BBA 71028

Sodium- and calcium-dependence of threshold potential in frog skin excitation

In a recent publication describing some effects of calcium on the electrical excitation of frog skin, FISHMAN AND MACEY[1] have reported the unexplained observation that at low external Ca^{2+} concentrations (4–500 μM) the skin is inexcitable in Ringer's solution (115 mM NaCl), while excitability is restored when 90% of the NaCl of the low calcium solution is replaced by an osmotically equivalent amount of sucrose. According to ref. 1 these differences in excitability may be related to differences in the ionic strength of the outside medium, but are probably not accompanied by threshold changes.

The experiments described below were undertaken to investigate the role of threshold changes in this phenomenon more closely. They show that the threshold potential (V_s) of the epithelium increases with increasing Li^+, Na^+, choline, or Tris concentration of the outside medium. This increase of V_s is also found at high Ca^{2+} concentrations, but is more pronounced at low Ca^{2+} concentrations where V_s is generally larger. The findings explain the inexcitability at the combination of low Ca^{2+} concentration and high ionic strength in terms of an increased potential and current threshold.

By "threshold potential" is meant that voltage (V_s) measured across the skin, which corresponds to the current peak in the skin's N-shaped dynamic current–voltage curve. V_s can not be determined accurately from a record of skin potential *versus* time, but it is easily read off a "dynamic diagram"[2,3] plotting $\dot{V}$ (the first time derivative of the transepithelial voltage) *versus* V. Fig. 1 depicts some ($\dot{V}$,V)-plots obtained with subrheobase and above rheobase rectangular inward going current pulses.

Fig. 2 shows results of a typical experiment, where V_s was recorded for outer Na^+ concentrations between 1 and 80 mM at Ca^{2+} levels of 0.05 mM (upper curve), 1 mM and 20 mM (lowest curve). In this experiment, the intermediate Na^+ concentrations were obtained by mixing 80 mM sodium gluconate, which also contained the specified concentration of $CaSO_4$, with a 140 mM sucrose solution of the same Ca^{2+} concentration. Similar results were obtained when the sucrose was omitted. It is apparent from Fig. 2, that V_s increases with the external Na^+ concentration and decreases with the external Ca^{2+} concentration. When at low Ca^{2+} concentrations the Na^+ concentration is increased further, V_s climbs into the voltage range which activates the "rectification process". This strongly time-dependent process normally counteracts the resistance increase (rising phase of the spike) when the spike potential has sufficiently developed, and thus causes the falling phase. When V_s is large, the resistance increase is counteracted earlier or prevented completely, and excitability is diminished or lost. However, in marginal cases where V_s has just reached the critical voltage range, the potential threshold can still be demonstrated by using large current pulses. As seen in the right diagram of Fig. 1, the voltage response is dominated by rectification (trajectory bends downwards) when intermediate current densities are used, while larger current pulses still cause excitation. Here the potential changes are apparently fast enough to temporarily overrule the time-dependent rectification process.

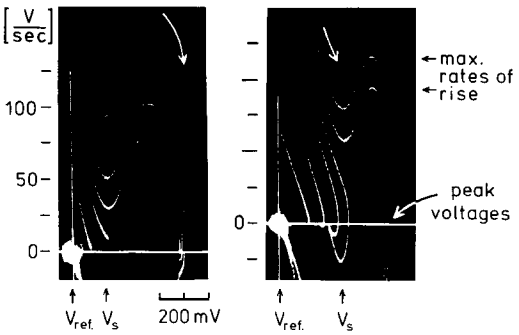

Fig. 1. Oscilloscopic ($\dot{V}$,V)-plots of the rising phase of skin responses to square inward going current pulses. The voltage across the epithelium was measured as potential of outer skin surface *minus* that of inner surface *minus* the resistive voltage drop (iR drop) at layers of saline external to the epithelium, and plotted on the abscissa against its rate of change (ordinate). Direction of time is indicated by two unlabeled arrows. Interval between unblanking pulses of each trace: 100 μsec. The potential threshold V_s is read off as the voltage which separates trajectorial sections of positive and negative slope in the first quadrant, counting from an arbitrary reference voltage $V_{ref.}$ (zero transepithelial resting potential). Thus changes of the skin's resting potential are not included in changes of V_s. Left: Na^+ concentration of outer solution 4 mM, Ca^{2+} concentration 20 mM. 7 current pulses of 10 msec duration at intervals of 30 sec were used. The smallest current pulse (60 $\mu A/cm^2$) was below threshold (see shortest line in first quadrant). Density of larger pulses was varied from 120 to 270 $\mu A/cm^2$ in steps of 30 $\mu A/cm^2$. Right: Na^+ concentration of outer solution 48 mM, Ca^{2+} concentration 1 mM. Duration of current pulses 20 msec. Current densities and type of response, counting trajectories from left to right: 90 and 150 $\mu A/cm^2$, almost linear trajectories (exponential rise of voltage); 210 and 240 $\mu A/cm^2$, exponential rise followed by time-dependent rectification (trajectories bend downwards); 270 and 300 $\mu A/cm^2$, exponential rise (negative slope) followed by excitation (positive slope), followed by rectification (downwards bend).

