## Heart Action Potential: Dependence on External Calcium and Sodium Ions

Abstract. *The height of the overshoot of the action potential recorded from frog ventricles is markedly less sensitive to lowering the external sodium concentration than predicted by the sodium-hypothesis of excitation, and it is surprisingly sensitive to changes in the external calcium concentration. These observations are explained by a mechanism in which there is competition between sodium and calcium ions, in the excitable membrane, for anionic sites which control the inward current of sodium ions at the crest of the action potential.*

It is well known that the contractile strength of the heart depends critically on the concentration of calcium and sodium ions in the external fluid (*1*); it increases on raising the external calcium, $[Ca]_o$, or reducing the external sodium, $[Na]_o$, but it remains unaltered when the ratio of $[Ca]_o$ to $[Na]_o^2$ is kept constant (*2*). These effects have been explained on the assumption that Ca and Na ions compete for specific anionic sites in the cell membrane which in the Ca-combined form activate contraction, probably by facilitating cellular uptake of calcium; contractile strength would thus be related to the concentration of these Ca-combined sites (*3*). In the study reported here it has been found that associated with these effects there are changes in the amplitude of the action potential which suggest that the hypothetical sites, when occupied by calcium, promote the inflow of ionic current through the excitable membrane.

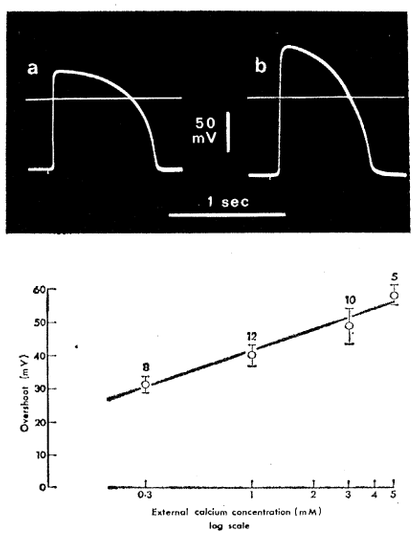


Fig. 1. Effect of varying the external calcium concentration, $[Ca]_o$, on the action potential. Top: intracellular records of action potentials from a strip of frog ventricle in the presence of (*a*) 0.3 m*M* Ca-Ringer, and (*b*) 5 m*M* Ca-Ringer. Horizontal line: level of "zero" potential. Bottom: height of the "overshoot" plotted against $[Ca]_o$. Same preparation as used for records *a* and *b*. Numbers indicate numbers of different cells; vertical bars, ± standard deviation of the mean. The means are fitted by a regression line with slope of 21 mv for a tenfold change in $[Ca]_o$.

Intracellular action potentials have been recorded from quiescent strips of frog ventricles stimulated at intervals of at least 1 minute. It was found that the size of the overshoot—that is, the difference between the amplitudes of the action and resting potentials—could be increased by up to 50 mv by raising the external Ca concentration, the change in overshoot being approximately linearly related to the log of $[Ca]_o$ in the range from about 0.1 to 5 m*M*, with a slope of about 20 mv for a tenfold change in concentration (Fig. 1). Strontium produced effects similar to calcium, increasing both the overshoot and the strength of contraction, while magnesium in comparable concentrations had little effect.

In order to observe these effects of Ca and Sr on the action potential, it is necessary to use very low frequencies of stimulation and only moderate concentrations of these ions, otherwise the effect becomes obscured by the known depressing action of Ca (see *4*). The maximum rate of depolarization, a measure of the inward current during the initial phase of the action potential, was little affected under the conditions of the experiments reported here (see also *5*), except in a range of rather high concentrations of the divalent ions, where the rate of depolarization tended to decline.

