Voltage Clamp Analysis of the Effects of Dopamine on the Transmembrane Ionic Currents Underlying the Action Potential of Sheep Cardiac Purkinje Fibers

Jeremiah M. Gelles and Ronald S. Aronson

SUMMARY Dopamine shifted the voltage-dependence of the slow outward current that underlies spontaneous diastolic depolarization to a more positive voltage range and increased the rate constants of inactivation of this current at voltages negative to ~80 mV. Dopamine increased the magnitude of the slow inward current and the delayed outward current. Dopamine had an inconsistent effect on the positive dynamic current and the time-independent potassium current. These effects were consistent with effects on the slope of diastolic depolarization, plateau voltage, and action potential duration and are similar to those of epinephrine. The effects of dopamine on the slow inward current and on the delayed outward current were abolished by verapamil, a slow channel blocker. This finding substantiates that outward potassium currents are modulated by intracellular calcium.

DOPAMINE is a naturally occurring, endogenous catecholamine in the treatment of cardiac failure and shock. Dopamine increases myocardial contractility and, in relatively large doses, can cause tachyarrhythmias. The electrophysiological effects of dopamine when studied by conventional microelectrode techniques are generally similar to those of other catecholamines. Although information is available concerning the effects of other catecholamines on the ionic currents underlying the action potential of cardiac Purkinje fibers, no data are available concerning the effects of dopamine on the ionic currents underlying the cardiac action potential.

In the present study we used a two-microelectrode voltage clamp technique to correlate changes in the transmembrane action potential induced by dopamine with changes in some of the ionic currents which generate the action potential of cardiac Purkinje fibers. Although information is available concerning the effects of dopamine on the ionic currents underlying the action potential of cardiac Purkinje fibers, no data are available concerning the effects of dopamine on the ionic currents underlying the cardiac action potential.

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Methods

Sheep hearts were obtained from a local abattoir, immersed in cooled (4-10°C) Tyrode's solution, and brought to the laboratory. Purkinje strands (0.5-0.8 mm in diameter and 5-10 mm in length) were removed from the ventricles and mounted in a plexiglass tissue bath with a volume of 1.0 ml. The procedure for making short (1.6 mm) segments of Purkinje tissue suitable for studying by voltage clamp has been described in detail elsewhere along with a partial description of the electrical apparatus used. The tissue was superfused at a rate of 5-10 ml/min with modified Tyrode's solution of the following composition in millimoles per liter: [Na] = 153.8, [K] = 2.7, [Cl] = 145.9, [Ca] = 2.7, [Mg] = 0.5, [HCO₃] = 12, [PO₄] = 1.8, [glucose] = 5.5. The solution was equilibrated with 95% O₂ -5% CO₂ and this resulted in a pH of 7.4. The temperature of the fluid was maintained at 37 ± 0.5°C.

The voltage clamp and application of current pulses were achieved by means of a solid state high gain feedback circuit. A Philbrick 1022 FET operational amplifier served as the final stage of the feedback loop. In experiments performed on an equivalent circuit of the membrane with a time constant of 22 msec the voltage clamp circuit charged the equivalent membrane capacitance within a few microseconds. It has been shown that the membrane capacitance of Purkinje fibers can be charged within 5 msec. The capacitive current has a time constant one or two orders of magnitude shorter than that of the ionic currents under consideration.

External stimuli were isolated from ground (ISA 100, Bioelectric Instruments) and applied to the tissue by small coaxial electrodes (Rhodes Medical Instruments). The timing of stimuli was determined by a waveform generator (Tektronix type 162) that triggered a pulse generator (Tektronix type 161). The latter was used to select the amplitude and duration of the rectangular stimulating pulses and also was used to program the timing, duration, and amplitude of command signals. A DC balance control incorporated in the clamp circuit permitted adjustment of the holding potential. Photographic records were made with a Grass C4R camera or a Tektronix C12 oscilloscope camera.

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Received March 17, 1976; accepted for publication November 18, 1976.
Action potentials were recorded from preparations immediately before or after the corresponding voltage clamp records were obtained. In the experiments to be described each fiber serves as its own control so that changes induced by dopamine are expressed in terms of relative changes. Details of the method for studying the I_{K2} current are given in a previous paper along with some discussion of the theoretical limitations of the two-microelectrode voltage clamp technique. The study of currents associated with the early part of the plateau of the cardiac action potential was accomplished by holding the membrane potential at -40 or -50 mV so that the excitatory Na current was not activated during depolarizing clamps. Under those conditions, the inward current should be separate from the excitatory Na current. The current measured under these conditions has been called the "slow inward current." The amplitude of this inward current was taken as the difference between the maximum inward current and the current level at 250 msec.

