Effects of Norepinephrine, Calcium, and Rate of Discharge on $^{42}$K Movements in Canine Cardiac Purkinje Fibers

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SUMMARY We studied the effect of norepinephrine, calcium concentration, and rate of discharge in the presence of different [Ca$^{2+}$], on radioactive potassium movements in cardiac Purkinje fibers. The following results were obtained: (1) norepinephrine increases potassium uptake in quiescent fibers and in fibers driven at constant rate, but more in the latter; (2) norepinephrine also increases potassium uptake in quiescent fibers depolarized at the plateau; (3) increasing [Ca$^{2+}$], increases potassium uptake in fibers driven at constant rate; (4) increasing [Ca$^{2+}$], may decrease K$^+$ uptake in quiescent fibers; (5) increasing [Ca$^{2+}$], decreases the rate of loss of tissue radioactivity in quiescent fibers and increases it in a driven fiber; (6) increasing the driving rate increases potassium uptake in low and high [Ca$^{2+}$],; (7) high [Ca$^{2+}$], increases K$^+$ uptake, especially at low rates; (8) norepinephrine is less effective in increasing K$^+$ uptake in the presence of a high [Ca$^{2+}$],. We conclude that: (a) norepinephrine increases potassium uptake by different mechanisms; (b) calcium affects potassium movements when it is allowed to enter the cell, presumably by affecting potassium conductance; (c) the effect of an increased rate of discharge on K$^+$ uptake may involve stimulation of active K$^+$ uptake and may include a calcium-dependent component which is largest at high [Ca$^{2+}$], and a slow rate of drive; (d) simultaneous application of two interventions results in a summation that is smallest when one of the mechanisms is already substantially activated.

CATECHOLAMINES increase potassium$^{17}$ and calcium uptake$^{8-13}$ in different cardiac tissues. Whether the catecholamine-induced increase in potassium uptake is causally related to that of calcium is not known. It is conceivable that the increased calcium uptake in the presence of catecholamines might result in an increased intracellular calcium concentration during systole as the contraction becomes stronger. In several tissues, an increased [Ca$^{2+}$], has been shown to increase potassium conductance of the surface membrane (see Romero and Whittam$^{14}$ and Vassort$^{15}$ for references). Suggestions have been made that a similar mechanism also may operate in Purkinje fibers.$^{16-18}$ Calcium increasing the potassium current $i_{k}$, but not $i_{a}$. In addition, catecholamines increase the plateau current $i_{x}$, which is carried predominantly by potassium ions.$^{21}$ Therefore, it is possible that catecholamines increase $K^+$ movements by increasing at the same time both the inward calcium movement (and therefore $i_{k}$) and the plateau current $i_{x}$. Furthermore, catecholamines could stimulate active $K^+$ transport,$^{5,6}$ in addition to their effects on calcium movements and on $i_{x}$.

One way to approach these problems was to study the effect of norepinephrine on $K^+$ uptake in quiescent and in active Purkinje fibers. In quiescent fibers, the potential is negative to the threshold for both the plateau current $i_{x}$ and the slow inward current $i_{a}$ carried by calcium. In fact, it has been shown that catecholamines increase calcium uptake in an active$^8$, $9$, $12$ but not in a quiescent fiber.$^8$, $10$, $13$ If catecholamines increase potassium uptake only by increasing the intracellular calcium concentration and the plateau current $i_{x}$, norepinephrine should increase potassium uptake in an active but not in a resting fiber.

An increase in [Ca$^{2+}$], also increases calcium uptake$^{25-26}$ and, in an active fiber, the [Ca$^{2+}$], during systole. In a resting fiber calcium could increase potassium conductance either by acting extracellularly or through the Ca-Na exchange. With regard to the first possibility, it should be noted that an extracellular action has been shown only in active fibers.$^{27}$ As to the second possibility, in the presence of a constant [Na$^+$], an increased Ca$^{2+}$-Ca$^{2+}$ exchange due to an increased [Ca$^{2+}$], would not lead to an increased [Ca$^{2+}$]. Therefore, a calcium-induced increase in potassium conductance may not occur in a resting fiber.

Calcium inward movement in a unit of time also may be increased by an increase in the rate of discharge. It has been shown that, during activity, potassium movements increase substantially in cardiac Purkinje fibers.$^{28-29}$ If the enhanced potassium movements are related to an increased calcium influx to any extent, the effect of rate changes on potassium uptake should be affected by different levels of [Ca$^{2+}$].

