THE N-SHAPED CURRENT-POTENTIAL CHARACTERISTIC IN FROG SKIN

II. KINETIC BEHAVIOR DURING RAMP VOLTAGE CLAMP

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ABSTRACT Previous step voltage-clamp measurements on frog skin showed the presence of an N-shaped current-potential (I-V) relation in excitable skin. However, the collection and reconstruction of I-V data using discrete step changes of skin potential was tedious because of the long refractory period (up to 1 min) in frog skin. A direct and rapid (5 msec) method for recording the N-shaped I-V characteristic in real time is presented. Ramp functions are used as the command to the clamp system instead of a step function. Consequently the skin potential is forced to change in a linear manner (as commanded) and the skin current can be recorded as a continuous function of the controlled change of skin potential. With the ramp clamp, a low-resistance membrane state ($< 10\ \Omega \cdot \text{cm}^2$) resembling a breakdown phenomenon was observed at high skin potentials (>300 mv). Entry into the low resistance state resulted in a collapse of the N-shaped I-V relation to a nearly linear function. The utility of the ramp measurement is demonstrated by predicting (1) that the maximum rate of rise of the spike occurs at a voltage corresponding to the valley (local minimum) in the N-shaped I-V curve, (2) that the rate of rise of the spike increases with increasing clamp currents, (3) the voltage peak of the spike, and (4) the time course of the rising phase of the spike.

INTRODUCTION

Studies of electrical excitability of membranes frequently require a series of repetitive measurements; ideally each measurement is made after the membrane has completely recovered from previous measurements. In the case of the frog skin, the long refractory period (up to 1 min) not only makes these measurements tedious, but often precludes studies where the skin characteristics are changing due to slow spontaneous drifts or to experimental manipulation of the environment.

In an earlier paper (Fishman and Macey, 1968 *b*) it was suggested that some of the excitation properties of frog skin are reflected in isochronal current-potential

(I-V) plots obtained from step voltage-clamp measurements. In particular, there was a complete correspondence between the appearance of a negative slope in the I-V plot and the capacity of the skin to respond with all-or-none voltage spikes when excited by constant currents above a threshold value. Further, the negative slope developed abruptly and relatively early and seemed to persist in a quasi-steady state for several milliseconds before suddenly disappearing. These results suggest that the salient features of the I-V plot can be obtained from experiments where the voltage-clamp command signal sweeps through the entire voltage range within a few milliseconds. This would not only permit a direct display of the I-V characteristic in real time, but in many instances it would obviate the necessity of repetitive measurements. This possibility is explored in this paper by use of a ramp voltage clamp[1] (i.e., the voltage command signal is a linear function of time). Comparison of I-V plots obtained in step voltage-clamp experiments with corresponding plots obtained using a ramp clamp show good agreement. The I-V data obtained by ramp clamp measurement also enables us to predict several spike properties.

At high potentials (i.e. >300 mv) the skin passes into a low resistance state which resembles a breakdown phenomenon. Entry into this low resistance state initiates the collapse of the characteristic N-shaped I-V relation to a nearly linear function. These results are used in further interpretations of skin excitation.

METHODS

The abdominal skin of grass frogs (*Rana pipiens*) was mounted between two chamber halves made from Lucite (Fishman and Macey, 1968 *b*). A 1 cm^2 area of skin was exposed to Ringer's solution (115 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 8 mM Tris and 2 g/liter glucose, pH = 8.0) on both sides. The skin potential control system used was described previously (Fishman and Macey, 1968 *b*) and the stability considerations discussed. However, the command potentials to the clamp system used in these experiments were ramp pulses (either a saw-tooth or a symmetrical triangular pulse). To obtain an I-V characteristic, the skin potential was first clamped to zero (short-circuited) and held there in the quiescent state. A single ramp pulse applied to the control amplifier input forced the control system to increase the skin potential (outside skin surface becomes more positive) from zero in a linear manner. Upon reaching the end of the linear potential excursion, the skin potential was returned to zero either rapidly (0.1 msec), by the "flyback" portion of the saw-tooth pulse, or at the same constant rate as the forward excursion (symmetrical triangular-ramp pulse). A direct I-V plot was obtained by applying the skin current to the vertical input and the skin potential to the horizontal input of an oscilloscope.

