

# ADRENERGIC MODULATION OF THE DELAYED RECTIFIER POTASSIUM CHANNEL IN CALF CARDIAC PURKINJE FIBERS

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**ABSTRACT** We have investigated the modulation of the delayed rectifier potassium channel in calf cardiac Purkinje fibers by the neurohormone norepinephrine. We find that  $0.5 \mu\text{M}$  norepinephrine increases this K channel current by a factor of 2.7. A maximal increase of about four was found for concentrations of  $1 \mu\text{M}$  and above. Norepinephrine produced a small ( $<5 \text{ mV}$ ) and variable shift of the K channel reversal potential toward more negative values. The kinetics of the potassium channel are well described by a two-exponential process, both in the absence and presence of norepinephrine. However, norepinephrine substantially decreases the slower time constant with no significant effect on the fast time constant. Potassium channel activation curves in the presence of norepinephrine are very similar to control curves except at large positive potentials. A simple sequential three-state model for this channel can reproduce these data both with and without norepinephrine. The logarithms of the rate constants derived from this model are quadratic functions of voltage, suggesting the involvement of electric field-induced dipoles in the gating of this channel. Most of the kinetic effects of norepinephrine appear to be on a single rate constant.

## INTRODUCTION

Modulation of ion channels by neurohormones is a topic of great interest in neurobiology and cellular physiology. Neurohumoral modulation is particularly evident in cardiac tissues where several aspects of cardiac function are under parasympathetic and sympathetic control (for review see Watanabe and Lindeman, 1984). Sympathetic nervous activity increases heart rate and, to allow adequate diastolic filling time and normal impulse conduction, it shortens the duration of the action potential in ventricular cells and in Purkinje fibers (Quadbeck and Reiter, 1975; Carmeliet and Vereecke, 1969).

Control of the action potential duration in these cells is due, in part, to two norepinephrine-sensitive currents: calcium channel current ( $I_{\text{Ca}}$ ) and a delayed rectifier potassium channel current (Kass and Wiegers, 1982; McAllister et al., 1975; Beeler and Reuter, 1977; DiFrancesco and Noble, 1985). Considerable efforts have been made to unravel the mechanism(s) underlying  $\beta$ -adrenergic modulation of Ca channels (reviewed by Reuter, 1983; see also Bean et al., 1984); however, regulation of  $I_{\text{K}}$  has not been systematically addressed.

We have recently made a quantitative study of the ionic selectivity and kinetics of the delayed rectifier potassium channel (Bennett et al., 1985b). This ionic channel has a

high selectivity for potassium ions ( $P_{\text{Na}}/P_{\text{K}} \approx 0.02$ ) with inward rectification properties. Therefore we will refer to this current as  $I_{\text{K}}$ , consistent with the nomenclature suggested by McDonald and Trautwein (1978) (see also Noble, 1984; DiFrancesco and Noble, 1985).

The time course of the decay of the current through this channel is well fit by the sum of two exponential functions of time. These data and the voltage-dependent activation of the K channels are consistent with a single population of channels. The behavior of these channels is quantitatively predicted by a three-state model with a single conducting state. The transitions among the states of this model are governed by voltage-dependent rate constants (Bennett et al., 1985b).

Our long range goal is to provide a detailed description of the mechanism by which norepinephrine modulates this potassium current in heart cells. The present study was designed to determine the norepinephrine-induced changes in several properties of  $I_{\text{K}}$  in calf Purkinje fibers. We have analyzed these results within the framework of the three-state model previously used to describe the behavior of these channels in the absence of norepinephrine. We find that norepinephrine dramatically increases the magnitude of K channel current, but has little or no effect on the channel selectivity for  $\text{K}^+$  ions or on the voltage dependence of channel activation. The time-course of the decay of  $I_{\text{K}}$  in the presence of norepinephrine is still described by two exponentials, but the neurohormone induces some changes in the channel gating kinetics. The slow time

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constant is significantly decreased by norepinephrine, with little or no effect on the fast time constant. The kinetic effects of norepinephrine-modulated channels are well described by the three-state model for  $I_K$ , with most of the changes confined to one voltage-dependent rate constant.

A preliminary report of some of these results was presented at the annual meeting of the Biophysical Society (Bennett et al., 1985a).

## METHODS

The methods used here have previously been described in detail (Bennett et al., 1985b). Small calf heart Purkinje fibers were voltage-clamped using conventional two-microelectrode techniques. The applicability of these methods for measurement of  $I_K$  is discussed in Bennett et al. (1985b).

Voltage-clamp pulses were generated by a 12-bit digital-to-analog converter controlled by a laboratory microcomputer of our own design. Membrane currents were sampled (typically at 7-ms intervals) by a 12-bit analog-to-digital converter also controlled by the microcomputer. The membrane current signal was filtered at 20 or 40 Hz with an 8-pole low-pass Bessel filter before it was sampled. The  $I_K$  time constants were too slow to be affected by this low-pass filter, but the capacity transient, which usually lasted 1–2 ms, was distorted to 50–60 ms by the filter. Consequently, the data sampled during this period were omitted from analysis, and, as a result, some resolution of only the fastest  $I_K$  time constants (near 40 ms) may have been compromised.

Three basic pulse protocols were used to provide data for the determination of the voltage dependence of activation, the time course of activation, and the instantaneous current-voltage relation of K channels. In each of these protocols an activating prepulse was applied from a holding potential near  $-30$  mV and the amplitude of the K channel tail current measured during a subsequent test pulse (voltage protocols shown in Noble and Tsien, 1969; Bennett et al., 1985b). Time constants for the decay of the current during the test pulse were obtained using nonlinear least squares fitting procedures as described in Bennett et al. (1985b). This fitting procedure also allowed the determination of the instantaneous value of  $I_K$  by extrapolating the tail current to the time of voltage transition.

The standard Tyrode's solution contained (millimolar): 150 NaCl, 4 KCl, 5 glucose, 1.8 CaCl<sub>2</sub>, 0.5 MgCl<sub>2</sub>, and 10 Tris (pH 7.4). A temperature of 37°C was used for all experiments. Aliquots of a concentrated KCl solution were added to K-free Tyrode's solutions to produce solutions with higher than normal potassium concentrations. Solutions containing norepinephrine (Sigma Chemical Co., St. Louis, MO) were made for each experiment. These solutions contained 0.5  $\mu$ M ethylenediamine-N,N'-tetraacetic acid (EDTA). In some experiments the calcium channel antagonist nisoldipine was used to block calcium-dependent currents. This drug could be applied at very low concentrations (50–100 nM) because of the relatively depolarized holding potentials used in the present experiments (Bean, 1984; Sanguinetti and Kass, 1984).