The mechanisms by which catecholamines, high [Ca$^{2+}$], and increased frequency of discharge may affect potassium movements across the cell membrane should be different. However, the simultaneous exposure to two of these interventions may result in interference of effects depending on the degree of activation of one or the other mechanism. For example, norepinephrine-induced K$^+$ uptake may vary as a function of [Ca$^{2+}$].
The present experiments were carried out on cardiac Purkinje fibers and were aimed at determining whether: (1) norepinephrine increases K⁺ uptake when it increases neither Ca²⁺ uptake nor the plateau current iₒ; (2) [Ca²⁺]ᵢ affects potassium movements similarly in quiescent and active fibers; (3) the rate-dependent increase in potassium uptake is modulated by extracellular calcium concentration; and (4) norepinephrine and calcium interact in their effects on K⁺ uptake.

A preliminary report has appeared in abstract form.²⁰

Methods

Mongrel dogs of either sex (12–22 kg) were anesthetized with sodium pentobarbital, 28 mg/kg, intravenously. Free-running strands of Purkinje fibers were dissected from the ventricles and were perfused with Tyrode’s solution (37°C) in a small tissue bath located in close proximity to a β-probe (Tracerlab Scintillation Detector P20D; B in Fig. 1). The composition of the oxygenated (97% O₂ and 3% CO₂) Tyrode’s solution (in mm/liter) was as follows: NaCl, 136.9; KCl, 5.4; NaHCO₃, 11.9; NaH₂PO₄, 0.45; MgCl₂, 0.5; CaCl₂, 2.7; glucose, 5.5. In different experiments, the composition of the solution was modified as indicated. The Tyrode’s solution was perfused at a rate of 0.76 ml/min by means of an infusion withdrawal pump (Harvard Apparatus; model 950). Norepinephrine (Levophed Bitartrate; Winthrop Laborato ries) was diluted in nonoxygenated Tyrode’s solution to the selected concentration and infused by means of a portable infusion-withdrawal pump (Harvard Apparatus; model 1100). Nonoxygenated Tyrode’s solution was infused also in the absence of norepinephrine administration to maintain the total flow constant. A modification was introduced in the infusion of norepinephrine with respect to the diagram shown in Figure 1. Namely, the tubing carrying the norepinephrine solution at a rate of 0.09 ml/min did not extend directly into the tissue bath but instead into the tubing carrying oxygenated Tyrode’s solution just before the latter entered the large water bath (A in Fig. 1).

The Purkinje strand (0.5–1 mm in diameter) was kept in place in the bath by means of two stainless steel pins insulated electrically except for the tip. The pins were connected to a Grass stimulator (model S4GRH) via a Grass Stimulus Isolation Unit (model SIU 4678). The rate of stimulation varied from 0 to 108/min in different experiments. The duration of the pulses was 2.5–3 msec and the voltage was set about twice threshold. The Purkinje strand (henceforth also referred to as the fiber) was generally stimulated at 90/min for 30 minutes in inactive Tyrode’s solution. The stimulation was continued at the same rate for about 210 minutes while the strand was perfused in radioactive solution (loading period). The radioactive Tyrode’s solution contained ⁴²K (chemical form KCl) obtained from IsoServe (Cambridge Nuclear Co.) and was perfused by means of a peristaltic pump (LKB Varioerpex Pump 12000) at a rate of 0.76 ml/min. About 30 minutes before the end of the loading period, the rate of stimulation was reduced to the value to be used in the remainder of the experiment. If the Purkinje fibers were to be kept quiescent or driven at a very slow rate during the experiment, the driving rate was halved at the beginning of the last hour of the loading period and reduced during the last 30 minutes of the loading period to the rate used subsequently. The stimulation rate during the ⁴²K load and the duration of the loading period were selected in view of the fact that the radioactivity of Purkinje fibers stimulated at 60/min reaches equilibrium in 140 ± 9.5 minutes.³ Therefore, after a total of approximately 4 hours, the preparations should have been in equilibrium as far as potassium, active and inactive, is concerned.

