Earlier investigations\(^1\)\(^2\) with mammalian heart muscle have shown that the resting potential drops only slightly when the temperature is lowered from 37 to 20°C, but decreases considerably upon further cooling. Coraboeuf and Weidmann\(^2\) suggested 3 possible explanations for the depolarization in the cold: (1) a rise of the Na permeability relative to the K and Cl permeabilities of the fiber membrane; (2) failure of active potassium reabsorption leading to a rise of the K concentration in the interspace; and (3) lowering of Na extrusion from the cells. Three groups of experiments were carried out in an attempt to test these hypotheses: (1) influence of Na-laek on temperature sensitivity; (2) rapid high-frequency heating of cooled fibers, to look for a possible time lag between the rise of temperature and the rise of potential; and (3) influence of metabolic inhibitors on temperature sensitivity.

**Methods**

Sheep or calf hearts were obtained at the slaughterhouse and carried to the laboratory in cool (4°C) Tyrode solution. False tendons of the left ventricle or muscle strips of the right ventricle were held by 2 loops of silk thread on the bottom of a small (14 X 3 mm.) Lucite chamber (fig. 1). The temperature of the bathing solution could be varied between 37 and 0°C by adjusting the rate of influx of cold and warm solution.

The resting and action potentials were recorded between 2 Ling-Gerard electrodes\(^3\) filled with 3M KCl.\(^4\) One of them was in contact with the Tyrode bath; the tip of the other electrode was introduced into a single fiber. Symmetrical cathode-follower input stages of the type described by Copeland\(^5\) worked into a balanced D.C. amplifier.

Microelectrodes may have considerable tip potentials\(^6\) which were found to change appreciably as a function of temperature. Pairs of electrodes were therefore selected, which gave a potential change of less than 3 mV, when the bath was cooled from 37 to 0°C.

The temperature was usually measured by a thermistor (Stantel F 2311/300). The method was simple and gave satisfactory results when the temperature was varied slowly, but it failed to record the true fiber temperature during a rapid temperature change. In some experiments, therefore, the tip of a third microelectrode was placed into the connective tissue just outside the fiber under investigation. A current was passed through a 100 Ω resistor and through the electrode into the bath, giving a voltage drop of about 100 mV across the microelectrode. The largest portion of the resistance of a microcapillary is located in a segment of a few micra closest to the tip. Thus, by recording the electrode resistance, the temperature in the immediate vicinity of the fiber could be obtained. While this method was more satisfactory for establishing temporal correlations, the measurement of the temperature was less accurate; calibration readings made before and after the experiment differed by as much as 5°C. The temperature was displayed on the second beam of a Dumont double-beam oscilloscope, type 333.

A rapid rise of the temperature throughout the fiber bundle could be obtained by high-frequency current, 14 megacycles/second. This was applied longitudinally to the fiber axis, through a pair of platinum electrodes. The output impedance of a 100 watt amateur transmitter was matched to the impedance of the muscle chamber by means of a Collins antenna coupler.

It was impossible to record the membrane potential during the flow of high-frequency current. Therefore, brief high-frequency pulses of constant duration were repeated regularly by an automatic key, and the membrane potential was recorded in the intervals between these pulses. In order to reduce a relatively long-lasting artifact following the heating, the grids of the cathode-followers were earthed while the high-frequency field was applied. Two high-speed relays were used for this purpose; they were driven by the screen current of the final stage of the transmitter.
Results

Effect of Temperature on the Resting Potential

Figure 2 shows the variation in resting potential that could be observed when Purkinje fibers were cooled to 0°C and subsequently rewarmed. Average values obtained with 10 Purkinje fibers (108 experiments) and 10 ventricular fibers (66 experiments) are given in figure 3A. The potential change observed in the temperature range from 37 to 20°C was roughly proportional to the absolute temperature, as would be expected if the membrane resting potential followed the laws of a diffusion potential. However, below a critical temperature, varying slightly from 1 preparation to another and estimated at about 20°C, the slopes of the curves (fig. 3A) were about 3 times greater.

Sodium-Free Solution

It is generally accepted that the sodium permeability of excitable membranes increases as the membrane potential is lowered from its normal resting value. 7, 8 It seemed possible, thus, that the slight depolarization brought about by cooling might result in an increase of Na-inward current and thereby lead to further depolarization. Sodium-free solutions were prepared by substituting choline chloride (Hoffmann-La Roche) for NaCl and by omitting the bicarbonate-phosphate buffer of Tyrode solution; atropine sulfate, 20 mg./L, was added. Figure 3B indicates that, in the absence of extracellular sodium, cooling still had a large effect on the resting potential and, therefore, an increase of Na-inward current is unlikely to provide the explanation for the large depolarization in the cold. The increase of the resting potential in a Na-free solution is to be expected if the resting membrane is somewhat permeable to Na ions. 9

High-Frequency Heating

Heart muscle fibers are depolarized if the extracellular potassium concentration is increased. 10 There is, at present, no conclusive evidence whether all K ions enter the cardiac fibers passively, or whether a metabolically driven “pump” is responsible for part of the K-influx. 11 Thus, failure of K-reabsorption in the cold might be another reason for depolarization.