As to the effect of Na ions, we found that the replacement of NaCl with osmotically equivalent sucrose caused the overshoot of the action potential to decline (Fig. 2). But, although an effect of this kind is in accordance with the sodium hypothesis of excitation, its magnitude—that is, a decrease of about 18 mv for a tenfold reduction in $[Na]_o$—is only about one-third of that predicted by

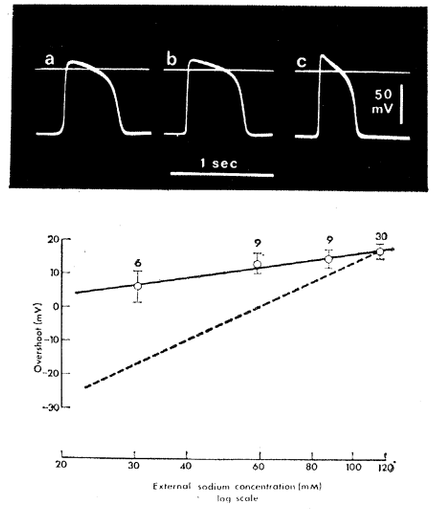


Fig. 2. Effect of decreasing the external sodium concentration, $[Na]_o$, on the action potential. Top: intracellular records of action potentials in the presence of (*a*) 30.5 m*M* Na, (*b*) 59 m*M* Na, and (*c*) normal Na, 116 m*M*. (Sucrose substituted for NaCl in *a* and *b*). All fluids contained reduced $[Ca]_o$ (0.1 m*M*), but normal $[K]_o$ (3 m*M*). The increase in duration of the action potential at low $[Na]_o$ was observed only at low $[Ca]_o$ but the decline in overshoot due to low $[Na]_o$ was similar at low and normal $[Ca]_o$ (1 m*M*). Bottom: height of overshoot plotted against $[Na]_o$. Numbers indicate numbers of different cells; vertical bars, ± standard deviation of the mean. Smooth line, regression line with slope of 18 mv; dashed line, theoretical line with slope of 58 mv for a tenfold change in $[Na]_o$.

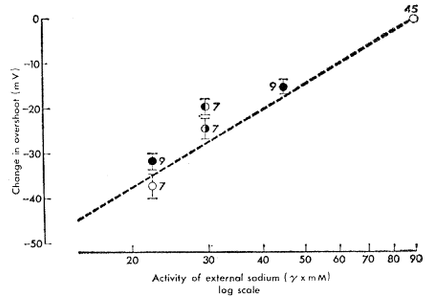


Fig. 3. Change in overshoot resulting from decreasing the external activity of sodium, $\gamma_{Na}$ $[Na]_o$, the ratio between activity of calcium and the square of sodium activity, $\gamma_{Ca}[Ca]_o/(\gamma_{Na}[Na]_o)^2$, being kept constant at $2.3 \times 10^{-4}$. Difference between the mean overshoot in low $[Na]_o$ and at normal $[Na]_o$ plotted against the external sodium activity. Results from four ventricle strips, each shown by a different symbol. Numbers indicate numbers of cells sampled at low $[Na]_o$. Vertical bars indicate ± standard error of the difference between the means. Dashed line with slope of 58 mv for a tenfold change in $\gamma_{Na}[Na]_o$. Control fluid contained 3 m*M* Ca, 116 m*M* Na (sucrose substituted for NaCl in the fluids with low $[Na]_o$).

the theoretical Nernst equation which satisfactorily describes results from nerves and vertebrate skeletal muscles (*6*). When choline chloride, in the presence of sufficient atropine to counteract its "cholinergic" effect, was used to replace NaCl there was little change in the height of the action potential even with Na concentrations as low as 25 percent normal, the overshoot usually increasing somewhat on application of these fluids. In contrast with these relatively small effects on amplitude, the maximum rate of depolarization changed quite strikingly as it does in other excitable cells, the rate decreasing in low $[Na]_o$, and decreasing to a similar extent when either sucrose or choline chloride was used as a substitute for NaCl. This finding confirms previous work with cardiac cells (see *7*) in suggesting that Na ions are the main carriers for inward current during the initial phase of the action potential.