As shown in Figure 1, the radioactive solution was allowed either to perfuse the tissue or to recirculate in its own reservoir bottle. Thus, the speed of the peristaltic pump was left unchanged throughout the experiment, the flow being directed by setting the two 3-way stopcocks in the appropriate position. At the end of the loading period, inactive Tyrode’s solution was perfused at a rate of 7.8 ml/min for 5 minutes in order to wash the radioactive potassium from the tubing, the tissue bath, and the extracellular space. The procedure adopted to estimate K⁺ uptake was similar to that introduced by Keynes³¹ and used in cardiac tissues by a number of other investigators.²⁻⁵,²⁻³ The principle of the method involves the measurements of the increase in tissue radioactivity at the end of an uptake period interpolated between two efflux periods. At the end of the 5-minute fast wash, the flow rate of inactive Tyrode’s solution was reduced to 0.76 ml/min and the tissue radioactivity counted for 1 minute every 3 minutes by means of a β probe (B in Fig. 1) connected to a Scaler spectrometer (Tracerlab model SC530). Fourteen counts were taken, and then the Purkinje fibers were exposed to the radioactive solution for 30 minutes. During the ensuing efflux period, tissue radioactivity was measured as during the first efflux period. The two efflux curves were extrapolated to the end of the uptake period, and the increase in tissue radioactivity was taken as a measure of potassium uptake (see Fig. 2). The whole procedure (efflux, uptake, efflux) constituted the control period. The same procedure was repeated in the presence of norepinephrine (or other variables under study) and once more during the recovery period. The change in tissue radioactivity during the test period was expressed as a percentage change with respect to the average value of the increment in radioactivity during the control and recovery periods. In most of the experiments, the whole procedure thus far outlined (control, test, recovery) was repeated during the second part of the experiment to study the effect of the experimental changes introduced (e.g., see Fig. 2). Other details of the methods have been reported recently.²⁻⁶,²⁻⁹ It should be pointed out that the procedure adopted underestimates the K⁺ influx, since no correction is made for K⁺ effluxing during the loading period (see Carmeliet²⁸).

In a series of experiments, transmembrane potentials of Purkinje fibers were recorded by means of microelectrode technique under several different conditions. The details of the intracellular recording technique have been described.³⁻⁴ In a series of five control experiments from this laboratory,⁷ Purkinje fiber preparations were loaded with radioactive potassium to equilibrium, and then six
uptake periods were carried out in succession in the absence of test procedures. The first uptake was equated to 100, and the following five uptakes were expressed as a percentage with respect to the first. With the exception of a single uptake, the values differed from control by 2.9% or less. This test suggests that the preparations were in a steady state throughout the experiment, as far as potassium uptake was concerned. Also, the action potential configuration and magnitude were similar at the beginning and at the end of this type of experiments (see Fig. 1 in Ref. 5 and Fig. 2 in Ref. 7). Finally, conditions which caused an increased K⁺ efflux enhanced the K⁺ uptake as well. From these findings, we assume that the K⁺ content was in a steady state and that an increased K⁺ efflux would result in an increased K⁺ uptake.

The measurements of radioactivity were corrected for background and radioisotope decay and plotted on semilogarithmic paper. The curves connecting tissue radioactivity counts were drawn by eye and by the method of the least squares using a Wang 520 programmable calculator. Percentage changes in tissue uptake were calculated first from graphs drawn by eye and then from graphs drawn with regression lines. Both sets of values were fairly similar and the small differences were not statistically significant (P > 0.20). All results reported here are those obtained by curves drawn by eye. Statistical evaluation of
Effects of Norepinephrine on K+ Uptake of Quiescent and Active Fibers

If norepinephrine were to increase potassium uptake in a quiescent fiber, it would be unlikely that the mechanism of such an increase would involve an increase in [Ca2+]i, or in iKr. However, norepinephrine induces spontaneous activity in quiescent Purkinje fibers perfused in Tyrode's solution, and several approaches had to be tried to overcome this difficulty. In the procedure eventually selected, the norepinephrine concentration was 6.9 × 10^{-7} M and the extracellular potassium concentration was 10.8 mM. The protocol adopted was as follows (Fig. 2). During the first part of the experiment, the preparation was not driven and was quiescent. Norepinephrine was administered during the period indicated by the arrows in between the control and recovery uptakes. During the second part of the experiment, the preparation was driven continuously at 30/min, and administration of norepinephrine was repeated as in the first part of the experiment. During norepinephrine administration, the fiber remained quiescent and yet K+ uptake increased by 11.0%. As expected, K+ uptake increased with the initiation of electrical stimulation as shown by the comparison of the last uptake at rest with the first uptake during drive. The administration of norepinephrine caused an increment of 15.3% above the new control and recovery uptakes. In a total of five experiments, the average increase induced by norepinephrine in quiescent fiber was +7.4 ± 1.5% (mean ± se) (P > 0.01). When the same fibers were driven during the second part of the experiment, norepinephrine caused a more pronounced increase (+16.2 ± 3.3%; P > 0.01). The difference between the effect of norepinephrine in the same fibers at rest and during activity was statistically significant (P < 0.05).