RESULTS

Hysteresis

To interpret the ramp voltage-clamp measurements, it is advantageous for the command signal to sweep through the desired voltage range before the negative

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[1] A preliminary report of these measurements was given previously (Fishman and Macey, 1968 *a*).

slope I-V characteristic shows any substantial change—i.e. the I-V plot should be obtained in a quasi-steady state. To test this possibility, hysteresis measurements were made by using symmetrical triangular pulse commands. Fig. 1 shows this measurement for a ramp slope of 100 v/sec. A DC displacement of the forward and backward traces is expected because of the skin capacitance (See discussion). Thus at times when the ramp slope is altered (i.e. initially when the ramp is applied and at the peak of the triangular waveform) a DC shift should occur. Neglecting the DC shift, the shape of the forward and backward traces are quite similar; the negative slope in the I-V characteristic has remained reasonably stationary.

Notice that the skin potential in the data of Fig. 1 was not raised sufficiently to

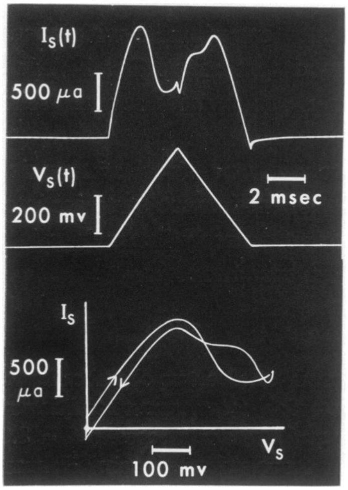

FIGURE 1 Hysteresis in the I-V plane. The lowest figure shows the skin current response (top) plotted as a function of the triangular ramp voltage (middle) across the skin. These data are displayed as an I-V plot in the bottom figure. The backward-trace negative slope is the same as the forward-trace negative slope for this ramp slope (100 v/sec). The negative slope is thus displayed in a quasi-steady state. The skin capacitance causes an upward displacement of the forward trace and a downward displacement of the backward trace so that the actual hysteresis is much less than is apparent. Notice that the peak voltage in the ramp was not sufficient to show the complete N-shape in the I-V relation.

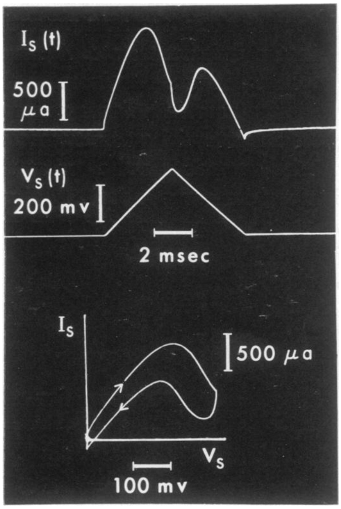

FIGURE 2 The same experiment as in Fig. 1; however, the ramp pulse amplitude has been increased slightly so that on the forward trace the current just begins up the far side of the valley before the skin potential is returned toward zero. Notice that in this case the backward trace begins to show significant hysteresis (change) in the negative slope region.

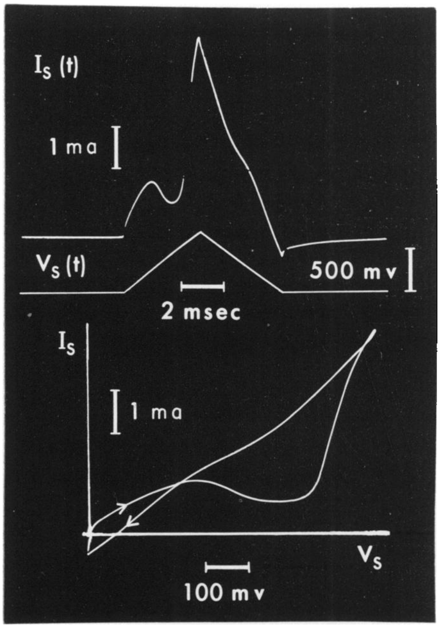

FIGURE 3 Continuation of the experiment of Figs. 1 and 2. The ramp pulse amplitude has been increased to display the full N-shape I-V curve on the forward trace. The backward trace in this case is completely different and the negative slope has disappeared in less than 2 msec after entry into the steep slope at high skin potential during the forward trace. Apparently a process occurs at high skin potential which results in the rapid collapse of the negative slope characteristic to a nearly linear function.

display the complete N-shaped I-V curve. When the amplitude of the triangular pulse is increased slightly (Fig. 2), the high-potential side of the N-shape curve just becomes visible; however, the backward-trace negative slope begins to show significant change. An increase of the triangular pulse amplitude to the point where the complete N shape develops on the forward trace (Fig. 3) resulted in the complete loss of the negative slope on the backward trace. The high-potential portion of the I-V curve shows the skin in a low resistance (reciprocal slope) state. Entry into this state initiates a process which results in the collapse of the N-shaped I-V characteristic to a nearly linear function.