In our previous study (Bennett et al., 1985b) characterizing the currents through K channels in the absence of norepinephrine we took care to demonstrate that these currents can be studied without artifacts produced by accumulation or depletion of potassium ions (Attwell et al., 1979). Norepinephrine substantially increases K channel currents that might exacerbate these artifacts, so it was important to reexamine this issue with norepinephrine present. We varied K channel current in the presence of norepinephrine by changing the amplitude or duration of a conditioning prepulse and measured  $I_K$  during a subsequent fixed test pulse. Two properties were measured under these conditions: K channel reversal potential and kinetics.

Fig. 1 A shows an experiment in which conditioning prepulses to 8 mV for 0.5 and 3.5 s were used. The instantaneous current is plotted as a function of the test pulse voltage. The larger currents produced by the longer prepulse reflect the increased number of K channels that had a

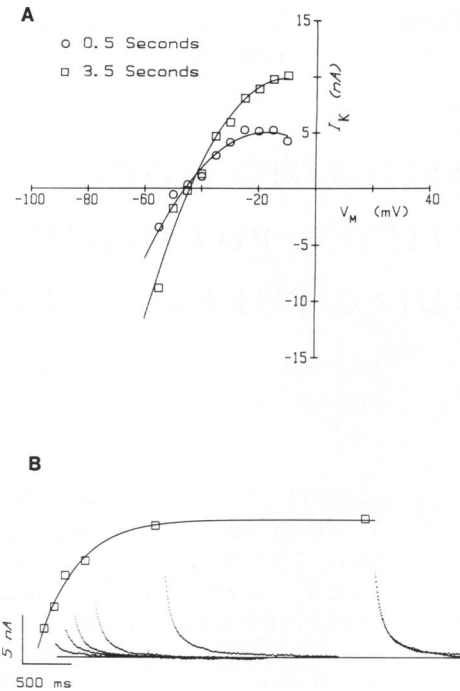


FIGURE 1 (A) Zero-time amplitudes of  $I_K$  tails plotted as a function of potential ( $V_m$ ) after a prepulse to  $+8$  mV for 0.5 (O) or 3.5 (□) s duration in 20 mM K<sup>+</sup> Tyrode's solution 1  $\mu$ M norepinephrine was present and the holding potential was  $-30$  mV. Expt. AR. (B)  $I_K$  tails after progressively longer activating prepulses. Tail currents were recorded at  $-30$  mV following 0.1–3.3 s activating prepulses to  $+11$  mV. The tail after a 1.2 s prepulse has been superimposed on the tail following the 3.3 s prepulse. The squares represent the zero-time amplitude of the tails estimated by fitting a biexponential function to each tail. The solid curve is the 'best fit' of a single exponential function with a time constant of 376 ms. 1  $\mu$ M norepinephrine was present and the extracellular potassium concentration was 4 mM. Expt. AU.

chance to open. The zero current potential,  $V_{rev}$ , was interpolated using a second order polynomial (solid line). In this experiment  $V_{rev}$  values of  $-46$  and  $-44$  mV were obtained for 0.5 and 3.5 s pulses, respectively.

Data from several other similar experiments are listed in Table I. The average shift in  $V_{rev}$  produced by lengthening the prepulse duration to 3.5 s was 1.6 mV. This is a small effect that would be consistent with only a 6% (1–1.2 mM) increase in extracellular K<sup>+</sup> concentration caused by the longest duration prepulse. To avoid even these small perturbations, prepulses were  $<1.5$  s in this study. Since the largest time constants of these channels in the presence of norepinephrine are near 0.5 s, a substantial amount of kinetic data can be obtained even with this constraint.

Table I also contains the results of an experiment in which the magnitude of the prepulse was varied, instead of the duration. No change in  $V_{rev}$  was found when the prepulse was changed from 8 to 35 mV. Thus,  $I_K$  can be measured using pulses up to at least 35 mV in amplitude. Furthermore, the voltage range can actually be extended to  $\sim 50$  mV for two reasons. First, the kinetics are faster at more depolarized potentials so shorter pulses can be used. In addition, the rectifying properties of these channels (seen in Figs. 1 and 3) tend to reduce the current flow at large potentials, at least compared to the case if there were no rectification.

Tests for ion accumulation were done with elevated concentrations of external K<sup>+</sup>, which facilitates the measurement of the reversal potential. A test of possible current-dependent artifacts in 4 mM external K<sup>+</sup> is illustrated in Fig. 1 B.

This figure shows tail currents in response to conditioning pulses to 11 mV lasting 0.1–3.3 s in the presence of norepinephrine (1  $\mu$ M). The tail

TABLE I  
TESTS FOR ION ACCUMULATION IN THE PRESENCE OF  
NOREPINEPHRINE

Expt.	$K_o/Ca_o$	P1	T1	$V_{rev}$	$\Delta V_{rev}$
	(mM)	(mV)	(s)	(mV)	(mV)
AL	16/1.8	10	0.6	-58	
		10	1.5	-55	+3
AP	16/0.9	15	0.5	-59	
		15	3.5	-56	+3
AR	20/1.8	8	0.5	-46	
		8	3.5	-44	+2
AS	20/1.8	10	0.5	-48	
		10	3.5	-49	-1
BU*	19/1.8	13	0.5	-49	
		13	3.5	-48	+1
PBB*	20/1.8	0	0.7	-55	
		0	1.0	-56	-1
		0	2.0	-53	+2
AR	20/1.8	8	0.5	-45	
		35	0.5	-45	0

\*Nisoldipine present.

current magnitude (measured at  $-30$  mV) reaches a near constant value after  $\sim 1$  s. Changes in  $K^+$  concentration caused by current flow during the conditioning pulse might be expected to produce alterations in the tail kinetics (Attwell et al., 1979). No such changes are apparent. Superimposed on the tail current following the 3.3 s pulse is the current after only 1.2 s. In fact the time constants of the tail current after a 0.3 s pulse (60 and 350 ms) are very similar to those obtained after pulses of 1.2 (65 and 306 ms) and 3.3 s (77 and 306 ms).

These tests demonstrate that K channel currents can be measured in the calf Purkinje fiber in the presence of norepinephrine without distortion due to ion accumulation and/or depletion.