Dopamine HCl (Armar-Stone) was used in all experiments. Verapamil HCl (Knoll) was supplied as the powder.

Results

EFFECTS OF DOPAMINE ON DIASTOLIC CURRENTS

The effects of dopamine on I_{K2}, the potassium current whose slow decay at diastolic levels of voltage is said to underlie spontaneous diastolic depolarization in cardiac Purkinje fibers, are shown in Figure 1. Figure 1A and B shows the change in transmembrane ionic current measured during three rectangular voltage clamp steps from -80 mV, the holding potential, to -84 mV, -93 mV, and -97 mV. The records in Figure 1A were obtained under control conditions and show that the currents recorded during the clamp steps slowly increase in an inward (negative) direction to reach a steady value. The onset of the gradual increase in inward current is preceded by a rapidly declining inward current transient. Following termination of the clamp steps slowly declining tails of inward current are recorded. The measured time course of the slow increase in inward current and the decay of the tail of inward current were plotted on semilog paper and could be fitted by single exponentials. The slowly increasing inward current actually results from the voltage-dependent deactivation of the slow outward K current, designated I_{K2} by Noble and Tsien. Similarly, the declining tail of inward current recorded following termination of the clamp step is the result of the voltage-dependent activation of I_{K2}.

Figure 1B shows records obtained after exposure to dopamine (10^{-5} to 10^{-4} M) for 18 minutes. In contrast to the control records, the time-dependent increase in inward current during the clamp and the slowly declining tail following termination of the clamp are almost entirely abolished during exposure to dopamine. The steady state inward current during the clamp steps decreased during exposure to dopamine.

The curve obtained by plotting the peak magnitude of each tail of current recorded after termination of the clamp (I_{K2}) as a function of the membrane potential (E_m) prior to termination of the clamp has been referred to as the steady state I_{K2} activation curve. Figure 2 shows such activation curves obtained for a preparation different from the one that provided the records in Figure 1. The value of current at -80 mV in Figure 2 corresponds to the holding current. The control activation curve has its usual sigmoid configuration. After exposure to dopamine (10^{-5} to 10^{-4} M) the half-maximum amplitude point of the activation curve is shifted 15 mV in a positive direction along the voltage axis but the amplitude and general configuration of the curve remain unchanged from control. A similar positive shift of the half-maximum amplitude point along the voltage axis in the presence of dopamine was found in eight experiments.

A consequence of this shift is that I_{K2} is deactivated at less negative potentials during exposure to dopamine. After removal of dopamine the activation curve returns to a position superimposable on the control curves. These results suggest that one effect of dopamine on I_{K2} is to shift...
the activation curve for the current in a positive or depolarizing direction. The activation curve is a steady state measurement and therefore does not provide information on the time-dependent properties of $I_{K_0}$. It was therefore of interest to determine the effect of dopamine on the time constant of change of $I_{K_0}$. In one experiment the voltage-dependent time constant of $I_{K_0}$ at $-93$ mV and $-97$ mV decreased in the presence of dopamine, whereas in another experiment that measured at $-80$ mV was unchanged.

**EFFECT OF DOPAMINE ON EARLY PLATEAU CURRENTS**

Records from an experiment in which the effects of dopamine on the slow inward current were studied are shown in Figure 3. Figure 3A shows an action potential recorded during the control period evoked during external stimulation at 1.3 Hz. Figure 3B shows that during exposure to dopamine ($10^{-3}$ M) the voltage level of the plateau measured 60 msec after the upstroke is shifted by 7 mV in the positive direction. The entire plateau up to the inflection separating phase 2 from phase 3 of the action potential is more positive during exposure to dopamine than during the control period.

Figure 3C and D shows voltage clamp records obtained from the same preparation that provided the action potentials shown in Figure 3A and B. The transmembrane potential was held at $-40$ mV, and rectangular clamp pulses to $-19$ mV were applied at 500-msec intervals after a period of quiescence in order to simulate a repetitive series of action potentials whose plateau level was at approximately $-19$ mV. Each depolarizing clamp lasted 500 msec. The first step shows an initial rapidly decaying outward current. The remaining clamps are superimposed on this initial clamp step and show a gradual decrease in the initial outward current and the development of a maximum inward current after several clamp steps. The rapidly decaying initial outward current that declines with repetitive clamps has been called the positive dynamic current; the inward maximum represents the slow inward current. The inward current is maximum at 60 msec. There is less outward current during the duration of the clamp after repetitive clamps. During the control period, (Fig. 3C) the decrease in outward current at 60 msec during repetitive clamps is 34 nA. The magnitude of the decrease in outward current during exposure to dopamine was 20 nA. In three experiments there was no consistent effect of dopamine on the positive dynamic current.