Comparison of Step and Ramp Voltage-Clamp Measurements

The hysteresis measurements have shown that for a ramp slope of 100 v/sec a quasi-steady state I-V plot is obtained. The relation between ramp clamp data and isochronal I-V curves can be compared in the same skin by superposing isochronal I-V data (Fishman and Macey, 1968 *b*) on the curve obtained with a 100 v/sec ramp clamp. This is done in Fig. 4. The similarity between the curves is quite good even without correcting the ramp clamp curve for the DC level which the capacity introduces. This comparison was made on four different skins. The data on all four skins matched as well as that shown in Fig. 4. Thus, the 100 v/sec ramp voltage-clamp measurement gives approximately the same I-V information as the isochronal quasi-steady state obtained by step voltage-clamp measurement. Furthermore, it gives this information rapidly (5 msec) and directly.

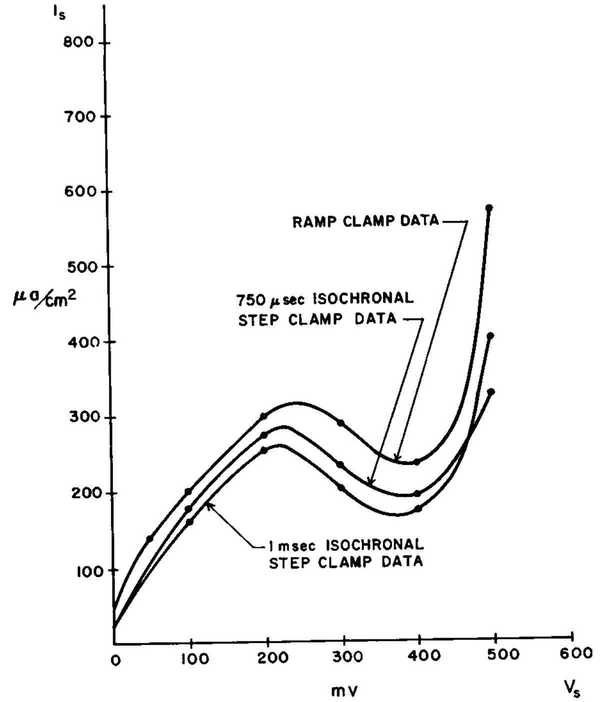

FIGURE 4 A comparison of I-V data obtained by ramp voltage-clamp and quasi-steady state isochronal I-V data obtained from step voltage-clamp data on the same skin.

Kinetic Behavior of I-V Characteristic at High Skin Potential (Refractoriness)

Information about refractoriness and the kinetic behavior of the skin I-V characteristic was obtained with the ramp clamp. In these experiments a potential ramp (saw-tooth pulse) was applied to a "resting" (120 sec) short-circuited skin to obtain its fully recovered I-V characteristic. A second ramp pulse was applied 1 sec after the end of the first ramp pulse and the I-V curve obtained. A third ramp pulse was applied 5 sec after the second ramp pulse ended and so on for times of 10, 20, 30, and 50 sec after the end of each preceding ramp pulse. The composite I-V data is shown in Fig. 5. The effect of the first ramp pulse is to cause a collapse of the negative-slope I-V characteristic of resting skin as a result of entry into the low resistance state. 1 sec after the first ramp pulse, the skin still does not have a negative slope. With each succeeding ramp pulse the recovery of the negative slope toward the resting characteristic is seen. The negative slope does not reappear until a 5 sec period is allowed between ramp pulses. Since the negative-slope characteristic is apparently necessary for spike generation (Fishman and Macey, 1968 *b*) this suggests that this skin should be absolutely refractory (no spike generation under current clamp) up to 5 sec after the first above-threshold current stimulus.

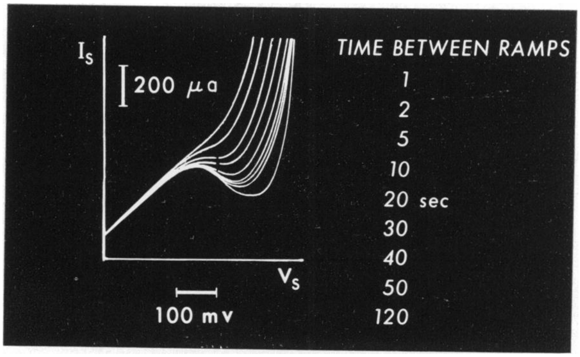

FIGURE 5 The kinetic behavior of the skin I-V characteristic after entry into the low resistance ("breakdown") state (high potential region of N-shaped curve). A "resting" skin (120 sec) is ramped with a saw-tooth pulse at zero time and the I-V curve displayed. 1 sec later it is ramped again, 2 sec after the second ramp it is ramped again, etc. at intervals of 5, 10, 20, 30, 40, and 50 sec. The recovery of the negative-slope characteristic after its collapse is thus seen. The period in which the skin has no negative slope corresponds to the absolutely refractory period for spike generation.

