The Effect of High Sodium Concentration on the Action Potential of the Skate Heart

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ABSTRACT It already has been well documented that the maximum rate of depolarization and amplitude of action potentials are directly dependent on \([\text{Na}^+]_o\) in the vertebrate myocardium. Almost all studies have been carried out at low sodium concentration ranges by substituting NaCl for other substances. Action potentials should be demonstrable in higher sodium concentrations, but cells are inevitably damaged by osmotic changes. The blood of elasmobranchs is nearly isosmotic with sea water, but NaCl accounts for 54.5% of the osmotic pressure and 38.7% of it is maintained by urea molecules. Utilizing this special situation in elasmobranchs, the effect of high sodium concentration was studied up to 170% of normal sodium concentration, while still retaining isosmotic condition. The rate of depolarization, amplitude, and duration of the myocardial action potential all increased in direct proportion to \([\text{Na}^+]_o\), and no depressant effect on transmembrane action potentials was observed in solutions of high sodium concentration. With regard to depolarization rate, the regression curve fitted by the least squares method passed through zero within two standard errors. At high sodium levels, the overshoot changed as expected theoretically, but at lower ranges it deviated from the theoretical values. \([\text{Na}^+]_i\) and \([\text{K}^+]_i\) in this tissue have been determined, and these data are explained on the basis of the Na theory.

INTRODUCTION
A linear relationship between both the maximum rate of rise and amplitude of action potentials in a variety of tissues and sodium concentrations in the medium \([\text{Na}^+]_o\) has been well demonstrated (Overton, 1902; Hodgkin and Katz, 1949; Nastuk and Hodgkin, 1950; Hodgkin, 1958; Brady and Woodbury, 1960). Almost all this evidence was obtained by experiments in various low sodium solutions, prepared by substituting sodium chloride for choline chloride, sucrose, and Tris chloride in various proportions. However, in experiments at high sodium concentration levels, this linearity could not be definitely demonstrated because of the marked increase in osmotic pressure. To avoid the hypertonic effect of sodium, careful attention was given to the
ionic composition of the Ringer's solution of marine elasmobranchs, in which NaCl and urea are the major osmotic solutes (Smith, 1929; Prosser and Brown, 1961). As urea, in fact, maintains 38.7% of the total osmotic pressure, it is possible by replacing urea with NaCl to prepare sodium solutions under isosmotic conditions which are 1.7 times higher in sodium concentration than that in normal Ringer's solution. In this study it was found that this proportionality could also be maintained at high sodium levels by using the myocardium of elasmobranchs.

**METHODS**

**Materials**  Skates *Dasyatis akajei* (Müller and Henle) weighing 0.5-1.5 kg which are common in the Inland Sea of Japan were used in the experiments. Immediately after the skate had been sacrificed by pithing, the heart was isolated from the body and placed in cold Ringer's solution kept in a vacuum bottle. The specimen was not examined until about 1 hr after being isolated, because of the time required to transport it back to the laboratory. Atrial strips about 0.5 cm in width, 1.5 cm in length, and about 1 mm in thickness were used in measuring the transmembrane potential. In the experiment to measure the extracellular space and intracellular ion contents, skates were kept alive in natural sea water while being transported to the laboratory. Immediately after the heart had been isolated, the atrium was divided from the ventricle by cutting at the atrioventricular groove.

**Solutions**  The standard solutions used by Lowenstein, Osborne, and Wersäll (1964) were employed in all experiments with the solution temperature maintained at 20°C ± 1°C. The values used in preparing the solutions of various concentrations (Table I) were based on the table of Hodgkin and Katz (1949) and the cryoscopic data in International Critical Tables.

