Exposure of the Isolated Frog Skin to High Potassium Concentrations at the Internal Surface

II. *Changes in epithelial cell volume, resistance, and response to antidiuretic hormone*

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ABSTRACT Isolated frog skin epithelia undergo marked, but reversible swelling when the external skin surface is bathed with conventional NaCl Ringer's and the internal surface with KCl Ringer's solutions. In 2 hours, epithelial thickness increased by over twofold. When NaCl Ringer's was replaced on both sides of the skin, volume returned to control levels in less than 1 hour. When sulfate, rather than chloride, was the predominant anion, exposure of the internal surface to high potassium concentrations did not evoke changes in epithelial cell volume. With both KCl and K_2SO_4 Ringer's, an immediate drop in DC resistance across the skin occurred. This was followed by partial recovery. Both the immediate drop and partial recovery were unrelated to changes in volume. A slow, sustained secondary drop in resistance was observed with KCl but not K_2SO_4 Ringer's. This slower drop was associated temporally with swelling. When epithelial cell swelling occurred (*i.e.* with KCl Ringer's), the characteristic response of the skin to vasopressin was abolished. However, with sulfate as anion, vasopressin elicited an increase in short-circuit current and/or in cell volume despite high internal potassium concentrations. It is concluded that epithelial swelling increased the permeability of the sodium-selective barrier at the external surface of the cells; and the possibility exists that stretching of cell membranes altered dimensions of pathways through which Na and water move, thereby mimicking the effects of vasopressin.

In a preceding paper (1) it was demonstrated that when the sodium ions in the Ringer's solution bathing the internal surface of the isolated frog skin are partly or completely replaced by equimolar concentrations of potassium, neither the skin potential nor the ability of the skin to maintain a short-circuit current is abolished. Moreover, transepithelial sodium transport continued from the outside to the inside bathing solution, and although this transport occurred "downhill" (*i.e.* down an electrochemical gradient) it could be shown to be active. Thus some of the most characteristic electrochemical properties of the skin are retained when Na in the internal bathing solution is replaced by potassium.

However, this treatment modifies the biological properties of the preparation in several respects. Thus there may be excessive swelling of the epithelium and the high K, low Na environment is also associated with characteristic changes in electrical parameters of the skin and with modifications of the reaction to antidiuretic hormone (ADH). The present studies are concerned with the nature of these reactions.

METHODS

The changes in volume of the frog skin epithelium were studied using the microscopic technique described by MacRobbie and Ussing (2). The apparatus used for these measurements was constructed so as to allow for the determination of the potential difference across the skin and the short-circuit current (2). The values for DC resistance were obtained either by dividing the open-circuit potential difference by the short-circuit current, or from the quantity of applied current necessary to produce a 10 mv change in the potential. The two methods usually agreed closely, although occasionally deviations of about 10 per cent were observed. However, discrepancies of this magnitude are insignificant in view of the very large alterations in resistance that were observed in these experiments. In the experiments in which measurements were made of the effects of ADH on potential and short-circuit current, the apparatus described by Ussing and Zerahn (3) was used. Further details of methodology were described in the previous paper (1).

The composition of the NaCl Ringer's solution was as follows: Na^+, 114 ± 2 meq/liter; K^+, 0 to 2.4 meq/liter; Ca^{++}, 1.2 mM; Cl^-, 117 ± 4 meq/liter; HCO_3^-, 2.4 meq/liter. The KCl Ringer's solution had the same composition except that the sodium was replaced by equimolar concentrations of potassium. In the Na_2SO_4 and K_2SO_4 Ringer's solutions, the concentrations of Na^+, K^+, Ca^{++}, and HCO_3^- were the same as in the foregoing media, but all of the chloride was replaced by sulfate. By virtue of the substitution of the divalent sulfate ion for the monovalent chloride ion, the sulfate Ringer's solutions were slightly hypotonic to the conventional solutions.