Another way to avoid the onset of activity on exposure to norepinephrine was to use ventricular muscle fibers. In a cat ventricular trabecula ([K+]o = 5.4 mM), norepinephrine (1.4 × 10^{-7} M) caused an increase in K+ uptake of 7.3% with the preparation at rest and of 14.9% with the preparation driven at 12/min. Thus, the experiments suggest that norepinephrine increases K+ uptake in a quiescent as well as in a driven fiber, but the increase is about half in the former.

Effects of Norepinephrine on Fibers Depolarized to the Plateau Range

The action of norepinephrine on K+ uptake was also studied in Purkinje fibers kept quiescent at a membrane potential within the plateau range (see inset in Fig. 3). The low membrane potential was obtained by increasing [K+]o, to 45 mM. The sodium concentration in the Tyrode's solution was suitably reduced to keep the osmotic pressure constant. As shown in Figure 3, norepinephrine (1.4 × 10^{-4} M) increased the potassium uptake by 9.2% while the preparation remained quiescent. In a total of three experiments, norepinephrine increased potassium uptake by 8.0 ± 0.9% (P < 0.02). When the same concentration of norepinephrine was administered to preparations stimulated at 60/min in 5.4 mM K+ Tyrode's solution, the increase in potassium uptake was +11.8 ± 1.6% (five experiments, P < 0.01).

Effects of Calcium on Potassium Movements

If norepinephrine is more effective in an active fiber because it increases calcium influx, a higher [Ca2+]i, in itself might increase potassium uptake. This was tested in the following manner. The fibers were stimulated at 42/min and the potassium uptake was measured in 0.9 (control), in 2.7 (test), and again in 0.9 (recovery) mM Ca2+ Tyrode's solution. The value of the test uptake was expressed as a percentage of the combined value of control and recovery. In three experiments (Fig. 4), increasing calcium to 2.7 mM resulted in an obvious increase in potassium uptake.

FIGURE 3 Effect of norepinephrine on K+ uptake in a quiescent fiber depolarized at the plateau range of potentials. For the other explanations of the legend, see Figure 2. The inset (taken from another experiment) shows the transmembrane potential recorded from a Purkinje fiber perfused with 45 mM K+ Tyrode's solution, superimposed on the record obtained during perfusion with 5.4 mM K+ Tyrode's solution. The vertical and horizontal bars are the voltage (50 mV) and the time (400 msec) calibration, respectively.

FIGURE 4 Effect of [Ca2+]i on potassium uptake in three experiments. C = average value of the uptakes preceding and following the test uptake. T = percent increase in potassium uptake during the test period. The calcium and norepinephrine concentrations during the test period are indicated at the top of each panel. The same symbols identify the same preparation in different panels. Average values ± standard error are shown (x).
increase in K\(^+\) uptake (+9.2\%) and this increase was somewhat higher (+11.2\%) when the preparation was perfused subsequently in 5.4 mM Ca\(^{2+}\) Tyrode's solution. Adding norepinephrine (1.4 x 10\(^{-4}\) m) to the high calcium solution only slightly changed the uptake (+12.8\%, see below).

The effect of high [Ca\(^{2+}\)]\(_o\), was studied also in resting fibers. In the protocol adopted to explore this point, the control and recovery uptakes were carried out in 0.9 mM [Ca\(^{2+}\)]\(_o\), and the test uptake in 8.1 mM [Ca\(^{2+}\)]\(_o\). The fiber was kept quiescent throughout the three uptakes by using a 6.75 mM K\(^+\) Tyrode's solution. As shown in Figure 5, increasing [Ca\(^{2+}\)]\(_o\), did not increase and even decreased K\(^+\) uptake. In contrast, when the same fibers were driven continuously at 90/min during the second part of each experiment, exposure to high [Ca\(^{2+}\)]\(_o\), consistently increased the uptake (+17.3 ± 1.4\%; \(P < 0.005\)).

The effect of high [Ca\(^{2+}\)]\(_o\), on the rate constant of the tissue radioactivity loss in resting and driven fibers was studied as follows. The preparation was loaded to equilibrium with \(^{40}K\) and then washed in inactive Tyrode's solution, while the tissue radioactivity was measured for 1 minute every 3 minutes. During the first part of the experiment, the fiber was quiescent and the tissue radioactivity was measured while the fiber was exposed to 0.9, 8.1, and 0.9 mM Ca\(^{2+}\) Tyrode's solution. As shown in Figure 6, the rate constant of tissue radioactivity loss decreased (-16.7\%) when [Ca\(^{2+}\)]\(_o\), was increased. In the second part of the experiment, the fiber was driven at 90/min and the presence of activity in itself increased the rate constant (+40.8\%), as expected.\(^{29}\) When [Ca\(^{2+}\)]\(_o\), was increased to 8.1 mM, the rate constant of tissue radioactivity loss increased by 20% with respect to the values preceding and following the exposure to high calcium. In a total of five experiments, on exposure to high Ca\(^{2+}\), the rate constant decreased in quiescent fibers by -12.6 ± 2.9\% (\(r < 0.02\)) and increased in driven fibers by +15.8 ± 2.1\% (\(P < 0.005\)).