**Recording of Transmembrane Potentials**  The atrium specimens were electrically stimulated at very low frequencies. Glass ultramicroelectrodes with resistance ranging from 16 to 20 MΩ measured in 3 M KCl solution were used for measuring transmembrane potentials. The flexibly mounted electrode system (Woodbury and Brady, 1956) was employed. The preamplifier used in the experiments was a negative-capacitance cathode follower type (MZ-3B of Nihon Koden Co., Tokyo). The intracellular action potentials were displayed on the upper channel of the Tektronix 502 type dual beam cathode ray oscilloscope. Differentiation was made with a RC circuit having a time constant of 50 μsec at the output of the voltage amplifier. The derivatives of the transmembrane potential were displayed on the lower channel of the cathode ray oscilloscope. The two tracings were recorded with a photokymograph camera.

**Estimation of Intracellular Potassium and Sodium**  After the skates had been sacrificed by pithing, the heart was carefully extirpated from the body, blotted with ashless filter paper (Toyo Roshi, No. 7), and put into microKjeldahl flasks. After weighing, each bottle was dried in an oven at 150°C for 1 hr. The total water content was determined by comparing the wet weight against the dry weight. The dried muscle was
heated for 15 min in acid mixture prepared by adding one part of $H_2SO_4$ to one part of $HNO_3$, which was then adequately diluted with distilled water. The resultant solutions were measured with a Model 139 Hitachi-Perkin Elmer electrophotometer with a flame photometer attachment. The measured concentration of Na and K in the heart ($C_m$) was expressed in millimoles per kilogram wet weight, and the intracellular concentration ($C_f$ in millimoles per kilogram fiber water) was computed by the following formulas (Boyle, Conway, Kane, and O’Relly, 1941)

$$C_f = \frac{C_m - 0.23C_o}{0.78 - 0.23} \text{ for the atrium}$$

$$C_f = \frac{C_m - 0.15C_o}{0.81 - 0.15} \text{ for the ventricle}$$

where $C_o$ is the concentration of the particular ion in the extracellular solution.

**TABLE 1**

<table>
<thead>
<tr>
<th>Compositions of Test Solutions</th>
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<tr>
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<tr>
<td>---</td>
</tr>
<tr>
<td>Na⁺ 50%</td>
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<tr>
<td>Na⁺ 100%</td>
</tr>
<tr>
<td>Na⁺ 150%</td>
</tr>
<tr>
<td>Na⁺ 170%</td>
</tr>
<tr>
<td>Na⁺ 200%</td>
</tr>
<tr>
<td>NO₃⁻ Ringer's</td>
</tr>
<tr>
<td>SO₄²⁻ Ringer's</td>
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**Estimation of the Extracellular Space** After blotting the heart with ashless filter paper and weighing the wet weight, the extracellular space was determined by immersing the heart in 1% inulin Ringer’s solution for 4 hr and electrophotometrically measuring the inulin content by the method of Ross and Mokotoff (1951).

**Estimation of the Urea Content of the Skate Heart** The atrium and ventricle were immersed in 170% Na Ringer’s solution containing no urea and the extrusion of urea into this solution was measured by a method modified from Ormsby (1942) and Kawerau (1946). After diluting the samples adequately with distilled water every hour except twice during the initial hour, 2 ml of diacetyl monoxime reagent, 3 ml of the acid mixture (one part of 60% $HClO_4$ and 4 parts of concentrated $HCl$), and 2 ml of distilled water were added to 2 ml of every sample solution. After boiling for 30 min they were cooled under running tap water for 3 min, and the volume was adjusted to 10 ml with distilled water. The developed yellow color was electrophotometrically measured at a wave length of 475 μm.
RESULTS

Relation between $[\text{Na}^+]_o$ and the Maximum Rate of Rise of the Action Potential