Figure 4 illustrates the results obtained with high-frequency heating. A sheep Purkinje fiber was stimulated once at 37°C, and an action potential was recorded. The shutter of the camera was then closed and the preparation cooled to 0°C. At the end of about 20 seconds, the inflow of Tyrode solution was stopped, the resting potential photographed, and high-frequency heating started. Five brief heating periods (0.25 seconds each) brought the temperature back to about 37°C. It can be seen that the membrane potential increased in distinct steps and that no major change took place towards the end of the recording intervals (1.25 seconds each). The small “hooks” at the beginning of the recording...
RESTING POTENTIAL DURING HYPOTHERMIA

Figure 3
Resting potentials at 0, 20, and 37 °C. A, from Purkinje fibers, mean of 108 measurements (●) and from ventricular fibers, 66 measurements (○); B, from Purkinje fibers in Tyrode solution (●) and same fibers in choline-Tyrode (○), 65 measurements, 4 preparations; C, from Purkinje fibers in Tyrode solution (●) and same fibers after monoiodoacetate poisoning in O₂-free solution (○), 58 measurements, 4 preparations. Vertical bars refer to the standard error. Lowest line in all graphs indicates theoretical curve for a diffusion potential (resting potential proportional to absolute temperature).

periods must be interpreted as artifacts for the following reasons: (a) they were found to have the same shape when both microelectrodes were leading off from the extracellular space (fig. 4), and (b) their direction would be either positive or negative. Due to these artifacts, it is impossible to say whether there was a time lag of the order of 1/10 second between the rise of temperature and the rise of potential. It can be stated with confidence that there was none of the order of 1 second. From tracer data it may be inferred that reabsorption of potassium from the bulk of the extracellular space into the fibers would require considerably longer times.

The temperature was not measured during high-frequency heating. The number and strength of the high-frequency pulses could be adjusted so as to obtain action potentials of the same duration before and after hypothermia. This was taken to indicate that the temperature at the end of heating was again close to 37 °C. No obvious damage was produced by the rapid temperature changes. The experiment could be repeated several times.

Metabolic Inhibitors

Evidence is available showing that Na ions are extruded from living cells against an electrochemical gradient. For giant nerve fibers of the squid, Na extrusion is much more sensitive to temperature than the "passive" ionic movements across the fiber membrane. Thus, if in mammalian Purkinje fibers Na-outflow contributed to the resting potential, a considerable slowing of such an "electrogenic pump" would offer an explanation for the observed potential drop during hypothermia.

In an attempt to deprive the Na-pump of its energy supply, use was made of metabolic inhibitors. Unfortunately, the sensitivity of different preparations varied considerably, and there was only a narrow margin between no effect and irreversible depolarization. Monoiodoacetate (2 mM) combined with oxygen lack (5 per cent carbon dioxide in nitrogen) gave more satisfactory results than 2,4-dinitrophenol. When fibers were poisoned to a degree that at 37 °C a slight drop of their resting potential was observed (fig. 3C), the temperature sensitivity of the resting potential was in fact reduced almost to that of a simple model membrane system. The observation that at 0 °C the membrane potential was somewhat higher in poisoned fibers than in normal fibers cannot easily be explained. The finding might indicate that monoiodoacetate had additional effects, e.g., an increase of the membrane permeability to K ions.
Rise of the membrane potential as a result of high-frequency heating. The temperature was increased in 5 steps, each heating period lasting for 0.25 seconds. The zero reference potential was recorded on the same frame. On the right, artifacts that were obtained with both microelectrodes outside the fiber.

Discussion

On the basis of the present results, certain explanations for the drop of the resting potential during hypothermia can be excluded: (1) a relative increase of the Na permeability of the surface membrane, and (2) accumulation of K ions throughout the interspace.

It has been shown by Hannou14 that oxygen consumption decreases sharply when rat hearts are cooled below 20 C. A pronounced slowing of a 'pump' depending on metabolism and ejecting Na ions seems to be, at present, the most probable reason for the potential drop during hypothermia.

Summary

Between 37 and 20 C, the resting potential of sheep ventricular and Purkinje fibers was found to be proportional to the absolute temperature. From 20 to 0 C, the potential drop was 3 times greater than that predicted from the theory of a concentration potential. Rapid heating by high-frequency current of previously cooled preparations, revealed no time lag between the rise of potential and the rise of temperature. Fibers kept in a Na-free solution still showed a large temperature sensitivity. With poisoned fibers, the resting potential was almost proportional to the absolute temperature. The results appear to contradict the hypothesis that extracellular K accumulation in the cold is the cause of the potential drop. They also exclude the hypothesis that a relative rise of the Na-inward current is the reason for depolarization. They support the idea that 'active' extrusion of Na ions normally contributes to the resting potential, and that the pumping rate decreases considerably in the cold.

Acknowledgment

The author is indebted to Professor S. Weidmann for his constant help and advice.

Summario in Interlingua

Esseva constatate que a temperaturas de inter 37 e 20 C, lo potential de reposo de fibras ventricular e de fibras de Purkinje ab oves esseva proportional al temperatura absolute. Inter 20 e 0 C, le reduction del potential esseva 3 vices plus grande que le prediction basate super le theoria de un potential de concentration. Le rapide ro-calafaccion del previousmente hypothermizate proparatos per medio de un currante de alto frequentia rovelava nulle retardo del augmento del potential in relation al augmento del temperatura. Fibras mantente in un solution libero de natrium manifestava nonobstante un marcante sensibilitate thermic. In le casos de fibras invenenate, le potential de reposo esseva quasi proportional al temperatura absolute. Iste constatationes pare contradicer al hypotheso que le accumulation extracellular de kalium que occurre durante le reduction del temperatura es le causa del reduction in le potential. Ils etiam elimina le hypotheso que un augmento relative del currente intorsio de natrium es le causa del dispolarisation. Le constatationes supporta le idea que un extrusion 'active' de ions de natrium contribuo normalmente al potential de reposo e que le frequentia del pumpation declina rapidemente a basso temperaturas.

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Circulation Research, Volume VIII, May 1960


Possible Reasons for Drop of Resting Potential of Mammalian Heart Preparations During Hypothermia

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Circ Res. 1960;8:553-557
doi: 10.1161/01.RES.8.3.553

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