Dopamine increased the maximum inward current developed during repetitive stimulation by 16 nA. This was a consistent finding in all experiments although the concentration of dopamine required for this effect varied from preparation to preparation.

To determine the effect of dopamine on the slow inward current without interference from the positive dynamic current it is necessary to clamp to potentials more negative than about $-20$ mV. Figure 4 shows an experiment in which the transmembrane potential was held at $-50$ mV and the preparation was repetitively depolarized to $-36$ mV for periods lasting 25-600 msec at a frequency of one clamp per second. The corresponding current records were superimposed. The inward tails of current recorded on termination of each voltage step increased in magnitude as the duration of the step increased to about 100 msec. An inward tail persists for as long as 600 msec (Fig. 4C). The envelope of tails of inward current gives the time course of activation of the slow inward current at $-36$ mV. During the control period (Fig. 4A) the peak inward current tail follows the termination of the 90-msec step and is $-36$ nA with respect to zero current. During exposure to dopamine (Fig. 4B) the maximum inward tail increases to $-40$ nA and follows the 60-msec step. In addition, during exposure to dopamine, the holding current becomes more negative during repetitive clamps (Fig. 4D).

**Figure 3** Effect of dopamine on plateau currents. A: control action potential. B: action potential during exposure to dopamine, $10^{-3}$ M. Dotted line shows 0 membrane potential. C: repetitive voltage clamp steps with currents superimposed. D: similar voltage clamp program as in C during exposure to dopamine, $10^{-3}$ M. (See text for complete description.)

**Figure 4** Effect of dopamine on the slow inward current in sheep cardiac Purkinje fibers. A: series of voltage clamp pulses from a holding potential of $-50$ mV to $-36$ mV. The pulses vary in duration from 25 msec to 600 msec. Only the current record is displayed. The current tails have been retouched to eliminate the inward capacitive artifacts. The repetition rate of clamp steps is sec$^{-1}$. B: similar clamp program to that in A during exposure to dopamine, $10^{-4}$ M. The thickness of the holding current trace indicates an increase in inward holding current during repetitive clamps. C: similar clamp program to that in A and B after removal of dopamine.
4B) although to a smaller extent this can also be seen in Figure 4A and C. This may be due to a portion of inward current activated on depolarization to -36 mV which is not completely inactivated on repolarization to -50 mV. After removal of dopamine, the maximum inward tail returns toward the control value (Fig. 4C) and follows the 40-msec step.

**EFFECT OF Dopamine ON LATE PLATEAU CURRENTS**

To distinguish the effects of dopamine on the outward currents activated during the plateau of the Purkinje fiber action potential from its effects on outward currents recorded at diastolic levels of potential it is necessary to maintain the transmembrane potential at levels positive to about -50 mV at which the I_K1 current discussed earlier is completely activated.\(^{14-17,18}\) The results of such an experiment are shown in Figure 5. The transmembrane potential was held at -40 mV and the preparation was repetitively depolarized to -10 mV after inactivation of the positive dynamic current. Each depolarizing clamp was somewhat longer than the last so that a gradually increasing outward current was recorded. On repolarizing, at the end of each clamp, a tail is recorded that has an initial rapid inward component followed by an outward component that decays slowly to the holding current. The envelope of outward tails reflects the voltage-dependent time course of activation of the slowly increasing outward current superimposed on the voltage-dependent time course of inactivation of the slow inward current. The "delayed" outward current has been called I_K2, and is thought to be responsible in part for termination of the plateau in cardiac Purkinje fibers.\(^{17-19}\)

When the outward current is plotted on semilog paper with respect to time, two exponentials are found, one with a time constant of 57 msec that reflects the inactivation of slow inward current, and one with a time constant of 515 msec that reflects the activation of I_K2. During exposure to dopamine (Fig. 5B) the time constant of inactivation of slow inward current becomes 42 msec while the time constant of I_K2 activation becomes 462 msec. The outward current magnitude recorded at the end of the longest clamp increased from 32 nA during the control period (Fig. 5A) to 52 nA during exposure to dopamine (Fig. 5B).