The mean maximum rate of rise of the action potential of the skate heart was $9.5 \pm 0.7$ v/sec (mean $\pm$ se) in normal Ringer’s solution. This value was remarkably lower than $560 \pm 80$ v/sec (mean $\pm$ se) for the Purkinje fiber of sheep (Weidmann, 1955) and one-third of $30 \pm 1.5$ v/sec (mean $\pm$ se) for the frog ventricle (Brady and Woodbury, 1960). The increment in the maximum rate of depolarization was directly proportional to the increase of $[\text{Na}^+]_o$ up to $150\% \ [\text{Na}^+]_o$ (Figs. 1 and 2). The increase in the maximum rate of depolarization was remarkably sensitive to $[\text{Na}^+]_o$ and was not saturated even when $[\text{Na}^+]_o$ was two times greater than normal Ringer’s solution.
In 170 and 200% [Na⁺]₀ the maximum rate of rise was much higher than expected from the line extrapolated from the least squares method.

**Relation between [Na⁺]₀ and the Amplitude and Half-Duration of the Action Potential**  The amplitude of the action potential in normal Ringer's averaged 92 ± 2.6 mv (mean ± 2 SE). In Fig. 3, the amplitude increased proportionally with [Na⁺]₀, so that in 200% Na Ringer's solution it was 1.14 times larger than that in normal Ringer's solution. According to the least-squares method the line intersected the ordinate at 78.9 mv. The half-duration of the action potential, defined here as duration measured at half-amplitude, was also prolonged with increase in external sodium concentration (Fig. 4). The configuration of the action potential resembled the square shape in 200% sodium concentration. As shown in Fig. 1, the rate of fall of the action potential was also enhanced with higher [Na⁺]₀.

**Resting Potential in Various Sodium Concentrations** In Fig. 5, the resting potential changed with Na, though K remained constant. The measured resting potential in 200% Na was 14 mv higher than in 50% Na. Three possible causes for this unexpected change in resting potential could be considered. The first possibility is the transient effect of changing $E_{cl}$ (Hodgkin and Horowicz, 1959). However, this effect could not be the major cause, because the resting potential did not change significantly when excess chloride was replaced with nitrate and sulfate. The resting potential of 66 ± 2.6 mv (mean ± 2 se) in 170% Na Ringer's solution is very close to 67 ± 2.6 mv (mean ± 2 se) in NO₃ Ringer's solution and 66 ± 2.6 (mean ± 2 se)
in SO₄ Ringer's solution. Moreover, to avoid this transient effect, the experiments were carried out 20 min after changing the soaking solutions.

A second possibility is concerned with the change in potassium equilibrium potential, $E_K$. The permeability of urea through the cell membrane is known to be very high (Bozler, 1959). Intracellular $K^+$ will increase because of extrusion of urea together with water and cause $E_K$ to increase. Therefore, the concentration and rate of extrusion of urea from the myocardium were studied (Fig. 6). The concentration of urea inside the cell was calculated by measuring the urea concentration in 170% Na solution 8 hr after equilibrium and subtracting the extracellular urea concentration from the measured con-
centration. It was 8.2 mg/g wet weight in the atrium and 8.6 mg/g wet weight in the ventricle. Since the experiments in 150% [Na⁺]₀ were performed within 1 hr and the test solution contained 28% urea of normal Ringer's solution, the extrusion of water from the cell should be smaller than the predicted values of Fig. 6 at 1 hr.

In 170% [Na⁺]₀ Ringer's solution, experiments were carried out within 1-2 hr. As this solution does not contain urea molecules, the extrusion of urea from the cell will be about two-thirds of the total intracellular urea concentration as can be predicted from Fig. 6. If the assumption can be made that the osmotic pressure inside the cell is equal to that of the extracellular fluid, the intracellular K will relatively increase in 170% Na Ringer's solution, and this might affect the membrane resting potential. Moreover, 200% [Na⁺]₀ solution is calculated to be 17% hypertonic to the normal solution, and the experiments were done within 2-3 hr. Therefore, the increased intracellular K should be enhanced. The increase in resting potential in 200% [Na⁺]₀ solution could be attributed to this reason.