For the interpretation of these results it is important to note that the maximum of the action potential is reached some 20 to 30 msec after its moment of fastest rise—that is, at a time when probably, as in other heart cells (*8*), the initial increase in permeability to Na ions has subsided toward a much lower but longer-maintained level. It is thus possible that at this time ions other than Na participate in determining the height of the overshoot. The possibility that Ca is involved is suggested by the finding that Ca influx is facilitated in the presence of either Ca-rich or Na-depleted fluids (*9*). However, the extra influx of ionic calcium during an action potential determined in frog ventricles with $Ca^{45}$-labeled fluids was only $\leq 0.2$ picomole/$cm^2$ surface membrane, which would not contribute much to the height of the potential. It seems more probable that associated with the movement of Ca ions there is an inward current of Na which is also facilitated by the action of Ca on the hypothetical membrane sites. The degree of the permeability change responsible for this delayed Na-current would thus depend on the external Ca and Na concentrations (or on the activities of the Ca and Na ions). To test this hypothesis the dependence of the overshoot potential on external Na activities has been examined, keeping the ratio $\gamma_{Ca}[Ca]_o/(\gamma_{Na}[Na]_o)^2$ (that is, the concentration of Ca-occupied sites) constant. Under these conditions, the results are fairly close to the theoretical line (Fig. 3) and thus support the idea that the inward current which causes the overshoot is mainly carried by sodium ions.

R. K. ORKAND*
R. NIEDERGERKE
*Department of Biophysics,*
*University College London,*
*London, W.C.1*

**References and Notes**

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* Postdoctoral fellow of the National Institute of Neurological Diseases and Blindness. Present address: Laboratory of Neurophysiology, Harvard Medical School, Boston, Massachusetts.

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## Spawning of Starfish: Action of Gamete-Shedding Substance Obtained from Radial Nerves

Abstract. *An extract of the radial nerves of the starfish,* Asterias amurensis, *acts on the ovary in two ways: it induces meiosis and brings on spawning. Contraction of the gonadal wall, the driving force for spawning, does not happen until this gamete-shedding substance acts to liberate the eggs that adhere to each other or to the inner surface of the gonadal wall.*

When injected into the coelomic cavity, a water extract of starfish radial nerves induces the shedding of eggs or sperm from the mature gonads (*1*). The active extract can be obtained from both male and female starfish and seems to be identical in the two sexes. Although there may be some species differences in detail, the active substances seem to be chemically analogous among starfishes; the nerve extract obtained from one species acts similarly in several species (*2*). The active substance obtained from *Asterias amurensis* was reported to be a polypeptide with a relatively small molecular weight (*3*). Moreover, neurosecretory cells were revealed in the radial nerves of *A. glacialis* (*4*). This was confirmed in Japanese starfishes, including the species used in this study (*5*). These facts suggest that the active element responsible for the spawning of starfishes is a product of neurosecretion. Described in this report are some experimental effects of the spawning factor, obtained from the radial nerves, on the ovary and ovarian eggs; a possible mechanism of spawning in *A. amurensis* is suggested.

In the first series of experiments, an ovary was removed and torn with fine forceps so that the eggs were released into sea water. The germinal vesicles broke down after about 20 to 30 minutes, and the first polar bodies appeared after 70 to 80 minutes at 18°C. Discharge of the second polar bodies began after 110 to 120 minutes. Maturation of the starfish egg in sea water is said to be promoted by calcium ions (*6*). Isolated ovarian fragments were thoroughly washed with calcium-free sea water, and eggs thus obtained were suspended in calcium-free sea water before being transferred to artificial sea water containing calcium in various concentrations. Figure 1 shows that breakdown of the germinal vesicles became more frequent as the concentration of calcium rose. Maturation did occur, however, even in the absence of calcium, when these eggs were treated with calcium-free sea water containing nerve extract (*7*) desalted on a Sephadex G25 column (Fig. 2).

Under natural conditions, the initiation of meiosis seems to be due to the action of the gamete-shedding substance, since the eggs within isolated ovaries do not undergo maturation in sea water, and since most of the germinal vesicles of eggs spawned from the gonopores disappear by the time of spawning. Meiosis proceeded in isolated ovarian fragments when they were ligated and then placed in nerve extract. After 2 hours of such treatment, polar bodies were observed in most of the eggs within the ovary. Without the ligature, an isolated gonad fragment immersed in nerve extract shed its eggs vigorously from the cut surface after a certain period, and discharge of polar bodies occurred in sea water.

In the next experiment, the nerve extract was applied locally. The distal part of an isolated ovary was immersed in sea water containing the nerve extract; its proximal part, in sea water alone (Fig. 3*A*). After 40 minutes,