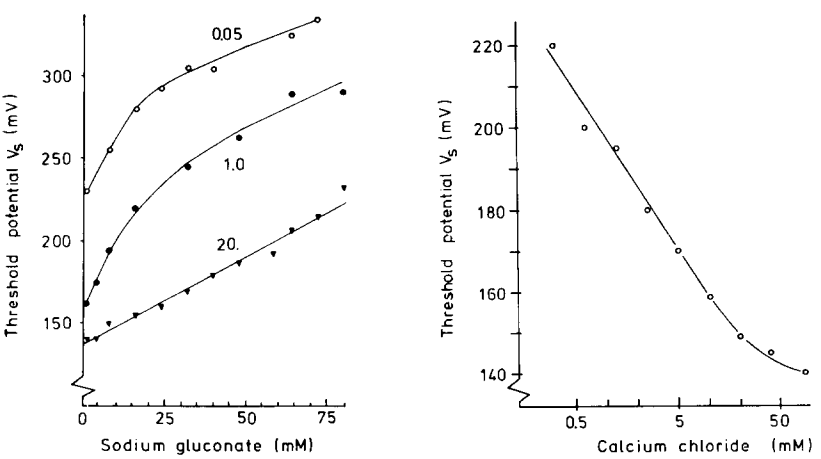

Fig. 2. Variation of threshold potential (V_s) with sodium gluconate concentration of outside bathing medium. Parameter: $CaSO_4$ concentration of outer medium (given in mM on curves). Exposure to each test solution was less than 1 min. Effects of such brief exposures in the presence of poorly penetrating anions are considered to be due to changes in the outer membrane, which has been shown to be the excitable one[4]. Inside bathing medium: sulfate Ringer's solution.

Fig. 3. Variation of V_s with Ca^{2+} concentration of outer medium, which contained a background concentration of 10 mM NaCl. Inside bathing medium: gluconate Ringer's solution.

The increase of V_s with the external Na^+ concentration may be partly or entirely independent of Na^+ as an ionic species. For instance, it may be caused by a decrease of the partial current of Ca^{2+} in the unstirred layer outside of the excitable membrane. To check for ionic specificity the effect of Na^+ on V_s has been compared to that of Li^+, choline and Tris. With the latter two ionic species, a small background concentration of Na^+ or Li^+ (0.2–0.5 mM) had to be used since choline and Tris ions did not by themselves support excitation. At a Ca^{2+} concentration of 0.5 mM it was found that choline or Tris increase V_s just like Li^+ or Na^+. However, the effect of Na^+ exceeded that of the other three species, suggesting that there is an Na^+-dependent component in the shift of V_s. Apart from this, we may summarize that V_s increases with the ionic strength and decreases with increasing Ca^{2+} concentration of the outer medium. Both effects may be mediated by a change of the same variable (like the Ca^{2+} concentration in or at the excitable membrane or the partial current of Ca^{2+}) which in turn influences V_s.

Of the many possibilities to explain the effect of Ca^{2+} on the threshold potential, a blockage of Na^+- and Li^+-specific pores by Ca^{2+} will be considered. The Na^+ and Li^+ current may be channeled through a limited number of sites in the membrane, which can also be occupied by Ca^{2+}. For reasons of ionic specificity, Ca^{2+} may be unable to leave the sites in the direction of current flow, while at high membrane potentials, the electrical field will also prevent Ca^{2+} from leaving the sites against the direction of current flow. The resulting blockage of Na^+ current at high membrane potentials is reflected in the negative slope segment of the membrane's current–voltage curve. At increased resting Na^+ conductance (influence of vasopressin[5,6]), current threshold and negative slope would be larger. Because of the Na^+–Ca^{2+} competition, the onset of effective blockage would occur at lower potentials (small V_s) when the Na^+ concentration is small or when the Ca^{2+} concentration is large. A Ca^{2+}-valve model was discussed for nerve by FRANKENHAEUSER AND HODGKIN[7], but rejected mainly because an e-fold change in Ca^{2+} concentration shifted the threshold potential by less than 12.5 mV. In frog skin, however, a slope of 18 mV per e-fold change of Ca^{2+} concentration is found (Fig. 3). This makes the Ca^{2+}-valve model appear worth a more detailed consideration.

This work was made possible by a grant (Li 126/3) from the Deutsche Forschungsgemeinschaft.

2nd Department of Physiology,
Saarland University,
665 Homburg/Saar (Germany)

B. LINDEMANN

1 H. M. FISHMAN AND R. I. MACEY, *Biochim. Biophys. Acta*, 150 (1968) 482.
2 U. FRANCK, *Z. Physik. Chem. Frankfurt*, 3 (1955) 183.
3 H. JENERICK, *Biophys. J.*, 3 (1963) 363.
4 B. LINDEMANN AND U. THORNS, *Science*, 158 (1967) 1473.
5 B. LINDEMANN, *Biol. Bull.*, 129 (1965) 391.
6 B. LINDEMANN AND J. KUTSCHERA, *Arch. Ges. Physiol.*, 283 (1965) R10.
7 B. FRANKENHAEUSER AND A. L. HODGKIN, *J. Physiol. London*, 137 (1957) 218.

Received July 29th, 1968