As for the third possibility, the variation in tip potential might cause a difference in resting potential in various solutions of remarkably different electrolyte composition. The method employed to measure the electrode tip potential was similar to Adrian's system (1956): Ag-AgCl·agar-test solution·test solution·microelectrode 3 m-KCl·agar-test solution·Ag-AgCl. As shown in Fig. 7, the tip potentials of several electrodes in 200% Na were evidently lower than those in 50% Na.
The measured resting potential reported here might possibly be affected markedly by this significant difference in tip potential in 50, 100, and 150% [Na⁺]. It should be noted that although the tip potential of the electrodes was approximately 5 mv in normal Na solution, there was a considerable change in the potential according to the concentration of the Na solutions (Fig. 7). In 170 and 200% Na solution, the tip potential is relatively small compared to that in lower Na solutions. The hyperpolarization in these higher Na solutions may be ascribed to a relative increase of [K⁺], accompanied by extrusion of urea from the cells. These experiments suggest that the major difference in the resting potential might be due to the variation in the tip potential and the increase in $E_K$.

**Overshoot** Overshoots of action potentials were computed, assuming that 66.5 mv, the value based on the intercept of the least squares method
in 150% [Na⁺], plus 3 mv compensation as shown in Fig. 5, is the true resting potential and that the resting potential would not change significantly in various [Na⁺]. The overshoot values showed a linear relationship with the logarithm of the extracellular sodium solution at concentrations higher than those of normal Ringer's solution, but at concentrations below this the overshoot deviated from the theoretical line. The sodium equilibrium potential, $E_{Na}$, at normal sodium concentration was 28.5 mv in the atrium but dropped to zero in 22% sodium concentration of normal Ringer's solution (Fig. 8).

**Figure 7.** The effect of various sodium Ringer's solutions on the tip potential of five electrodes sampled at random. Abscissa is Na in per cent. Ordinate is the potential of the inside of the electrode with respect to the surrounding solution in millivolts. The resistance of microelectrodes measured in 3 KCl is 10 MΩ in A, 20 MΩ in B, 20 MΩ in C, 21 MΩ in D, and 16 MΩ in E.

**Measurement of the Intracellular Concentration of Sodium and Potassium** The ionic composition of the skate heart was found to be characterized by extremely high sodium concentration when compared to the values for the frog ventricle (Hajdu, 1953; Danielson, 1964) and for the cat myocardium (Robertson and Dunihue, 1954) and by potassium values slightly lower than those of other species (Table II). This fact coincides well with the finding that the rate of rise of depolarization is lower than the data of frog or cat myocardium, and is considered to be due to the smaller electromotive force of Na. The sodium concentration of the striated muscle of the skate was 50% of that of the myocardium, but the potassium concentration in the striated muscle was 150% of that of the myocardium. Since these results conflicted with those of the foregoing references, these methods were repeated with other tissues such as those of the frog ventricle, frog striated muscle, and skate striated muscle. The results obtained were almost identical with those given in the references (Desmedt, 1953; Robin, Herschel, Murdaugh, and Weiss, 1964).
DISCUSSION

These experiments were significant in that at high [Na\(^+\)]\(_o\), the maximum rate of depolarization, amplitude, and duration of the skate myocardium were all found to increase in direct proportion to [Na\(^+\)]\(_o\). Previous workers (Hodgkin and Katz, 1949; Nastuk and Hodgkin, 1950) have reported that the maximum rate of depolarization did not increase in proportion to [Na\(^+\)]\(_o\).