**Effect of Rate of Discharge on K\(^+\) Uptake at Two Different Values of [Ca\(^{2+}\)]\(_o\).**

Repeated depolarizations of Purkinje fibers, induced either by voltage clamp\(^{28}\) or by electrical stimulation,\(^{29}\) increase potassium uptake and loss. If this change is associated in part with calcium entry during the action potential, the rate-dependent changes in K\(^+\) uptake may be a function of [Ca\(^{2+}\)]\(_o\).

The percent increase in potassium uptake when the preparations were driven at different rates at two different external calcium concentrations is illustrated in Figure 7. The preparations were kept either in low (0.9 mM/liter) or high (8.1 mM/liter) calcium throughout the experiment, including the initial loading period. The fibers were kept quiescent during each control and recovery period ([K\(^+\)]\(_o\) = 6.75 mM/liter) and were stimulated at the rate of 12, 36, 108 impulses/min during test periods interposed between control and recovery. In low calcium solution (filled circles), driving the fiber at 12/min produced a small increase in potassium uptake (+5.6 ± 0.3\%). Increasing the driving rate to 36/min and 108/min enhanced the uptake by 32.4 ± 8.5\% and 69.1 ± 5.2\%, respectively. In high calcium solution (open circles), driving the fiber at 12/min produced an increase in K\(^+\) uptake of 26.7 ± 2.4\%. Increasing the driving rate to 36/min and 108/min enhanced the uptake by 52.0 ± 1.6\% and 83.8 ± 8.5\%, respectively. While an increase in the driving rate increased K\(^+\) uptake in both calcium solutions, there are marked differences in the two sets of results. Thus, at the lowest driving frequency, the increment in K\(^+\) uptake was far larger (+376.7\%) in high than in low calcium. At 36 and 108/min, the increment of potassium uptake was also larger in high than in low calcium, but progressively less so (+60.4\% and +21.2\%, respectively).
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Figure 7 Effect of rate of discharge on potassium uptake at two different calcium concentrations. The ordinate shows the percent increase in potassium uptake during drive with respect to the control and recovery uptakes measured with the fibers at rest. The abscissa shows the three driving rates studied, in beats per minute. The filled circles show the values obtained in 0.9 mM and the empty circles those in 8.1 mM Ca²⁺ Tyrode's solution. The standard error is shown by the vertical lines. The number of experiments for each point is indicated in parenthesis.

Thus, while the increase in rate gave the expected increment in potassium uptake, the present experiment shows that the calcium concentration gradient is important in determining the magnitude of the change, especially at a low driving rate.

If calcium increases potassium uptake by entering the cell membrane through the slow channel, then it should be possible to antagonize such an effect by using the slow channel-blocker Verapamil. In a quiescent fiber exposed to 8.1 mM [Ca²⁺]ₐ, driving at 12/min led to an increase in K⁺ uptake of 20.9%; when the same procedure was repeated in the presence of Verapamil (4 x 10⁻⁷ M), the uptake was +8.2%. This suggests that blockade of the slow channel reduces the stimulatory effect of high [Ca²⁺]ₐ on K⁺ uptake. Still, there was the possibility that calcium might act by interacting with adenylate-cyclase as norepinephrine does (see Robison et al.34). In a quiescent fiber exposed to 8.1 mM [Ca²⁺]ₐ, driving the preparation at 108/min led to an increase in K⁺ uptake of 92.4%, with respect to the combined value of control and recovery determined with the fiber at rest. When the drive was repeated in the presence of propranolol (4 x 10⁻⁷ M) during the second part of the experiment, the result obtained was practically unchanged (+85.2%), suggesting that adenylate cyclase, while involved in the stimulatory action of norepinephrine in K⁺ uptake, is not involved in the stimulatory action of calcium.

Effect of Norepinephrine in Low and High [Ca²⁺].