To determine whether the dopamine-induced increase in maximum inward current (Figs. 3 and 4) was related to a specific dopamine-induced increase in slow inward current (Fig. 5A and B), verapamil was used to block the slow inward current channel.\(^{20,21}\) The same preparation that provided the records shown in Figure 5A and B was exposed to verapamil, 2 mg/liter, after the effect of dopamine recorded in Figure 5B had been washed out. Figure 5C shows the same voltage clamp program applied to the fiber 2 minutes after beginning exposure to verapamil. The initial inward current minimum is almost completely eliminated and the outward current recorded during the superimposed clamps of increasing duration is increased. The outward current tails are decreased and the slow increase in outward current seen in Figure 5A is diminished in magnitude, the entire current trace assuming a more rectangular appearance. Exposure of the preparation to dopamine (10\(^{-3}\) m) during the verapamil effect (Fig. 5D) had minimal influence on the verapamil effect on the slow inward and delayed outward currents.

**Discussion**

**EFFECT OF Dopamine ON I_K2**

The increase in the rate of spontaneous diastolic depolarization caused by dopamine\(^{2}\) can be attributed to its effects on I_K2. By shifting the potential range in which I_K2 is deactivated to more positive potentials, dopamine increases the amount of I_K2 available for deactivation at voltages positive to the maximum diastolic potential. Dopamine also accelerates the rate of deactivation of I_K2 at potentials negative to -80 mV. Because the curve relating the time constants of I_K2 activation to voltage is U-shaped,\(^{14}\) the time constant increases at voltages positive to -80 mV (the trough of the curve) and decrease at voltages negative to -80 mV when the curve is shifted in a positive direction along the voltage axis. At -80 mV, the result may be either an increase or a decrease in the time constant of I_K2.\(^{3}\) Epinephrine has been shown to have the same effect on I_K2 as dopamine.\(^{5}\)

The steady state inward current appears to be less during exposure to dopamine than during the control period because the holding current is more inward during exposure to dopamine than during the control period. This is so because more I_K2 is deactivated at -80 mV during exposure to dopamine than during the control period (Fig. 2). The change in holding current observed during these experiments was accounted for by the change in I_K2 and there was no evidence that dopamine affected the time-independent potassium current, I_K1. This is consistent with Tsien's results with epinephrine.\(^{5}\)
EFFECT OF DOPAMINE ON EARLY PLATEAU CURRENTS

Depolarization by voltage clamp of cardiac Purkinje fibers to about -25 mV or more positive values of membrane potential results in an initial transient outward current that declines with a time constant of about 100 msec.16 Dudel et al.19 proposed that this current might be responsible for the notch seen in Purkinje fibers driven at slow rates because this current is reactivated in a time-dependent manner at potentials negative to about -50 mV. According to the studies of Fozzard and Hiraoka,22 this current is about 50% inactivated at a holding potential of -40 mV and is not reactivated at -40 mV after a depolarization from that level. The decrease in the outward current during the series of clamps in Figure 3 thus is probably due to the decrease in positive dynamic current remaining at the holding potential of -40 mV. Dopamine had a variable effect in three experiments on the change in positive dynamic current observed during the train of clamps (Fig. 3). However, dopamine consistently increased the inward current maximum. The increase in inward current observed was sufficient to account for the observed positive shift in plateau level during the action potentials recorded before and during exposure to dopamine (Fig. 3A and B). Reuter17 found a similar effect of epinephrine on the slow inward current recorded from cardiac Purkinje fibers. Other investigators have shown a similar effect of epinephrine on bovine Purkinje fibers6 and on atrial fibers.23

It was possible to distinguish the effect of dopamine on the slow inward current from the effects on the dynamic outward current by holding the membrane potential at -50 mV and depolarizing to -36 mV at which level of membrane potential the outward current is not activated (Fig. 4). Dopamine increased the slow inward current tail recorded on repolarization to the holding potential. Rapid inward tails of current similar to those shown in Figure 4 at relatively long times after onset of a depolarizing clamp have been recorded by others from Purkinje fibers24 and suggest that the slow inward current does not inactivate completely during depolarization. Inward tails of current were recorded as long as 1,300 msec after onset of the depolarizing clamp in some experiments. (The possibility that these inward tails represent to some extent inward movement of potassium ions accumulated in the extracellular space during depolarization, as has been shown in frog node of Ranvier,24 has not been fully evaluated.25)

In one experiment dopamine did not appear to accelerate the time course of activation of the slow inward current at -36 mV as determined by the time taken to reach maximum inward current (Fig. 4). Dopamine did shorten the inactivation time constant of slow inward current in the experiment shown in Figure 5. The fact that inward holding current increased more during repetitive clamps during exposure to dopamine may reflect a shift in the voltage-dependence of the inactivation of the slow inward current. However, several other explanations are plausible and experiments have not yet been done to clarify this point.