RESULTS

Changes in Volume and Resistance with KCl Ringer's Bathing the Internal Surface of the Skin. Twelve experiments were performed in which the external surface of the frog skin was in contact with NaCl Ringer's solution and the internal surface with KCl Ringer's solution. The time course of the volume response in a typical experiment is shown in Fig. 1. Control measurements were made with sodium Ringer's solution bathing both surfaces of the skin. Thereafter, the internal solution was replaced with potassium Ringer's solution and the volume changes were observed over a 2 hour period. During this interval, the epithelium increased in thickness from approximately 60 to 150 μ. Thus, the thickness of the average epithelial cell increased by almost threefold. Direct observation using the water immersion microscope suggested that the swelling involved all cell layers out to the cornified layer, and not just the stratum germinativum. This impression was reinforced by histologic examination of

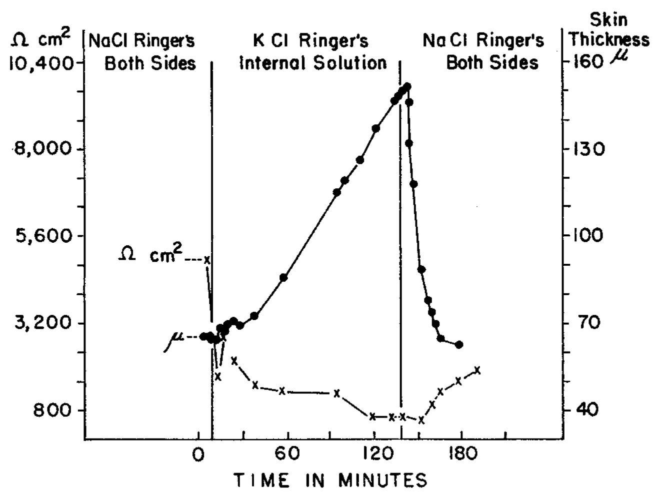

FIGURE 1. Change in the epithelial cell thickness and DC resistance of the isolated frog skin during exposure of the internal surface to KCl Ringer's solution. The external surface was maintained in contact with NaCl Ringer's throughout. The skin was short-circuited only during resistance measurements.

specimens which were fixed in formalin after exposure to the high potassium solution.

When NaCl Ringer's was replaced as the internal solution, the thickness of the epithelium decreased to the original level in less than an hour. The reversibility of the swelling phenomenon suggests that the epithelial cells did not sustain permanent damage despite the excessive deformation that occurred. When exposure to the high potassium Ringer's was prolonged, a state of quasi-equilibrium generally was achieved within 2 hours, and no further increase in cell volume occurred thereafter.

The DC resistance of the skin responded in a very characteristic manner to exposure to the KCl Ringer's solution. This response is exemplified in Fig. 1. Immediately after application of the high potassium solution, the resistance dropped precipitously (from 4800 to 1600 ohms cm^2 in the representative study). A rapid partial recovery then occurred, but this was interrupted by a further fall which continued throughout the high K period. When sodium Ringer's solution was replaced as the internal solution, the resistance increased, but did not return to control levels during a 60 minute period of follow-up measurements. It is of interest to note that the initial drop in resistance took place, and was terminated by the partial recovery, before there was any measurable change in the volume of the epithelium. The significance of this finding will be considered in the discussion.

Changes in Volume and Resistance with K_2SO_4 Ringer's Bathing the Internal Surface of the Skin. Because the frog skin epithelial cells are less permeable to

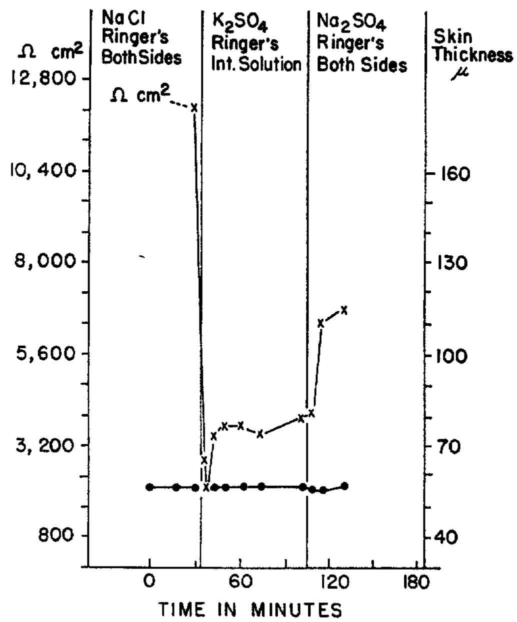

FIGURE 2. Change in the epithelial cell thickness and DC resistance of the isolated frog skin during exposure of the internal surface to K_2SO_4 Ringer's solution. The external surface was maintained in contact with Na_2SO_4 Ringer's throughout. The skin was short-circuited only during resistance measurements. The closed circles refer to skin thickness.