## RESULTS

### Norepinephrine Increases $I_K$ with Minor Changes in $V_{rev}$

Norepinephrine substantially increases current through K channels. This effect is illustrated in Fig. 2. Shown here are tail currents at  $-30$  mV in the absence (smaller) and in the presence of 500 nM norepinephrine. In this experiment norepinephrine increased the current by a factor of about 4. The average increase in the current measured near  $-30$  mV in five similar experiments was  $2.7 \pm 0.4$  (mean  $\pm$  SEM). Currents were increased by factors of  $4.1 \pm 0.2$  ( $n = 5$ ) in  $1 \mu\text{M}$  NE and  $3.8 \pm 0.4$  ( $n = 11$ ) with  $2 \mu\text{M}$  NE. In one experiment 150 nM norepinephrine increased  $I_K$  by 1.3-fold.

The enhancement of current measured at  $-30$  mV by norepinephrine was independent of the external concentration of K. The average increase produced by  $2 \mu\text{M}$  norepinephrine was  $3.6 \pm 0.6$  ( $n = 4$ ) in 4 mM  $K_o$  and was  $3.9 \pm 0.6$  with elevated  $K_o$  (five experiments in 12 mM  $K_o$ , and one each in 16 and 20 mM  $K_o$ ). Due to the slight norepinephrine-induced shift of  $V_{rev}$  (see below), the increase in current magnitude has an apparent voltage-dependence.

Instantaneous K channel tail current values in control

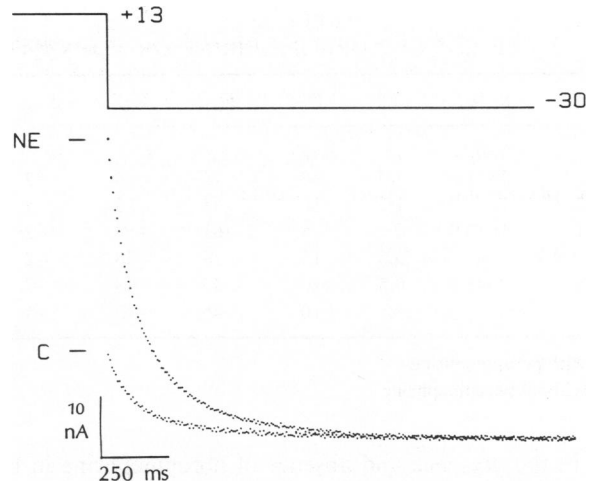


FIGURE 2 Effect of norepinephrine on  $I_K$ . Tails were recorded at  $-30$  mV following a prepulse to  $+13$  mV for 0.85 s in the absence (C) and presence (NE) of norepinephrine (500 nM). The bath solution contained 4 mM  $[K]_o$  and 50 nM nisoldipine. The asymptotic current was 4.8 and 2.5 nA for the control and NE data, respectively. Expt. BU.

and in the presence of norepinephrine are shown as a function of test potential in Fig. 3. In this experiment, norepinephrine causes not only an increase in K current tail amplitude, but also a very slight hyperpolarization of the  $I_K$  reversal potential. To determine whether this was a genuine norepinephrine-induced change, we measured  $V_{rev}$  in norepinephrine in several experiments. The average reversal potentials in 16 and 20 mM external  $K^+$  in the presence of norepinephrine were  $-55 \pm 3.5$  mV ( $n = 3$ ) and  $-49.3 \pm 3.9$  mV ( $n = 4$ ). These values are slightly more negative than those obtained in our previous study (Bennett et al., 1985b) without norepinephrine:  $-50.5 \pm 1.6$  mV ( $n = 6$ ) and  $-45.7 \pm 3.9$  mV ( $n = 3$ ).

The apparent differences in these means are not statistically significant, perhaps due to variations among different fibers. To address this issue more carefully, we measured

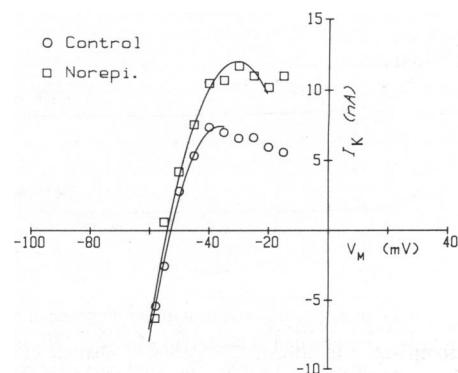


FIGURE 3 Zero-time amplitudes of  $I_K$  tails plotted as a function of potential after a prepulse to  $+10$  mV for 1.5 s in the absence (O) and presence ( $\square$ ) of norepinephrine. Third order polynomial fits to the data yielded  $V_{rev}$  values of  $-51$  and  $-55$  mV for control and norepinephrine data, respectively. Holding potential was  $-30$  mV. The bath solution contained 16 mM  $K^+$  and  $2 \mu\text{M}$  norepinephrine. Expt. AL.

TABLE II  
EFFECT OF NOREPINEPHRINE ON  $V_{rev}$

Expt.	$K_o/Ca_o$	T1	P1	$V_{rev}$	$V_{rev}$	$\Delta V_{rev}$
	(mM)	(s)	(mV)	(-)	(+)	(mV)
11-1	16/1.8	1.5	30	-46	-48	-2
AL	16/1.8	1.5	10	-51	-55	-4
AP	16/0.9	0.5	15	-61	-59	+2
		3.5	15	-58	-56	+2
AS	20/1.8	0.5	10	-45	-48	-3
		3.5	10	-45	-49	-4

(+) with norepinephrine  
(-) without norepinephrine

$V_{rev}$  in the presence and absence of norepinephrine in the same fiber. Table II summarizes the results of these experiments. A hyperpolarizing shift was found in three out of four experiments, however, the largest shift was only -4 mV.

