rine per kilogram resulted in a further decrease in blood pressure.

Mammalian studies beginning with that of Alquist (*4*) have led to the classical concept of alpha and beta receptors for the reaction of animals to catecholamines. Usually, stimulation of alpha receptors causes vasoconstriction, and stimulation of beta receptors causes vasodilatation. Epinephrine will stimulate both receptors; isoproterenol stimulates only the beta receptors. Epinephrine and isoproterenol elicited similar responses in the species of sharks studied. However, the depressor response to epinephrine was not obtained until after a maximum pressor response occurred. In mammals, the depressor response to epinephrine precedes the pressor effect.

The epinephrine-induced depressor effect in sharks was unusual. The response was only to lower blood pressure to the control value. The only circumstance in which epinephrine elicited a decrease in blood pressure to below control values was in the animals first treated with dibenzyline, an alpha-receptor blocking agent. This observation suggests that the depressor effect is mediated in part, at least, by a beta-like receptor. Similarly, blockade of the pressor response may result from blockade of alpha-like receptors.

The prolonged pressor response to epinephrine may be a reflection of a deficiency of inactivation mechanisms. However, amine oxidase and *O*-methyltransferase are present in shark tissues (*5*), and sharks have chromaffin cells containing epinephrine and norepinephrine (*6*). Apparently, then, epinephrine is not foreign to the cardiovascular system of the animals studied.

The effect of epinephrine on heart rate was small. Large doses did have a negative chronotropic effect, confirming previous observations (*7*). However, the isolated heart of *Squalus acanthius* reacts to epinephrine and norepinephrine with positive inotropic and chronotropic effects (*6, 8*).

Thus, in both lemon and nurse sharks the basic qualitative reactions to epinephrine and isoproteronol suggest the presence of alpha- and beta-like receptor areas.

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References and Notes

1. B. R. Lutz and L. C. Wyman, *Biol. Bull.* **62**, 17 (1932); J. C. Mott, in *Physiology of Fishes*, M. E. Brown, Ed. (Academic Press, New York, 1957), vol. 1, p. 102.
2. These studies were carried out at the Lerner Marine Laboratory of the American Museum of Natural History, Bimini, Bahamas.
3. L. J. Hale, *Biological Laboratory Data* (Wiley, New York, 1958), p. 92.
4. R. P. Alquist, *Amer. J. Physiol.* **153**, 586 (1948).
5. E. G. Trams, presented at the meeting of the Shark Research Panel, American Institute of Biological Sciences, Washington, D.C., 26 April 1968.
6. E. Ostlund, *Acta Physiol. Scand.* **31** (Suppl.), 112 (1954).
7. J. W. Burger and S. E. Bradley, *J. Cell. Comp. Physiol.* **37**, 389 (1951).
8. Ostlund also reported that epinephrine "sometimes" had an initial inhibitory effect on the isolated dogfish heart which could be blocked by atropine.
9. We thank R. Mathewson, director of the Lerner Marine Laboratory, and his staff for technical advice and guidance. From the Bureau of Medicine and Surgery, Navy Department, Research Task No. MR005.04-0112. Supported by contract 104-037 from the Office of Naval Research.
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Sodium Current in Ventricular Myocardial Fibers

Abstract. *Membrane currents were measured in thin bundles of dog ventricular myocardium under voltage-clamp conditions. A rather large initial inward current which had an equilibrium potential at about + 55 millivolts could be recorded. When the external sodium concentration was reduced, the equilibrium potential for this current was shifted by the amount predicted theoretically for a current carried solely by sodium ions. The size of the sodium inward current (I_{Na}) was largely dependent on the preceding membrane potential. The I_{Na} was completely inactivated if the membrane potential was as low as − 45 millivolts. Sodium ions are the main carrier of charge during the rapid depolarization phase of the action potential.*

There is evidence suggesting that in cardiac Purkinje fibers, as in squid axon (*1*), the rapid depolarization phase of the action potential depends on a specific permeability increase of the membrane for sodium ions (*2*). The application of the voltage-clamp method to frog atrial (*3*) and mammalian ventricular (*4*) myocardial fibers showed that movement of sodium ions down their electrochemical gradient into the fibers might also be the basic mechanism for rapid depolarization in these excitable tissues. The experiments reported here were performed to obtain information about the sodium current in mammalian myocardial fibers.