sulfate ions than to chloride ions, it might be anticipated that the swelling would be less pronounced if the potassium Ringer's solution contained sulfate rather than chloride as the principal anion. Accordingly twenty-four experiments were performed in which control measurements were made with Na_2SO_4 Ringer's on both sides of the skin; then K_2SO_4 Ringer's was applied to the internal surface; and finally followup measurements were made with Na_2SO_4 Ringer's again in contact with both surfaces. A representative experiment is depicted in Fig. 2. No changes in the volume of the epithelium from the control level occurred either during the period of exposure to the high K solution or during the follow-up period.

Despite the lack of any volume change, however, the precipitous fall in resistance noted with KCl Ringer's solution still occurred with the sulfate Ringer's system. In the experiment shown in Fig. 2, the resistance dropped from 12,000 to 2,000 ohms cm². A partial recovery also occurred with the sulfate Ringer's solution; but in contrast to the pattern observed with KCl Ringer's the secondary drop in resistance did not occur, and values tended to stabilize at a low level. During the follow-up period, the resistance increased sharply, but it did not return to the control level.

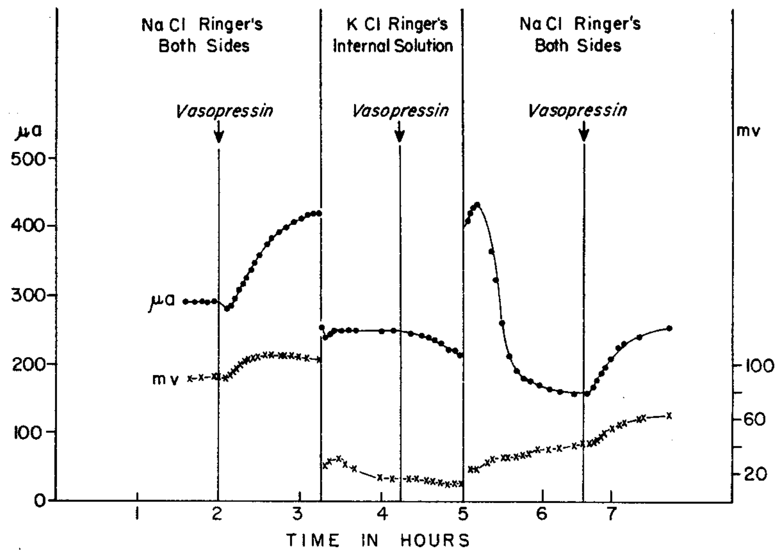

FIGURE 3. Effects of vasopressin (8 international units per 100 ml) on the isolated frog skin exposed to KCl Ringer's solution at its internal surface. The vasopressin was added first with NaCl Ringer's bathing both surfaces of the skin, then with KCl Ringer's as the internal solution and NaCl Ringer's as the external solution, and finally with NaCl Ringer's again bathing both surfaces of the skin. The short-circuiting was maintained except during the short intervals necessary for measuring the potential differences. Values for short-circuit current are expressed in microamperes (μa) per 7 cm² and potential difference in millivolts (mv). The ten studies of this design were performed with the skins mounted between the two symmetrical halves of a lucite chamber, and measurements of skin thickness were not performed.

The Effects of KCl Ringer's on the Response to ADH. The isolated frog skin, suspended in conventional frog Ringer's solutions, responds predictably to the addition of antidiuretic hormone to the internal medium with a pronounced increase in the short-circuit current and the skin potential (4). Fig. 3 depicts an experiment in which this typical response was elicited by vasopressin during the control period of measurements. Vasopressin was added again when KCl Ringer's was employed as the internal solution, and under these conditions no ADH response could be detected. However, in the follow-up period, with NaCl Ringer's again bathing both surfaces of the skin the vasopressin responsiveness was restored.