### Norepinephrine Alters $I_K$ Kinetics

Potassium channel tail currents in the absence of norepinephrine contain two exponential components (Bennett et al., 1985b). Currents recorded at -15, -30, and -45 mV in the presence of 0.5  $\mu$ M norepinephrine are shown in Fig. 4. Superimposed on each trace is a least-squares fit of a two-exponential function of time to the data. The data were not well described by a single exponential function. Potassium channel kinetics in the presence of norepinephrine are also biexponential. However, norepinephrine pro-

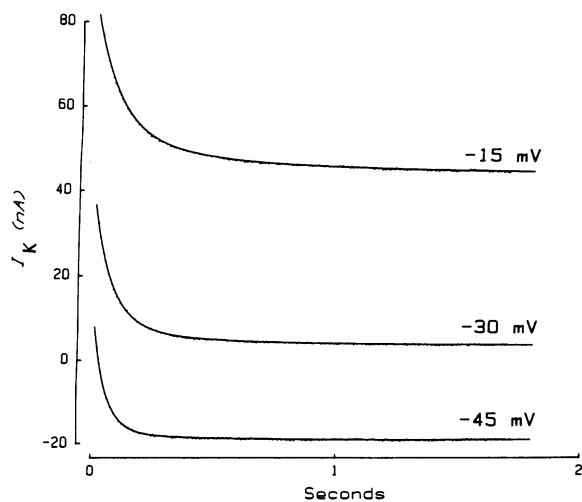


FIGURE 4 Time course of  $I_K$  deactivation in the presence of norepinephrine. Current tails were recorded at potentials of -15, -30, and -45 mV after an 800 ms prepulse to +14 mV. The solid curve superimposed on each trace is the nonlinear least squares fit of  $I_K = A \exp(-t/\tau_1) + B \exp(-t/\tau_2) + C$  to the data. The time constants for each record are: -15 mV:  $\tau_1 = 88$  ms,  $\tau_2 = 315$  ms; -30 mV:  $\tau_1 = 67$  ms,  $\tau_2 = 233$  ms; -45 mV:  $\tau_1 = 51$  ms,  $\tau_2 = 298$  ms. The holding potential was -30 mV and the bath solution contained 4 mM  $[K]_o$ , 500 nM norepinephrine and 50 nM nisoldipine. Expt. BT.

duces a substantial modification of the slow time constant as illustrated in Fig. 5.

Part A of this figure shows the voltage dependence of the fast time constant measured in an experiment on a single fiber, both with and without norepinephrine. There is no apparent effect of norepinephrine on this fast time constant. The slow time constant (part B), however, is considerably decreased by norepinephrine, especially at voltages more negative than -20 mV. This same result is seen in pooled data from many fibers as illustrated in Fig. 6.

The circles in the figure represent tail time constant data obtained in the absence of norepinephrine and the squares represent time constants measured with norepinephrine. The data in part A confirm the lack of effect of norepinephrine on the fast time constant at negative potentials

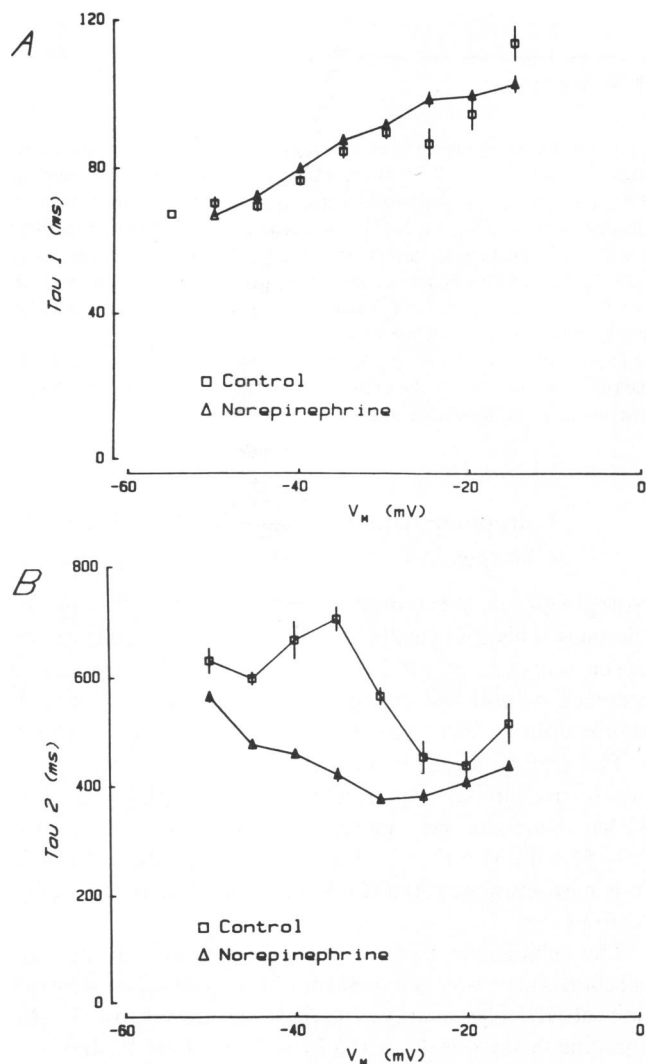


FIGURE 5 Voltage dependence of delayed rectifier time constants in the absence ( $\square$ ) and presence ( $\Delta$ ) of norepinephrine. Tails were recorded after an 0.85 s prepulse to +13 mV in 4 mM  $[K]_o$ , 500 nM norepinephrine and 50 nM nisoldipine. (A) voltage dependence of  $\tau_1$ . (B) Voltage dependence of  $\tau_2$ . Expt. BU.