Since high calcium in itself increases K⁺ uptake in active fibers (Fig. 5), it is possible that norepinephrine might be less effective in stimulating K⁺ uptake in a fiber driven at constant rate in the presence of high calcium. In two preliminary experiments, Purkinje fibers were driven at 60/min in normal (2.7 mM Ca²⁺) Tyrode's solution, and the action potentials were recorded. The calcium concentration was then decreased to 0.54 mM and action potentials were recorded for as long as 23 hours of perfusion. As shown in Figure 8, the lower calcium solution caused relatively minor changes in the electrical activity of the fiber. Having ascertained this, we carried out tests on potassium uptake in the following manner. The fibers were driven at 60/min throughout the experiments and were exposed to a lower Ca²⁺ (0.54 mM) in the first part of the experiment and to a higher Ca²⁺ (5.4 mM) during the second part of the experiment. Norepinephrine (1.4 x 10⁻⁶ M) was administered during the test uptake in each of the two halves of the experiments. As shown by the open symbols in Figure 9 (four experiments), the increase in K⁺ uptake induced by norepinephrine was larger in the lower calcium (lefthand panel) than in the higher calcium solution (righthand panel). In three additional experiments (Fig. 9, filled symbols), the effect of norepinephrine was also tested in 1.8 mM (lefthand panel).
Figure 9 Effect of norepinephrine at different external calcium concentrations. The ordinate shows the percent increment in potassium uptake induced by norepinephrine. C = average value of control and recovery uptake made equal to 100. Nor = percent increment in potassium uptake induced by norepinephrine. Empty symbols show the effect of norepinephrine in 0.54 mM [Ca\(^{2+}\)]\(_{o}\) (left panel) and in 5.4 mM [Ca\(^{2+}\)]\(_{o}\) (right panel). The filled symbols show the effect of norepinephrine in 1.8 mM [Ca\(^{2+}\)]\(_{o}\) (left panel) and 8.1 mM [Ca\(^{2+}\)]\(_{o}\) (right panel). The same symbol identifies the same preparation in the two panels. The average ± standard error for norepinephrine-induced potassium uptake in all experiments is shown (x).

High calcium does not increase K\(^+\) uptake in a quiescent fiber (Fig. 5). Therefore, norepinephrine should have its usual stimulatory effect on K\(^+\) uptake in a quiescent fiber, whether [Ca\(^{2+}\)]\(_{o}\) is high or low. In one experiment, a Purkinje fiber was continuously perfused with 13.5 mM K Tyrode's solution and remained quiescent throughout the procedures. In the first half of the experiment, [Ca\(^{2+}\)]\(_{o}\) was 1.8 mM, and norepinephrine (6.9 x 10\(^{-7}\)M) caused an increase in K\(^+\) uptake of 10.9%. In the second half of the experiment, [Ca\(^{2+}\)]\(_{o}\) was 8.1 mM, and the same dose of norepinephrine caused an increase of 13.3%.

Discussion

The present experiments show that: (1) norepinephrine increases K\(^+\) uptake both in quiescent and active fibers, but more so in the presence of activity; (2) in contrast, calcium increases K\(^+\) uptake in active but not in quiescent fibers; (3) the increase in K\(^+\) uptake induced by the onset of activity is greater when [Ca\(^{2+}\)]\(_{o}\) is larger and the rate is lower; and (4) there is an interaction among the effects caused by these different interventions.

Catecholamines may increase K\(^+\) uptake by different mechanisms. In spontaneously firing Purkinje fibers, catecholamines increase the rate of discharge, and this increase in rate would be expected to increase K\(^+\) movements. This rate-dependent increase would be expected on the basis that the induction of repeated depolarizing clamps\(^{36}\) or an increase in rate in electrically driven Purkinje fibers\(^{39}\) leads to a pronounced enhancement of potassium movements.

In Purkinje fibers, which are driven at a constant rate\(^{5,6,7}\) or remain quiescent (present results) during norepinephrine exposure, any increase in K\(^+\) uptake induced by norepinephrine is rate-independent. This rate-independent stimulation of K\(^+\) uptake by norepinephrine, in turn, may include several components. Thus, it has been postulated that catecholamines increase K\(^+\) movements by two rate-independent mechanisms: (1) an increase in K\(^+\) conductance,\(^{1,5}\) and (2) a stimulation of the activity of a sodium-potassium pump.\(^{5,6}\)

In reference to the first rate-independent mechanism (an increase in K\(^+\) conductance), the catecholamine-induced increase in potassium efflux\(^{1,3,5}\) could be due to the increment in the plateau current i\(_{Kp}\), an increment which has been shown in voltage-clamped Purkinje fibers.\(^{20}\) In the presence of a catecholamine-induced increase in calcium influx during systole,\(^{5,9,12}\) a shortening of the action potential suggests an increase of i\(_{Kp}\) also in nonclamped fibers. In addition, the increased calcium influx during systole should result in an increase of [Ca\(^{2+}\)]\(_{i}\) at the same time. Increasing [Ca\(^{2+}\)]\(_{i}\), increases i\(_{Ko}\),\(^{19}\) and an increase in [Ca\(^{2+}\)]\(_{i}\) increases potassium conductance in Purkinje fibers.\(^{22}\) Thus, the larger K\(^+\) uptake induced by catecholamines in active Purkinje fibers with respect to resting Purkinje fibers could be reasonably due to at least two factors: (1) an increase in i\(_{Kp}\); (2) an increase in [Ca\(^{2+}\)]\(_{i}\), during systole and therefore in i\(_{Ko}\). For the fiber to remain in a steady state, an increased loss would result in the increased uptake measured here.