The Effects of K_2SO_4 Ringer's on the Response to ADH. The effects of vasopressin were examined in ten experiments in which K_2SO_4 Ringer's was used as the internal solution. A typical experiment is shown in Fig. 4. Initially Na_2SO_4 Ringer's was maintained in contact with both surfaces of the skin. Thereafter, the internal solution was removed and replaced with K_2SO_4 Ringer's. Both the potential difference and the short-circuit current dropped immediately, then increased, and ultimately stabilized at values somewhat

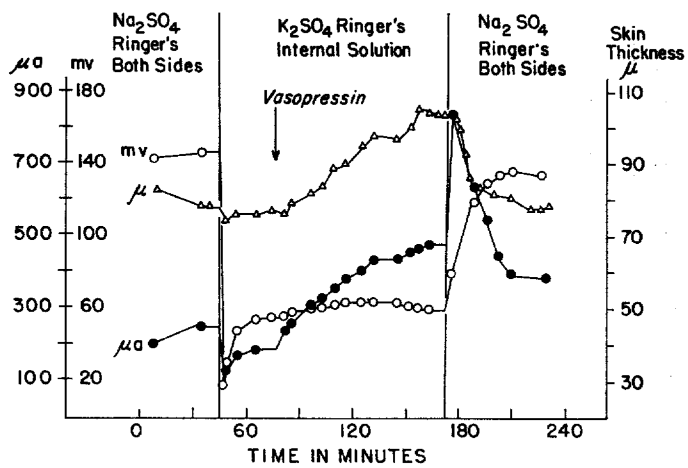

FIGURE 4. Effects of vasopressin (8 international units per 100 ml) on the isolated frog skin exposed to K_2SO_4 Ringer's at its internal surface. The initial observations were made with Na_2SO_4 Ringer's bathing both surfaces of the skin. After equilibration, the internal solution was replaced with K_2SO_4 Ringer's and approximately 30 minutes later vasopressin was added to the internal solution. After observing the vasopressin response, the K_2SO_4 Ringer's was replaced with Na_2SO_4 Ringer's. The skin was short-circuited intermittently. Values for short-circuit current are expressed in microamperes per 7 cm^2 (μa), and values for potential difference in millivolts (mv).

less than the control levels.[1] During this period of transients and the subsequent approach to steady-state values, the volume of the epithelial cells did not change. After the potential and current had stabilized, vasopressin was added to the internal bathing medium. The short-circuit current increased promptly and progressively and in association with this, the epithelium began to swell, increasing in thickness from 75 to 105 μ over a 90 minute period. The potential increased very slightly following addition of the vasopressin.

When the K_2SO_4 Ringer's was removed and fresh Na_2SO_4 Ringer's was added as the internal solution, the thickness of the epithelium decreased to approximately the initial (*i.e.*, control) values.

[1] The bioelectric properties and the patterns of Na transport of the isolated frog skin with Na_2SO_4 Ringer's as the external solution and K_2SO_4 Ringer's as the internal solution recently have been described in detail (5).

In nine of ten experiments, epithelial swelling occurred following the addition of vasopressin. The short-circuit current increased appreciably in eight experiments, and minimal although perceptible increments occurred in the other two experiments. The potential difference did not increase consistently. The fact that, with K_2SO_4 Ringer's, vasopressin induced a current and/or a volume increase in every experiment (increases in both occurred in eight experiments) indicates that high internal potassium concentrations *per se* do not abolish the responsiveness of the skin to vasopressin. Consequently, the lack of response observed with KCl Ringer's may relate to the swelling of the epithelial cells which preceded the addition of the vasopressin.

DISCUSSION

The changes in resistance that followed the application of the high K Ringer's solutions to the internal surface of the frog skin were of two types: The first occurred immediately, was characterized by a precipitous fall and a partial recovery, was seen with both KCl and K_2SO_4 Ringer's, and was independent of any swelling of the tissue. The second appeared after the partial recovery, was observed only with KCl Ringer's solution, and was associated temporally with the epithelial swelling.

