favor earlier repolarization of the action potential, while increase in the inward Ca^{2+} current would delay repolarization. It is tempting to suggest that the interplay of these two opposing factors explains the variable effect of NE on the cardiac action potential. Cell types or species could differ in their relative densities of I_{w} and I_{w} channels in the surface membranes. However, several problems with this idea can be mentioned. First, the voltage range in which the NE effects on I_{w} were seen is more positive than the cardiac action potential plateau. We used these positive steps to accentuate the current and to reduce any residual inward sodium currents, but NE effects are likely to be seen also at less positive potentials, where I_{w} is also evident. Second, I_{w} recovers slowly, so that it is a smaller current during action potentials occurring at a rate of 60–100/min. However, as is apparent in Figure 2, substantial I_{w} can be seen even after short recovery intervals. Since the total current during the action potential is small, even small changes could influence the timing of repolarization. Because of the multiple effects of NE on currents in intact cells, it is hard to resolve these questions at this time.

**Modulation of I_{w} by Norepinephrine**

The effect of NE on the canine Purkinje cell was to reduce the magnitude of the more rapidly decaying component of I_{w} and to increase the magnitude of the more slowly decaying component, while not affecting activation. Indeed, the overall results suggest that the current with fast decay kinetics was not simply inhibited, but rather altered into the slow kinetic form. The presence of these two components of current decay may offer some insight into the behavior of the channel responsible for I_{w}. This kinetic pattern could result from two populations of I_{w} channels with identical activation parameters but different inactivation ones. Alternatively, there could be two inactivated states, as suggested for the Na+ channel by Chiu.\(^33\) The effect of channel phosphorylation could be to convert the fast-inactivating population of channels to the slowly inactivating one or to alter the kinetics of the two inactivated states. Such a scheme raises the idea that some channels might be phosphorylated in the basal state, representing the slowly inactivating population. Kamayama et al.\(^32\) have shown a modest (20%) decline...
in I_w after cell perfusion with endogenous protein kinase inhibitor, supporting the idea that there may be some basal phosphorylation of the Ca^{2+} channels. Distinction between the two kinetic models and the role of phosphorylation is difficult at the level of whole cell currents but should be resolvable by single-channel analysis.

An alternative mechanism for membrane phosphorylation to affect inactivation is to alter the field in the membrane, thereby shifting voltage-dependent kinetic events. However, in the voltage range studied, the I_w inactivation kinetics were not sensitive to voltage changes (see Figure 5), so this mechanism seems unlikely.

In summary, we have found that the I_w in canine cardiac Purkinje cells that is independent of Ca^{2+} is substantially modified by NE. The effect of NE on I_w is reduction or abolition of the fast-decaying portion of current inactivation and increase in the slowly decaying portion. The NE action is via a cAMP-dependent pathway and may involve channel phosphorylation.

Comparison of I_w With the A-Current and Its Modulation

Many excitable cells have a transient outward current activated by depolarization and carried by K^+. This current is often called the A-current, or I_A. One similarity between I_w observed in cardiac cells and I_A in neuronal cells is that they are both blocked by the aminopyridines. In general, I_w has an inactivation time course more like the faster component of I_A.

There have been reports that I_A can be modified by various interventions that might be related to channel phosphorylation. Kaczmarek and Strumwasser reported that external application of cAMP reduced I_w in Aplysia bag cells, and Strong found that forskolin reduced I_w in the bag cells. These effects might be compared with the reduction we have seen in the fast component of I_w. Sakakibara et al have demonstrated reduced I_A in photoreceptor cells, resulting from intracellular injection of Ca^{2+}. They offer evidence that Ca^{2+} acts by activating a calmodulin-dependent protein kinase. None of these effects in neuronal cells exactly resembles those demonstrated here in cardiac Purkinje cells, but the evidence is strong that the transient outward current in a variety of cells can be regulated by intracellular processes.

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