It is unlikely, however, that norepinephrine affects potassium fluxes exclusively through an increase in the potassium conductances g\(_{Kp}\) and g\(_{Ko}\). Thus, the resting membrane potential is negative to the threshold for the activation of i\(_{Kp}\) and, therefore, an increment of this potassium current by catecholamines is unlikely. An increase in calcium influx and a consequent increase in the potassium current i\(_{Ko}\) is also unlikely, since the resting membrane potential is similarly negative to the threshold voltage for the activation of the inward calcium current. In addition, catecholamines do not increase the resting tension of quiescent cardiac tissues\(^{36}\) and decrease the resting potential of quiescent Purkinje fibers, a finding which does not suggest an increase in K\(^+\) conductance.\(^{36-38}\) Further evidence for little role of calcium in the norepinephrine-induced K\(^+\) uptake in quiescent Purkinje fibers is provided by the findings that, in quiescent fibers, norepinephrine has the same stimulatory action whether [Ca\(^{2+}\)]\(_{o}\) is normal or high, and that an increase in [Ca\(^{2+}\)]\(_{o}\) tends to decrease rather than increase potassium movements in a quiescent fiber (Figs. 5 and 6). The increase in K\(^+\) uptake induced by norepinephrine in fibers depolarized at the plateau level (Fig. 3) seems also unrelated to an increase in [Ca\(^{2+}\)]\(_{i}\), since catecholamines both abolish and prevent potassium-induced contractures, presumably by decreasing [Ca\(^{2+}\)]\(_{i}\) in depolarized quiescent fibers.\(^{36}\)
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The increased K⁺ movements in a quiescent fiber could be attributed to the decrease in membrane potential. However, the catecholamine-induced depolarization of a resting membrane is due to a shift in a depolarizing direction of the activation curve of i_K. Because of this mechanism, i_K cannot be greater than before the application of norepinephrine. In fact, i_K is likely to be smaller because, as a consequence of the depolarization, i_K should increase (although such an increase is restricted by anomalous rectification). An increase of i_K would reduce the magnitude of i_K, since the sum of i_K and i_K cannot exceed that prior to norepinephrine. On the contrary, the total current carried by K⁺ as a consequence of depolarization during norepinephrine should be somewhat less, since the inward leakage current should be decreased by the depolarization. Because of these reasons, the observed increment in K⁺ movements in quiescent fibers exposed to norepinephrine is unlikely to be secondary to the decrease in resting potential.

If the above findings seem to indicate that the action of norepinephrine is unlikely to be entirely mediated through an increase in potassium conductance, other findings support the concept of the existence of a second rate-independent mechanism, namely the stimulation of a sodium-potassium pump by catecholamines. Thus, norepinephrine counteracts the inhibition exerted by calcium on (Na⁺ + K⁺)-activated ATPase, and adenosine 3',5'-monophosphate (cyclic AMP) also stimulates the ATPase. The norepinephrine-induced K⁺ uptake is decreased or blocked by procedures (block of glycolysis, low temperature, Na⁺ lack, and strophanthidin) which either block the energy supply to the pump or the pump itself. Norepinephrine has little effect when the pump already has been stimulated prior to norepinephrine exposure. In addition, a stimulation of the pump would account for the increase in potassium content observed repeatedly in several tissues. Electrophysiological findings also suggest the stimulation of an electrogenic Na-K pump by catecholamines. Thus, in quiescent atrial fibers, epinephrine in appropriate concentrations increases a low resting potential without affecting the membrane resistance. In Purkinje fibers, the stimulation of an electrogenic sodium-potassium pump is suggested by the enhancement of the hyperpolarization induced by fast drive in the presence of norepinephrine.