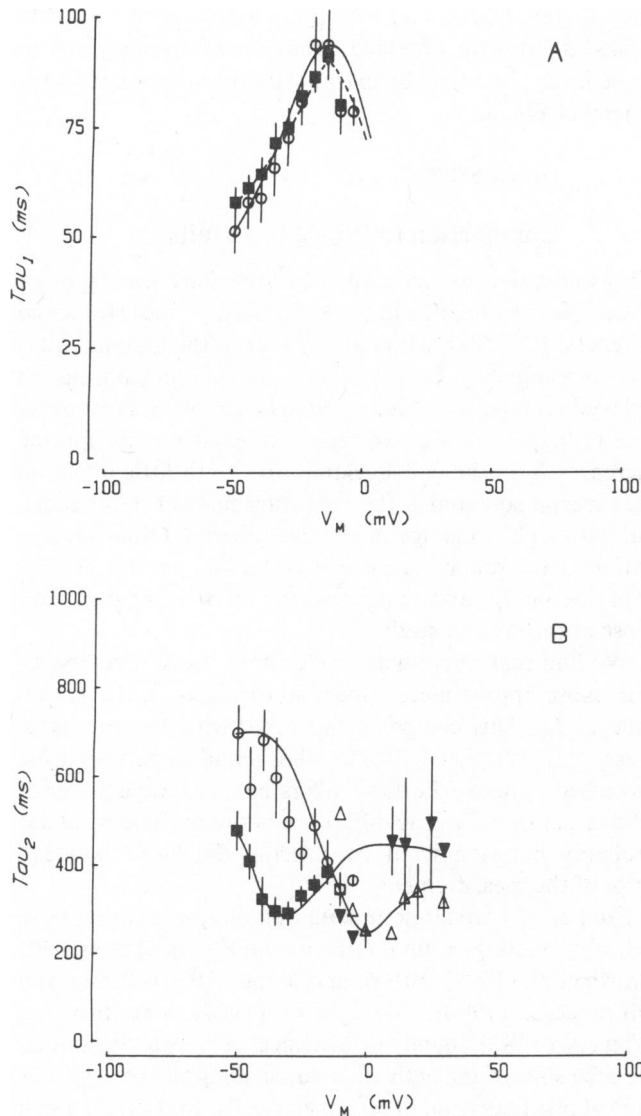


FIGURE 6 Pooled time constant data. (A) Voltage dependence of  $\tau_1$  (O, ■). (B) Voltage dependence of  $\tau_2$  (O, ■) and time constants from envelope of tails ( $\Delta$ ,  $\nabla$ ). Circles and upright open triangles are controls; filled squares and filled inverted triangles indicate the presence of norepinephrine. Time constants were determined by fitting the biexponential function (see Fig. 4) to  $I_K$  tails or by fitting a single exponential function to the envelopes of  $I_K$  tails (see Fig. 7). Data are shown as mean  $\pm$  SEM ( $n = 8$ , control;  $n = 5$ , norepinephrine). Time constants were determined in 4 mM  $[K]_o$ . The smooth curves were computed as described in Discussion.

seen in Fig. 5. Part B shows that norepinephrine substantially decreases the deactivation time constants at potentials between  $-50$  and  $-20$  mV, similar to that seen for the single fiber in Fig. 5. The lines in these figures are from the three-state model described in the Discussion section.

Also shown in Fig. 6 B are time constants obtained at potentials more positive than  $-10$  mV. It is difficult to use deactivating tail currents to obtain data at these positive potentials. Instead, these time constants were measured using a pulse pattern like that shown in Fig. 2. Another

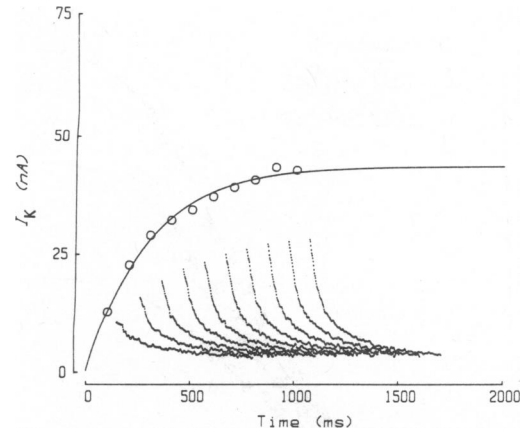


FIGURE 7 Time course of K channel activation in the presence of norepinephrine. Inset:  $I_K$  tails recorded at a  $-32$  mV holding potential after pulses of variable duration to  $+18$  mV. Pulse durations were 0.1–1 s in steps of 0.1 s. Graph: Zero-time amplitudes of  $I_K$  tails are plotted as a function of pulse duration. The solid curve is the best fit of one-exponential plus a baseline to the data. The fitted time constant was 302 ms. The bath solution contained 4 mM  $[K]_o$  and 2  $\mu$ M norepinephrine. Expt. AH.

example of this method is illustrated in Fig. 7. The instantaneous values of tail current (measured at  $-32$  mV) are plotted (open circles) as a function of the duration of the conditioning prepulse to 18 mV. These points represent the time course of activation of  $I_K$  at the prepulse potential. Also shown is the nonlinear least-squares fit of an exponential function with a time constant of 302 ms. Only the slow time constant is revealed by this technique because the fast time constant is expected to be  $<50$  ms at this potential (see Fig. 6 A), and thus cannot be resolved. Fig. 6 B shows that norepinephrine appears to slightly increase the slow time constant measured by this technique.

Fig. 7 shows that at a fixed potential, longer pulses cause larger K channel currents. Fig. 8 shows that at a fixed duration a larger depolarization produces more current—a reflection of the voltage dependence of channel opening. Part A of this figure illustrates the dose-dependent increase in  $I_K$  by norepinephrine over a broad voltage range. Norepinephrine does not appear to shift these activation curves along the voltage axis; rather, most of the effect is simply an increase in amplitude.

This is seen more clearly in Fig. 8 B, where data from several fibers in the absence and presence of norepinephrine are presented. The data have been scaled as described in the figure legend. The data in the presence of norepinephrine are similar to those in the control solution, and do not differ substantially at voltages near the midpoints of these curves. The norepinephrine data do, however, deviate somewhat from the control values at potentials more positive than 30 mV.

Very large membrane potentials are required to reach saturation of potassium channel currents in several dif-