Reuter23 found no effect of norepinephrine on activation and inactivation of the slow inward current. However, Vassort et al.23 found a small decrease in the time constant of inactivation of slow inward current in frog atrium. Because several currents overlap in cardiac fibers during the plateau phase of the action potential, final resolution of the effects of catecholamines on the kinetics of the slow inward current will have to await further experiments. It is unlikely that the ability of dopamine to shorten the action potential is related to its effect on the kinetics of the slow inward current.

EFFECT OF DOPAMINE ON LATE PLATEAU CURRENTS

Repolarization of cardiac Purkinje fibers is thought to be due to one or both of two mechanisms: (1) the activation of the delayed outward K-current, i_{Kd},17,19 and (2) the inactivation of the slow inward current.19,25 The results shown in Figure 5 show that dopamine augments the magnitude of the delayed outward current while decreasing the activation time constant from 562 msec to 415 msec. Tsien et al. have shown that cyclic AMP and norepinephrine increase the magnitude of i_{Kd} in a similar fashion. What is of most import, though, is the finding that verapamil, a relatively specific slow channel blocker,26,27 also blocked the dopamine-induced increase in i_{Kd}. This finding is consistent with that of Kass and Tsien,26 who have reported that “calcium antagonists” such as Mn and D 600 also inhibit i_{Kd}. Other investigators27,28 have suggested that cardiac cell membrane potassium permeability may be modulated by intracellular calcium concentration. Since the dopamine-induced increase in i_{Kd} (Fig. 5) was reversed by verapamil, a “calcium channel blocker,” the dopamine-induced increase in i_{Kd} may be secondary to the probable increase in calcium delivery during the enhanced slow inward current. The increase in i_{Kd} would cause the action potential to shorten.

Acknowledgments

We thank Dr. B.F. Hoffman for his valuable advice in carrying out this work. Dopamine hydrochloride and verapamil hydrochloride were kindly supplied by Arnar-Stone Laboratories, Inc., and Knoll Pharmaceutical Co., respectively.

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The Effects of Hyaluronidase on Coronary Blood Flow following Coronary Artery Occlusion in the Dog

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SUMMARY In an attempt to determine the mechanism by which hyaluronidase reduces myocardial injury following coronary artery occlusion, myocardial blood flow was studied in 20 open-chest dogs with occlusion of the left anterior descending coronary artery. Ten dogs served as controls, and 10 received hyaluronidase (500 NF units/kg) intravenously 20 minutes after occlusion. At 15 minutes and at 6 hours after occlusion, regional myocardial blood flow in the epicardial and endocardial halves of both ischemic and nonischemic zones were determined with radiolabeled microspheres. Mean arterial pressure, heart rate, and cardiac output were similar in the untreated and treated dogs through the 6 hours of the experiment. Moreover, regional blood flow to nonischemic myocardium (areas without epicardial S-T segment elevation 15 minutes after occlusion) was similar in the two untreated and treated groups 15 minutes and 6 hours after occlusion. Fifteen minutes after occlusion, the flow to the ischemic myocardium subjected to sites with S-T segment elevation exceeding 2 mV) in the untreated group was: transmural, 28.1 ± 2.2 (mean ± se) ml/min per 100 g; endocardial, 20.7 ± 1.8; and epicardial, 38.5 ± 3.1. The endocardial-epicardial flow ratio was 0.56 ± 0.04. Six hours after occlusion, the untreated group demonstrated a further decrease in blood flow to the ischemic myocardium: transmural, 15.2 ± 1.4 ml/min per 100 g; endocardial, 6.8 ± 1.1; and epicardial, 24.3 ± 1.9. The endocardial-epicardial flow ratio fell to 0.28 ± 0.04. In contrast, the hyaluronidase-treated dogs showed no further reduction in blood flow to ischemic myocardium 6 hours after occlusion: transmural, 30.3 ± 3.1 ml/min per 100 g; endocardial, 21.3 ± 2.5; and epicardial, 38.8 ± 3.8. These regional myocardial flows were significantly higher than those of the untreated dogs 6 hours after occlusion. Thus, salvage of damaged myocardium by hyaluronidase might be explained by its beneficial effect on collateral blood flow to the ischemic tissue, though this effect on collateral flow could be the consequence rather than the cause of this salvage.

IN 1959 IT WAS reported that intravenous hyaluronidase reduced the magnitude of S-T segment elevation both in animals with coronary artery ligation and in patients with acute myocardial infarction. This effect was attributed to the decrease in edema formation, but it was not suggested that the drug reduced the extent of myocardial damage. In 1969 it was reported that the amount of myocardial damage resulting from an experimental coronary artery
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doi: 10.1161/01.RES.40.6.561

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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