Thin ventricular trabeculae or very small papillary muscles (diameter, 0.5 mm or less; length, 5 mm or more) were isolated from dog heart. The preparations were pulled through tightly fitting holes in two rubber membranes which divided a chamber into three compartments. The middle compartment was continuously perfused with isotonic sucrose solution, and the two outer compartments were perfused with Tyrode's solution (*5*). In one of the outer compartments only about 1 mm of the fiber was exposed. When a voltage was applied between the two outer compartments, current flowed between these compartments through intracellular pathways of the fiber bundle, owing to the high resistivity of the sucrose solution and the relatively low intracellular resistance. The current flow through the membrane resulted in a homogeneous depolarization in the small portion of fiber exposed to Tyrode's solution. The membrane potential in this fiber portion was recorded between an intracellular and an extracellular microelectrode filled with 3*M* KCl, and the potential was controlled by a negative feedback circuit which used a Philbrick P85A operational amplifier. The gain and frequency response of the system were sufficient to produce a stepwise membrane-potential change within 0.5 msec. Contractions of the same fiber portion were measured with a force-displacement transducer (Grass FTO3C). Contraction, membrane voltage, and membrane current were recorded simultaneously on an oscilloscope (Tektronix 565). There are advantages and limitations to this kind of voltage-clamp method (*6*).

The data recorded were the membrane current and the contraction in response to depolarizing voltage-clamp steps (Fig. 1). The resting potential (−77 mv) was identical with the holding potential from which rectangular voltage steps of increasing amplitudes were applied to the membrane of the fiber bundle. Immediately after all potential steps, a surge of capacitative current flowed. At potential levels up to − 64 mv, constant outward current was recorded throughout the depolarizing pulse. At potentials beyond − 64 mv, however, the capacita-

tive surge was followed by an inward current exhibiting a steep voltage dependence and reaching its maximum amplitude at − 60 mv. This initial inward current decayed rapidly and turned into outward current within about 50 msec. With larger depolarizing pulses, the initial inward current decreased in amplitude. After correction for the capacitative surge, the potential at which the early current record showed neither an inward nor an outward hump is considered to be the equilibrium potential for the initial inward current (*1*).

If the initial inward current is carried solely by sodium ions, its equilibrium potential should change in a predictable manner when the external sodium concentration $[Na]_o$ is decreased to 31 percent of that in Tyrode's solution by replacing NaCl isotonically with sucrose or tris(hydroxymethyl)aminomethane-chloride. To test this prediction for a given preparation, the equilibrium potential for the initial inward current was first determined in normal Tyrode's solution. Next, a similar run was made in low sodium solution within 10 minutes after the bathing fluid was changed. The peak initial currents were plotted against membrane potential (Fig. 2). In normal Tyrode's solution, the amplitude of the initial inward current increased steeply in the potential range from − 70 to − 65 mv and reversed its sign, becoming outward current, at + 60 mv.

This current-voltage relation showed rectifier properties in the potential range from − 50 to + 50 mv. In sodium-poor solution, the amplitude of the initial inward current was reduced, and the equilibrium potential was shifted to + 30 mv. The Nernst equation, $E_{Na} = (RT/F) \times \ln ([Na]_o/[Na]_i)$, predicts a theoretical equilibrium potential shift of 30.6 mv under these conditions, if the initial current is carried solely by sodium ions. In five similar experiments, the equilibrium potential shifted by 29 ± 2.3 mv (mean and standard error of mean), showing that the initial inward current measured in dog myocardial fibers is carried by sodium ions.

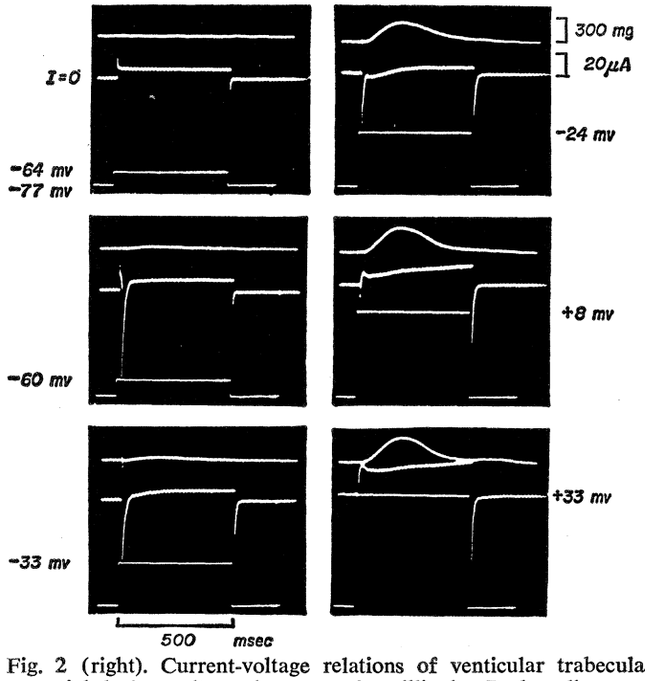
The net outward current in Tyrode's solution was measured at the end of a 300-msec depolarizing pulse for the same fiber (Fig. 2). As in cardiac Purkinje fibers, the current-voltage relation for outward current shows inward-going (anomalous) rectification and has a negative slope in the potential range from − 50 to − 25 mv (*7*).