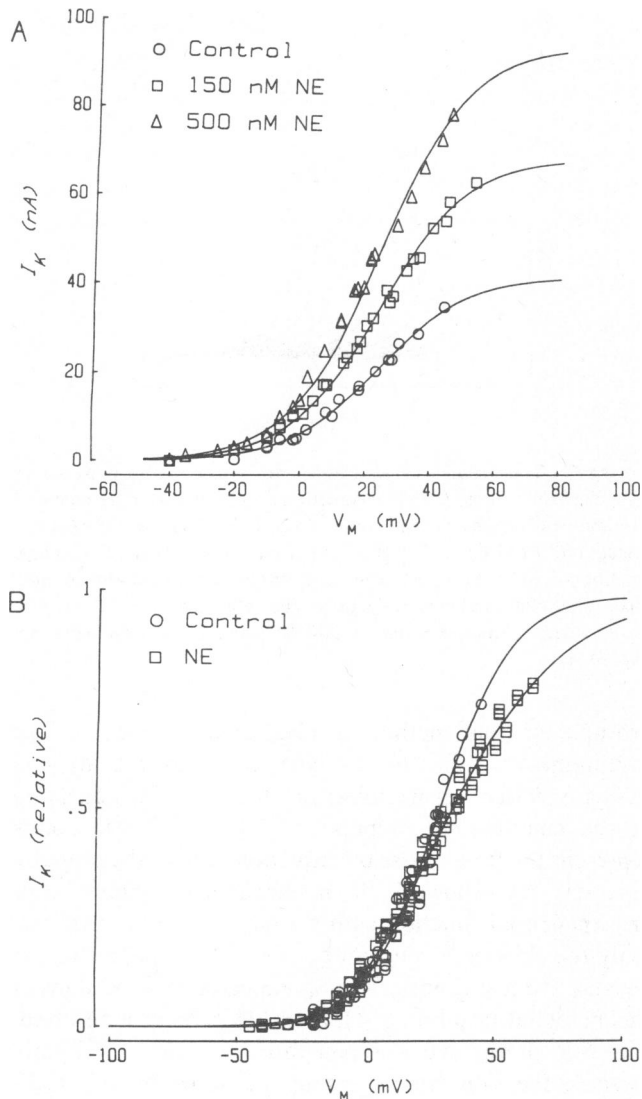


FIGURE 8 (A) Voltage-dependence of K channel activation in the absence and presence of norepinephrine.  $I_K$  tail magnitudes were recorded at  $-30$  mV after 800 ms prepulses to different potentials ( $V_m$ ). Measurements were in 4 mM  $[K]_o$ , 50 nM nisoldipine and 0 (○), 150 (□), or 500 nM (△) norepinephrine. A concentration of 2  $\mu$ M norepinephrine did not further increase the current. The solid curve through the control data was simply scaled by 1.64 and 2.25 to give the curves through the 150 nM and 500 nM norepinephrine data, respectively. Expt. BT. (B) Normalized activation curves from experiments in 4 mM  $[K]_o$ . Activation curves were scaled and normalized by fitting the data with a sigmoid function appropriate for a 3-state model:  $I(V) = \{I_{max}/1 + \text{EXP}[A_1 - V_m \cdot B_1]\} + \text{EXP}[(A_2 - V_m \cdot B_2)]$ . The data were then normalized by the best fit estimate of  $I_{max}$  in either control or norepinephrine. This procedure allowed an objective estimate of the saturation of the activation curves based on the data available. Circles are from four control experiments and the squares are from six experiments in norepinephrine (five in 500 nM, one in 2  $\mu$ M).

ferent biological preparations including isolated guinea pig myocytes (Bennett et al., unpublished observations), rat skeletal muscle (Beam and Donaldson, 1983a), and squid giant axons (Gilly and Armstrong, 1982). Limitations imposed by the use of microelectrodes prohibits the exten-

sion of the data of Fig. 8 to potentials beyond  $\sim 50$  mV. Consequently, we obtained estimates of the currents at these large potentials by extrapolation as described in the legend of Fig. 8.

## DISCUSSION

### Comparison to Previous Results

This study, the continuation of two previous investigations of delayed rectification in the calf Purkinje fiber (Kass and Wiegers, 1982; Bennett et al., 1985b), is the first quantitative investigation of the effects of norepinephrine on delayed rectifier potassium channel currents in heart cells. The principal findings we report are that norepinephrine increases  $I_K$  in the calf Purkinje fiber with little effect on the reversal potential or the activation curve of the channel, but with a clear change in channel kinetics. Other investigations have probed some aspects of the actions norepinephrine on  $I_K$ , and these results can be compared with those of the present study.

We find that micromolar concentrations of norepinephrine cause approximately fourfold increases in the amplitude of  $I_K$ . This compares favorably with the results of Kass and Wiegers (1982), who found norepinephrine increased  $I_K$  in calf Purkinje fibers by an average factor of  $4.98 \pm 2.3$  ( $n = 7$ ). The difference between these results is probably not significant considering the large standard error of the measurement.

Studies of adrenergic modulation of  $I_K$  activation have led to conflicting results. Pappano and Carmeliet (1979), Brown et al. (1975), Brown and Noble (1974), Brown and DiFrancesco (1980), Noma et al. (1980), and Kass and Wiegers (1982) found no evidence of a catecholamine-induced shift of the activation curve along the voltage axis in Purkinje fibers and other cardiac cells. In contrast Tsien et al. (1972) reported that epinephrine, theophylline, and mono-butyl cAMP all shifted the voltage dependence of  $I_K$  activation toward more negative potentials, so that at any given potential a greater proportion of K channels are activated after adrenergic stimulation.

Our results showed an increase in  $I_K$  at all potentials studied with little or no shift along the voltage axis, but the pooled data from several fibers reveal a slight difference in the shape of the activation curves with and without norepinephrine.

There is a small norepinephrine-induced hyperpolarization of the K channel reversal potential (about  $-3$  mV, see Table II). This could be due to an increase in the selectivity of the channel for  $K^+$  ions as suggested by Tsien et al. (1972) for the actions of mono-butyl cyclic AMP (McBcAMP). If this were the case in our experiments, then the  $P_{Na}/P_K$  ratio for the channel would have to change from 0.02 (Bennett et al., 1985b) in the absence of norepinephrine to 0.005 in the presence of norepinephrine.

Another possibility to account for this slight change in  $V_{rev}$  is that intracellular concentrations of  $Na^+$  and  $K^+$  are

changed through a norepinephrine-induced acceleration of the Na/K pump. Clausen and Flatman (1977) reported such an effect on the pump by catecholamines in rat soleus muscle and found a 53% decrease in  $\text{Na}_i$  and a 6% increase in  $\text{K}_i$ . If we fix the  $P_{\text{Na}}/P_{\text{K}}$  ratio at 0.02 as determined for control solutions in our previous study (Bennett et al., 1985b) and incorporate similar changes in intracellular ion activities, the K channel reversal potential is computed to change by about  $-2$  mV when extracellular  $\text{K}^+$  is 12–16 mM, an effect quite similar to our observations. While this interpretation is consistent with our measurements, it must be considered cautiously, since Gadsby (1983) finds no

catecholamine-induced increase in Na/K pump currents in canine Purkinje fibers.