Immediate Drop in Resistance. According to Share and Ussing (in preparation) the addition of the high K Ringer to the inside solution stimulates the skin glands which will secrete for about 20 minutes after which the secretion stops. It is known (3, 6) that when the glands are induced to secrete by adrenalin, the skin becomes temporarily leaky to ions. It is very likely, therefore, that the immediate drop in resistance is associated with this period of glandular activity.

One more event could enter into the immediate resistance drop. Koefoed-Johnsen and Ussing (7) have presented evidence previously that the inward-facing surface of the frog skin epithelium exhibits a high permeability to potassium, but a low permeability to sodium. It has been shown recently by Ussing and Windhager (8, 9) that the sodium-selective, potassium-impermeable boundary of the frog skin epithelium is located immediately beneath the cornified layer, which is only a few micra thick, and that the full potential develops stepwise through several layers of cells. From this and other evidence it was concluded that the cells are interconnected by resistive cell bridges. This view has been supported by the electron microscopic studies of Farquhar and Palade (10), who showed furthermore that the cell interspaces are closed to the outside by tight seals between the outermost layer of living cells. The inward-facing surface of the epithelium thus is composed of an interconnected array of cells rather than of a single cell layer. Nevertheless, there still would be a limiting continuous surface which would represent the potassium-permeable, sodium-impermeable membrane. If the increase in the potassium concentration of the internal solution were to increase the potassium conductance of this inward-facing cell membrane as high K does in nerve, the DC resistance of the epithelium would fall. This mechanism, however, probably could not account for the entire drop in resistance in view of the finding of Hoshiko (11) that most of the electric resistance is located in the outer half of the epithelium.

Secondary Fall in Resistance In contrast to the immediate resistance changes, the slow, sustained change occurred only with KCl Ringer's and thus was associated with swelling of the epithelia. It seems to be a general rule that epithelial swelling leads to a drop in resistance and increased active sodium transport, whereas shrinkage is associated with increased resistance and decreased sodium transport (9); moreover this obtains whether the volume changes are brought about by altering the osmolality of the bathing solutions, or by electrolyte shifts (9). In the present experiments, using KCl Ringer's as the internal bathing solution, the resistance dropped *pari passu* with the increase in volume of the cells. At the same time, the short-circuit current frequently increased. This increase in current, which it has been suggested is a function of active sodium transport (1), suggests that the secondary resistance drop might be located at the external, sodium-selective membrane of the skin. Thus, if most of the resistance in the sodium transport pathway normally is located at the external border, limiting transcellular sodium movements by limiting the entry of sodium ions into the cells, the concurrence of a fall in transcellular resistance and an increase in short-circuit current (and sodium transport) would point to an alteration at the external border.

The Changes in Response to Vasopressin According to current views, the sodium permeability of the external cell membrane is increased by vasopressin. It is of considerable interest, therefore, that when the internal surface of the skin was exposed to KCl Ringer's, no response to ADH occurred; whereas with K_2SO_4 Ringer's, a response to vasopressin was observed consistently. When chloride was the predominant anion, the epithelial cells swelled markedly; but when sulfate was the predominant anion, no change in volume occurred (prior to the addition of ADH). Thus, the possibility exists that the swelling evokes structural (and functional) changes in the external membrane which are similar to those produced by vasopressin. Another possibility is that the swelling opens new portals of entry into cells for sodium and tends to render insignificant the usual effects of vasopressin. The available evidence does not allow for a differentiation between these two possibilities.

The increase in epithelial cell volume induced by vasopressin when K_2SO_4 Ringer's was the internal solution and Na_2SO_4 Ringer's the external solution requires the entrance of sulfate ions (along with a cation) into the epithelial cells. The fact that this swelling occurred only in the presence of vasopressin, suggests that the hormone is required for sulfate to permeate the cell membranes. However, when the K_2SO_4 Ringer's solution was removed, and Na_2SO_4 Ringer's replaced in contact with the internal surface of the skins, the swelling reversed. Hence at least some of the sulfate ions which entered the cell water under the influence of ADH, must have left without the re-addition of hormone.

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