The inactivation effect of the membrane potential on the Na current was investigated by changing the potential in two steps (*8*). When the potential of the second step (V_2) is held constant, I_{Na} elicited at this level is strongly dependent on the potential of the initial, conditioning depolarization (V_1). The results are expressed as the fraction, h_∞ (*8*), of maximum I_{Na} which can be elicited at V_2, plotted as a function of the potential during the conditioning depolarization, V_1. Our experiments revealed that if V_1 is more negative than − 70 mv, peak I_{Na} is maximum, whereas if V_1 is more positive than − 45 mv, there is no detectable I_{Na} at V_2. The standard form for this curve (*2*, *8*) is

$$h_\infty = 1/[1 + \exp((V_1 - E_h)/S)]$$

Typical results from our experiments yielded E_h of − 55 mv, and a slope factor S of 4.5 mv. Weidmann (*2*) obtained a comparable inactivation curve for cardiac Purkinje fibers. The slopes for the curves from the two cardiac tissues are the same, but the curve obtained here is about 15 mv more positive than that for the Purkinje fibers. Similar relations have been described for other excitable tissue (*8*, *9*).

A second much smaller and slower net inward current can be recorded at − 24 mv (Fig. 1). At this potential, the steady-state contraction of the myocardial preparation is rather strong, whereas at more negative potentials



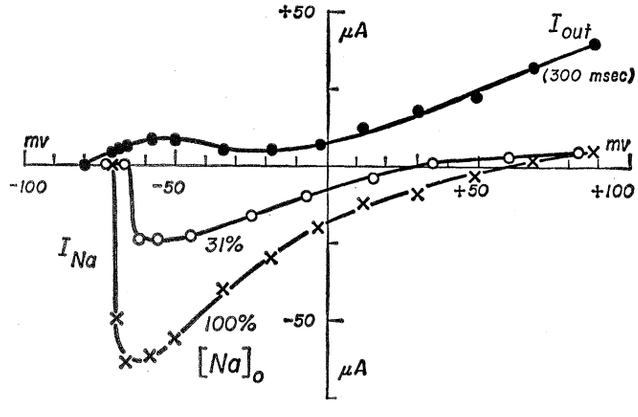


Fig. 1 (left). Voltage clamps of ventricular trabecula from dog heart in Tyrode's solution. Upper traces, isometric contraction; middle traces, membrane current (upward deflection from $I = 0$ indicating outward current, downward deflection indicating inward current); lower traces, stepwise displacement of membrane potential from its resting value at −77 mv. Figures beside records indicate the membrane potential during voltage-clamp step. Records show steady-state conditions for contraction after membrane potential had been clamped five or six times to each potential level; frequency of depolarizations was 0.3 per second.

Fig. 2 (right). Current-voltage relations of venticular trabecula (dog). Ordinate, ionic current in microamperes; abscissa, membrane potential during voltage-clamp step in millivolts. Peak sodium currents in normal Tyrode's solution (**X**) and in a solution containing 31 percent of the normal $[Na]_o$ (○) after isotonic replacement of NaCl by tris-Cl are plotted as negative (inward) currents. There is a shift of equilibrium potential (intersection of current with voltage axis) by 30 mv in sodium-poor solution. Positive (outward) current in Tyrode's solution (●) was measured at end of 300-msec clamp step.

(− 60 to − 33 mv), where I_{Na} is largest, the contractions are weak and are not influenced much by the membrane potential. Thus steady-state contraction seems to depend mainly on the flow of the second inward current. The ionic requirements for the second inward current, its dependence on the membrane potential, and its relation to activation of contraction are the subjects of another report (*10*).

Thus our experiments suggest that in dog ventricular myocardium the flow of sodium current is mainly responsible for the rapid depolarization during the action potential. Some of the kinetics of I_{Na} are quite similar to those in other excitable tissues.

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References and Notes

1. A. L. Hodgkin and A. F. Huxley, *J. Physiol. (London)* **116**, 449 (1952).
2. S. Weidmann, *ibid.* **127**, 213 (1955); J. Dudel, K. Peper, R. Rüdel, W. Trautwein, *Pfluegers Arch. Ges. Physiol. Menschen Tiere* **292**, 255 (1966).
3. O. Rougier, G. Vassort, R. Stämpfli, *Pfleugers Arch. Ges. Physiol. Menschen Tiere* **301**, 91 (1968).
4. G. Giebisch and S. Weidmann, *Helv. Physiol. Pharmacol. Acta* **25**, CR189 (1967).
5. R. L. Heppner, S. Weidmann, E. H. Wood, *ibid.* **24**, CR94 (1966); H. Reuter and H. Scholz, *Pfluegers Arch. Ges. Physiol. Mensch. Tiere* **300**, 87 (1968).
6. G. W. Beeler, Jr., and H. Reuter, in preparation.
7. W. Trautwein, J. Dudel, K. Peper, *J. Cell Physiol.* **66** (suppl. 2), 79 (1965); R. E. McAllister and D. Noble, *J. Physiol. (London)* **186**, 632 (1966); H. Reuter, *ibid.* **192**, 479 (1967).
8. A. L. Hodgkin and A. F. Huxley, *J. Physiol. (London)* **116**, 497 (1952).
9. B. Frankenhaeuser, *ibid.* **148**, 671 (1959).
10. H. Reuter and G. W. Beeler, Jr., *Science*, this issue.
11. Supported by research grant AHA 67-053 from the American Heart Association. H.R. is a career investigator visiting scientist of the American Heart Association.
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Calcium Current and Activation of Contraction in Ventricular Myocardial Fibers