**Figure 8.** Relationship between membrane potential overshoot and logarithm of extracellular sodium concentration. In the upper figure the relationship between the amplitude of action potential and [Na\(^+\)]\(_o\) in one experiment is illustrated. Solid line is the least squares fit: \(y = 0.165x + 68.5\). Dotted lines are two standard errors of estimate. Abscissa is [Na\(^+\)]\(_o\) in per cent and ordinate is amplitude of action potential in millivolts. In the lower figure the mean values of action potential overshoots are calculated under the assumption that from 50–150% [Na\(^+\)]\(_o\) the resting membrane potential is 66.5 mv and at 200% Na it is 4.5 mv more hyperpolarized. Top line is drawn according to the equation \(E_{Na} = 58 \log_{Na} \frac{[Na^+]}{[Na^+]}\) with [Na\(^+\)]\(_i\) = 91.7 mv. Bottom line is drawn through the mean calculated overshoot in normal sodium concentration with slope of 58 mv/decade. Vertical bars are ± 2 se. Abscissa is sodium concentration in per cent (logarithmic scale). Ordinate is membrane potential overshoot in millivolts.
at high \([\text{Na}^+]_o\), and they have suggested that this phenomenon might possibly be attributed to the hypertonic effect of high \([\text{Na}^+]_o\). Although the test solutions were slightly hypertonic due to the penetration of urea, the myocardium in these high sodium solutions responded to electrical stimulation and contracted vigorously for more than 5 hr, suggesting that these solutions were satisfactory for the experiments reported here. This is in accord with the results in skeletal muscles, where the physiological condition can be well maintained within 10% osmotic change (Dydynska and Wilkie, 1963). Although the rate of depolarization increased as the extracellular sodium concentration was increased, linearity was observed up to 150% \([\text{Na}^+]_o\), and beyond this concentration the maximum rate of depolarization increased far more than expected from the predicted line of the least squares method fitted between 50 and 150% \([\text{Na}^+]_o\). This increase in the maximum rate of depolarization might be caused by the hyperpolarization of the resting membrane potential. This proportionality is considered to be due to the large electromotive force generated by the great difference between intracellular and extracellular sodium concentration.

The dependency of the overshoot of the action potential on \([\text{Na}^+]_o\) was more evident in higher sodium solutions than in lower \([\text{Na}^+]_o\), where the data deviated from the line drawn through the mean overshoot in normal Ringer’s solution with a slope of 58 mv/decade (Fig. 8). This is in agreement with the findings of other workers (Orkand and Niedergerke, 1964; Hagiwara and Nakajima, 1965) and because the measured data have deviated from the theoretical values at 50% \([\text{Na}^+]_o\), as shown in Fig. 8, it is quite possible that other ions participated in lower Na concentrations.

### Table II

<table>
<thead>
<tr>
<th>Total concentrations</th>
<th>Extracellular space</th>
<th>Calculated intracellular concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na, mEq/kg wet weight</td>
<td>mL/100g wet weight</td>
<td></td>
</tr>
<tr>
<td>Atrium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>117.4±9.5</td>
<td>74.7±8.0</td>
<td></td>
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<tr>
<td>(21)</td>
<td>(21)</td>
<td></td>
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<tr>
<td>Ventricle</td>
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<tr>
<td>112.5±6.6</td>
<td>69.4±5.2</td>
<td></td>
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<tr>
<td>(21)</td>
<td>(21)</td>
<td></td>
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<tr>
<td>Skeletal muscle</td>
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<tr>
<td>65.9±3.0</td>
<td>105.4±5.4</td>
<td></td>
</tr>
<tr>
<td>(6)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
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<tr>
<td>284.5±7.4</td>
<td>7.0±0.5</td>
<td></td>
</tr>
<tr>
<td>(5)</td>
<td>(5)</td>
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Mean ± sd and number of samples given in parentheses.

* Data cited from Robin et al. (1964).
The regression curve fitted by the least squares method passed through zero within two standard errors. This coincides well with the result of Brady and Woodbury (1960) and suggests that the maximum rate of depolarization is dependent on an inward sodium current. However, according to Fig. 8, action potentials could be generated at less than 22% \([Na^+]\). Thus it was assumed that at lower sodium levels, the ratio of participation of other ions to that of the sodium ion becomes greater so that the electromotive force generated by the difference between intracellular and extracellular sodium concentrations plays a smaller role in the rate of rise and the amplitude of the action potential.

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