In conclusion, it appears that catecholamines can increase potassium movements in Purkinje fibers by: (1) increasing i_K; and (2) increasing i_K by shifting the plateau to more positive values. However, the catecholamine-induced depolarization of a resting membrane is due to a shift in a depolarizing direction of the activation curve of i_K. Because of this mechanism, i_K cannot be greater than before the application of norepinephrine. In fact, i_K is likely to be smaller because, as a consequence of the depolarization, i_K should increase (although such an increase is restricted by anomalous rectification). An increase of i_K would reduce the magnitude of i_K, since the sum of i_K and i_K cannot exceed that prior to norepinephrine. On the contrary, the total current carried by K⁺ as a consequence of depolarization during norepinephrine should be somewhat less, since the inward leakage current should be decreased by the depolarization. Because of these reasons, the observed increment in K⁺ movements in quiescent fibers exposed to norepinephrine is unlikely to be secondary to the decrease in resting potential.

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In conclusion, it appears that catecholamines can increase potassium movements in Purkinje fibers by: (1) increasing the rate of discharge, (2) an increase in i_K, (3) an increase in the slow inward calcium current and therefore of i_K, and (4) a stimulation of active transport. The present results provide evidence that high calcium also influences potassium movements. High [Ca²⁺], increases both K⁺ uptake (Figs. 4 and 5) and loss (Fig. 6) in active fibers, presumably by increasing [Ca²⁺], during systole. The mechanism of such an increase seems to be related to an increase in potassium conductance, as suggested also by the fact that high [Ca²⁺], shortens the action potential and increases maximum diastolic potential in Purkinje fibers (see Fig. 8 in Ref. 45). High calcium can affect potassium movements in an active fiber also by shifting the plateau to more positive values which, in turn, induces a greater activation of i_K; this in spite of a direct depressing action of high calcium on i_K. A component due to the stimulation of active transport is unlikely, for it is well known that calcium inhibits the sodium-potassium pump. As to the site of action of calcium (intracellular or extracellular), the results in Figure 5 suggest that calcium does not act on external sites to affect potassium movements, at least in quiescent fibers. However, this suggestion is open to the objection that the lack of an effect of high calcium in a quiescent fiber may be only apparent and could be due to a balance among opposing actions. Thus, high external calcium could still increase i_K but at the same time decrease i_K (due to a shift of the steady state activation curve of this current in a depolarizing direction) and decrease the function of the sodium-potassium pump. These opposing actions would have to be closely matched, since the resting potential is not affected by high [Ca²⁺].

In conclusion, high calcium (by enhancing the slow inward calcium current) can increase potassium movements by: (1) increasing i_K; and (2) increasing i_K by shifting the plateau to more positive values. There are similarities between the actions of norepinephrine and those of calcium. Thus, i_K is increased by catecholamines and may be increased by high calcium through a more positive plateau. In addition, i_K is increased by high calcium and should be increased by norepinephrine by increasing calcium at the cell membrane. However, the modality by which high calcium and norepinephrine initiate an increase in potassium movements is different. Norepinephrine increases calcium and potassium movements by acting on a beta-receptor. High [Ca²⁺], alone also increases calcium and potassium (Fig. 5) movements. However, this action of calcium on i_K uptake does not involve a beta-receptor, since it is not modified by propranolol. That high calcium acts by increasing calcium influx is suggested by the reduction of its effect by Verapamil.

An increase in the rate of discharge also increases potassium movements, but this is accomplished by other mechanisms yet. During each action potential, K⁺ efflux is larger as i_K becomes larger and the slow plateau currents i_K and i_K are brought into play. As sodium influx is increased, active transport is also stimulated. The increased K⁺ loss is reflected in the increased K⁺ uptake. At high rate of stimulation, more action potentials are present in a unit of time and therefore K⁺ movements increase. The role of the slow inward calcium movements in the increased K⁺ loss with a higher rate of drive is demonstrated by the results obtained with an increase in rate at two different calcium concentrations (Fig. 7). The mechanism by which high calcium increases potassium movements more at low drive rate is probably related to the fact that calcium influx is likely to be smallest when the rate of discharge and [Ca²⁺], are lowest. Therefore, increasing [Ca²⁺], at the lowest rate is bound to provoke a more marked effect than when calcium influx (and therefore calcium at the cell membrane) is already enhanced at a higher rate.

If the mechanisms by which norepinephrine, high cal-

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cium, and increased rate of discharge are different, there is clearly an overlap in the factors brought into play to increase potassium movements. Therefore, it is not surprising that the effect of one of these interventions should be smaller when the effect of another is already conspicuous. Thus, norepinephrine is less effective in increasing potassium movements when \([\text{Ca}^{2+}]\) (Fig. 9) or the rate of discharge is high; and the effect of the rate of discharge is less pronounced when \([\text{Ca}^{2+}]\) is high (Fig. 7). It is of interest that a high \([\text{Ca}^{2+}]\), reduces also other actions of norepinephrine, such as the increment of contractility. 

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