Abstract. *In thin bundles of dog ventricular myocardium, a slow inward current (distinct from the sodium inward current) could be recorded under voltage-clamp conditions. This inward current was influenced by changes in external calcium concentration, but it was not dependent on external sodium concentration. Therefore, this current which contributes an appreciable amount of charge transfer during the plateau of the action potential, is carried by calcium ions. In sodium-free solution, the flow of calcium ions into the fiber is directly related to activation of contraction. In sodium-containing solution, however, calcium inward current serves primarily to fill up some intracellular stores from which calcium can be released by moderate depolarization.*

In cardiac Purkinje fibers, calcium ions contribute to the total membrane current during rather strong depolarizations (*1*). Evidence has been obtained that, during depolarizations in sodium-free solution, calcium ions carry a charge across the membrane of ventricular myocardial fibers isolated from sheep and calf hearts. Under these conditions, activation of contraction seemed to be directly related to the inward movement of calcium ions (*2*). In order to get more information about the importance of calcium inward current for the action potential as well as for excitation-contraction coupling in mammalian cardiac muscle, we performed voltage-clamp experiments and simultaneously measured contraction in thin trabeculae and papillary muscles excised from dog hearts.

We used the method already described (*3*). In that report, apart from excitatory sodium current (I_{Na}), a much smaller and slower net inward current was described as flowing in dog ventricular fibers during depolarization to potential levels between −30 and −20 mv. Steady-state contraction became large in the same potential range. In the presence of I_{Na}, the slow inward current could not be accurately resolved, but I_{Na} could be completely inactivated with a conditioning depolarization of the membrane to −45 mv. Therefore, we investigated the second inward current in greater detail after the sodium system had been inactivated.

Double-step voltage clamps recorded from a dog ventricular trabecula soaked in Tyrode's solution are seen in Fig. 1. The duration of the first depolarization step, V_1, from −77 mv (resting potential) to −38 mv was 420 msec; that of the second, superimposed step, V_2, was 210 msec. During the first step, which was always of constant amplitude, there was a transient inward sodium current (*3*). The V_1 was sufficiently large and long to inactivate I_{Na} completely at V_2. The second step of depolarization, V_2, was varied in amplitude. A small depolarization, from −38 to −33 mv, produced a slight increase in outward current. Stronger depolarization produced a flow of slow inward current that was maximum in this preparation at −7 mv. Net inward current could be recorded at potentials up to +7 mv. A precise determination of the equilibrium potential for this current was unattainable, because during depolarizations to potentials beyond +30 mv the outward current increased steeply, making it impossible to separate the slow inward current with sufficient accuracy.

The slow inward current was influenced by the external calcium concentration $[Ca]_o$. It became extremely small when $[Ca]_o$ was low (0.1 mmole/liter), and its amplitude increased with increasing $[Ca]_o$ up to 7.2 mmole/liter. The difference between the initiation and the maximum of the slow inward current (after correction for the capacitative outward current) was considered to represent an estimate of the total inward current and was plotted against membrane potential (Fig. 2). The slow inward current in $[Ca]_o$ of 1.8 mmole/liter increases steeply at potentials from −25 to −18 mv, and decreases again during depolarizing clamp steps above −10 mv. When $[Ca]_o$ was increased to 6.3 mmole/liter, the threshold for the slow inward current was shifted negatively by 8 mv and the amplitude of the current was larger, attaining its maximum at about −25 mv. In positive-potential regions, the two current-voltage relations are almost parallel, the separation between the curves being about 18 mv. Although there are uncertainties in this method of estimation, this tends to agree with a theoretical 16-mv shift of the equilibrium potential calculated from the Nernst equation under the assumption that the current is carried solely by calcium ions.

This assumption is strongly supported by the finding that the slow inward current was not much affected by the complete absence of sodium in the bathing solution, and also that it was sensitive to alterations of $[Ca]_o$ in sodium-free medium. The main difference between the two conditions seemed to be a shift of the threshold for the slow inward current by 10 to 20 mv toward more