Many of the effects on cardiac tissues of catecholamines, including norepinephrine, are mediated by changes in the concentration of intracellular cyclic AMP (for review see Tsien, 1977). The work of Tsien et al. (1972) has shown that exposure of calf Purkinje fibers to McBcAMP, a lipid soluble analog of cyclic AMP, increases  $I_{\text{K}}$ . Similarly, the work of Brum et al. (1983) showed that injection of the free catalytic subunit (C) of cAMP-dependent protein kinase increased  $I_{\text{K}}$  in isolated guinea pig ventricular cells. Thus, it seems likely that the

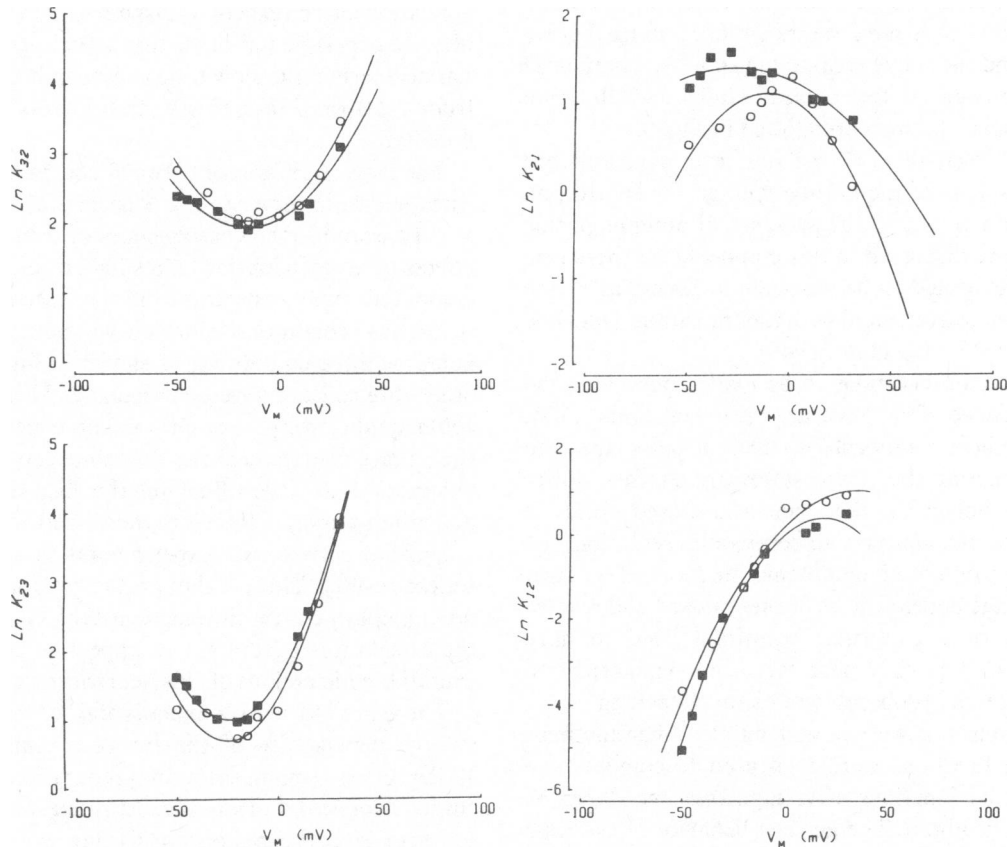


FIGURE 9 Voltage dependence of rate constants. Natural logarithms of the four rate constants are plotted as a function of membrane potential ( $V_m$ ). Control rate constants (O) and rate constants in the presence of norepinephrine (■) were determined from pooled  $I_{\text{K}}$  data (4 mM  $[\text{K}]_o$ ). Also shown are the best least squares fits of a quadratic function:

$$\ln k_{ij} = A + B \cdot V_m + C \cdot V_m^2$$

The fitted parameters for each rate constant were as follows:

		A	B $mV^{-1}$	C $mV^{-2}$
Control	$k_{32}$	2.17	0.0188	0.000656
	$k_{23}$	1.31	0.0511	0.001040
	$k_{21}$	1.05	-0.0127	-0.000520
	$k_{12}$	0.17	0.0474	-0.000682
Norepinephrine	$k_{32}$	2.07	0.0154	0.000489
	$k_{23}$	1.57	0.0478	0.000997
	$k_{21}$	1.26	-0.0109	-0.000197
	$k_{12}$	-0.01	0.0414	-0.001118

effects we report are also mediated by intracellular cAMP. However, in contrast to our results, Tsien et al. (1972) found that McBcAMP caused large hyperpolarizing shifts in  $V_{rev}$ , and that the activation gating of the channel was also shifted in the hyperpolarizing direction. These differences must be resolved before conclusions about the role of cAMP in modulation of  $I_K$  can be reached.

### A Kinetic Model

In a previous study we showed that the kinetics of the delayed rectifier K channel in Purkinje fibers were consistent with a simple three-state model (Bennett et al., 1985*b*). In that report we described a method for obtaining the rate constants governing the transitions among the two closed states and the single conducting state. We have used the same approach in the present study and the rate constants we have obtained are plotted in Fig. 9.

The natural logarithms of the four rate constants are plotted as functions of membrane voltage for control, no norepinephrine (○), and in the presence of norepinephrine (■). The data are displayed in this manner since these rate constants are expected to be exponential functions of the free energy change associated with the transitions from one state to the next (Eyring et al., 1949).

There are two observations to be made concerning the voltage dependence of the measured rate constants. First, the shapes of these relationships occur in pairs: the rate constants connecting the closed states are concave down, and the pair connecting the open and closed states is concave up. Second, contrary to common expectation, the rate constants need not be monotonic functions of voltage, nor do the voltage dependencies of the forward and reverse rate constants of a particular transition need to have opposite slopes. It is only necessary that depolarization leads to an increased probability of channel opening.

These rate constants were fit with cubic spline functions, and the spline functions were then used to compute the time constants as functions of voltage that are shown as smooth curves in Fig. 6. The general behavior of the time constants predicted by these rate constants is similar to that of many different types of voltage-dependent ionic channels.

Stevens (1978) has discussed the physical mechanisms of channel gating that predict a quadratic dependence of the log of the rate constants on voltage. In this model, the linear term includes interactions between channel permanent electric dipoles and the membrane electric field. This field may act on polarizable molecules in the protein channel to induce additional dipoles whose interactions with the applied field might give rise to a quadratic term in the expression for the log of the rate constants.

Thus, we fit the data of Fig. 9 with quadratic functions of voltage (solid lines, Fig. 9), and approximated the rate constants by the following equations:

$$k_{ij} = \exp(A + B \cdot V_m + C \cdot V_m^2). \quad (1)$$

The rate constants in the absence of norepinephrine in our earlier study (Bennett et al., 1985*b*) had this same voltage dependence. Fig. 9 shows that the rate constants in the presence of norepinephrine also have this form, including  $k_{21}$ , which is considerably different than the control rate constant.

We used the quadratic representation of the rate constants in the absence and presence of norepinephrine (smooth curves, Fig. 9) to compute the time constants and activation curves. These theoretical results, shown in Fig. 10, compare very favorably with the experimental data of Figs. 6 and 8. The model predicts that norepinephrine would have little effect on the fast time constant or on the activation curve (except at large positive potentials). Also, like the experimental data, this model predicts a substantial decrease in the slow time constant in the voltage range from  $-50$  to almost  $0$  mV and a cross-over at positive potentials.

The largest difference between control and norepinephrine quadratic curves in Fig. 9 occurs for the rate constant  $k_{21}$ . To examine the consequences of incorporating all the effects of norepinephrine on a single rate constant, we also computed time constants and activation curves under theoretical conditions in which we assumed that norepinephrine affected only  $k_{21}$  as shown in Fig. 9, but that all other rate constants were unchanged. These computations could qualitatively account for our experimental results (i.e., a large decrease in the slow time constant at negative potentials and little effect on the fast time constant or activation curve). However, there were quantitative discrepancies between our experimental data and these theoretical results. Thus, although the predominant effect of norepinephrine is on the rate constant  $k_{21}$ , modification of the other rate constants does appear to contribute to the overall kinetic actions of this neurohormone.

There are alternative explanations of the nonmonotonic voltage dependence of the rate constants. The work of Keller et al. (manuscript in preparation) has analyzed similar kinetic data from reconstituted sodium channels in terms of several types of models. For example, there may be more than three-states and, consequently, more than two system time constants. One or both of our exponential terms may be due to two time constants of similar magnitude. Our extracted rate constants then would be contaminated by the missing kinetic steps. We cannot exclude this possibility, but there are also no data to suggest the presence of more than three states of the K channel. Consequently, we have chosen the present model for our analysis.

### Mechanism of Norepinephrine Modulation

There are three general mechanisms by which norepinephrine could increase K channel current: an increase in the number of channels, an increase in the current through a single channel, or an increase in the probability of opening individual channels. Our modeling suggests that there is



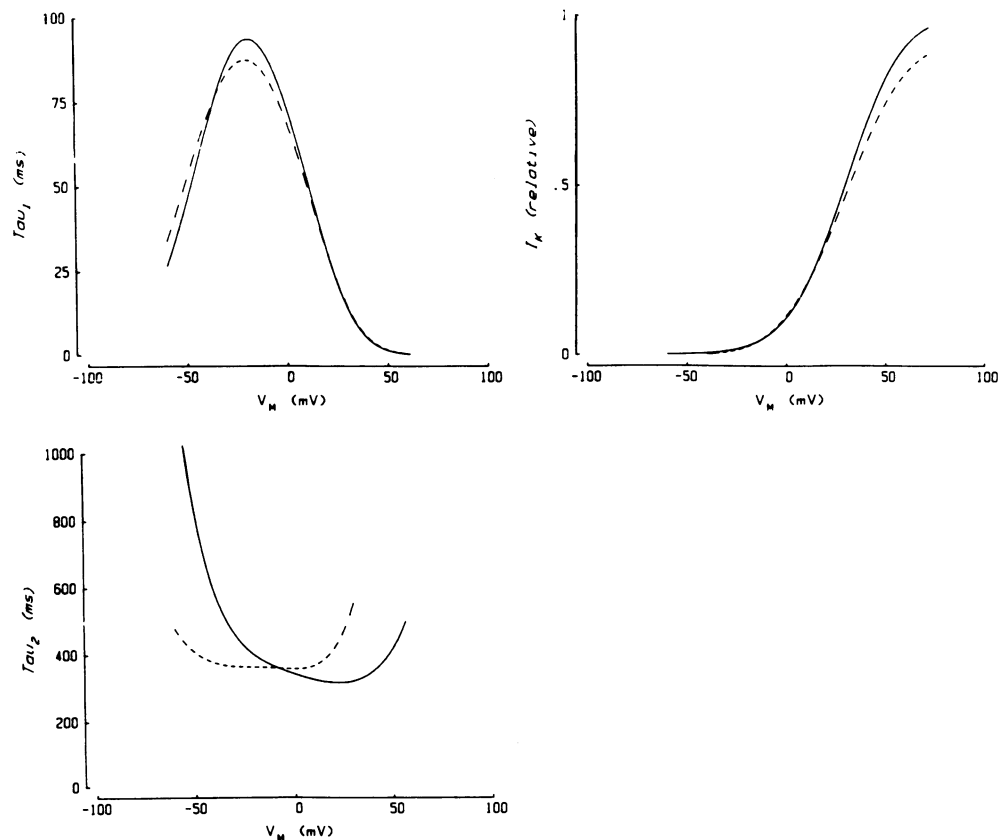


FIGURE 10 Predicted voltage dependence of the two time constants and steady state activation derived from the quadratic voltage dependence of the rate constants. The best fitting parameters for the quadratic voltage dependence of the rate constants (solid curves, Fig. 9) were used to recompute the two system time constants and the steady state probability of the channel occupying the conducting conformation for both the control (solid lines) and norepinephrine (dashed lines) conditions.

little effect on the opening probability (see Figs. 8 and 10). However, due to the limitations inherent in the use of microelectrode voltage-clamp of Purkinje fibers, we were unable to obtain accurate values of channel activation at large positive potentials. Consequently we are not able to distinguish among these possible mechanisms. These questions can be more directly addressed using ensemble fluctuation analysis or single channel recordings.

A related issue is whether the action of norepinephrine results in one or two channel populations: one with "normal" kinetics and one population with altered kinetics. If, in the presence of norepinephrine, there were two populations of K channels, then there should be at least three-system time constants. However, if norepinephrine produces the fourfold increase in current by increasing the number of channels, then these new channels would represent something like 80% of the total population. The techniques used in this study probably could not detect the remaining 20% of normal channels.

### Summary

The sympathetic neurohormone norepinephrine has very specific and simple effects on the delayed rectifier potassium channel in cardiac Purkinje fibers. The magnitude of

the current through this channel dramatically increased with little or no change in the selectivity of  $K^+$  over  $Na^+$ . The gating kinetics in the presence of norepinephrine are, as in the absence of norepinephrine, biexponential; norepinephrine decreases the slow time constant with little or no effect on the fast time constant. Our analysis of the results in terms of a simple three state kinetic model suggests that most if not all of the kinetic effects of norepinephrine may be on one rate constant. These results and interpretations should provide a beginning for a molecular level description of the actions of sympathetic modulation of this channel.

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