The skin also requires about a minute of rest (short-circuited, no potential excursion) in order to fully recover its resting I-V characteristic. Thus, after the absolutely refractory period (5 sec), a relatively refractory period (5–60 sec) should follow in which spike generation can occur. These predictions from the ramp-clamp data were confirmed by current-clamp data on the same skin.

Data on spike refractoriness (Finkelstein, 1964) showed that a spike in this interval is different in size and shape from that produced in a fully recovered skin. The I-V curves of Fig. 5 reflect this observation. Apparently, the recovery of the excitable system in the skin is relatively slow so that it is possible to obtain the skin in various excitable states (with negative slopes less than the fully recovered slope). This is probably the case in other excitable preparations such as eel electroplaques (Altamirano et al., 1955) where graded spikes are also observed in the relatively refractory period and for similar aspects of "decremental conduction" in treated nerve fibers (Deck, 1958; Mueller, 1958; Lorente de Nó and Condouris, 1959; Mauro's unpublished data in Lorente de Nó, 1961) and muscle fibers (Werman et al., 1961).

DISCUSSION

Ramp Voltage-Clamp Measurement

Previous isochronal I-V data (Fishman and Macey, 1968 *b*) obtained from step-voltage clamping were used to describe the development and disappearance of a negative resistance. However, in order to obtain these data on a fully recovered skin, the interval between successively applied steps was large (15–30 sec). The long refractory period (up to 1 min) in frog skin restricted the rate at which the steps could be applied. Thus the time interval to collect a complete set of current responses using discrete steps on frog skin was several minutes. This is obviously too long to allow observation of rapid changes in the skin I-V characteristic following externally imposed changes in the skin environment. Therefore, a ramp of linearly changing potential excursion was employed in the voltage-clamp system as a means of rapidly obtaining a direct display of the skin I-V characteristic. Trautwein et al. (1965) used a ramp-voltage clamp on muscle fibers and Bennett and Grundfest (1966) used it on gymnotid electroplaques. However, in both experiments the rate of change of the ramp voltage was slow compared with the rate of change of the membrane characteristic.

Problems associated with obtaining useful ramp-clamp data arise primarily from the nonlinear, time-varying properties of frog skin. The current response is a function of the rate of change of the potential ramp; if the rate of potential change is too slow, the skin will not show the negative-resistance character. On the other hand, a ramp which is too fast will elicit a response in the interval before the negative slope develops. The ramp slope which gives useful data is the one for which the negative slope has fully developed and is not changing significantly, i.e., a slope which elicits the quasi-steady state negative resistance. Slopes which do not meet this criterion will result in plots that are two-dimensional projections (the I-V plane) of the trajectory in a complicated three-dimensional space consisting of current, potential, and time. Such an empirical trajectory would be difficult to interpret.

Capacitive transients can introduce artifacts into the ramp clamp measurements. These transients affect both the current delivered to the skin and the voltage drop across the membrane resistance which is in parallel with the skin capacitance. These problems are illustrated by considering the response of the skin equivalent circuit (Fig. 6) to a voltage ramp. If we assume linear, time invariant circuit elements, then the current response to a ramp input $V_s = V_0 t$ is

$$I_s(t) = At + B(1 - e^{-t/\tau}) \tag{1}$$

where $\tau = \dfrac{R'_s R_m C_m}{R'_s + R_m}$, $\quad A = V_0/(R'_s + R_m)$ and $B = A\tau R_m/(R'_s + R_m)$.

Further, the corresponding voltage across C_m or R_m is

$$V_{R_m}(t) = \frac{V_0 \tau}{R'_s C_m}[t - \tau(1 - e^{-t/\tau})]. \tag{2}$$

For $t \gg \tau$, both I_s and V_{R_m} become proportional to the ramp voltage superimposed on a DC level given by B and $V_0\tau^2/R'_s C_m$, respectively. The time constant τ in frog skin ranges from 50–120 μsec (Fishman and Macey, 1968 *b*). Thus for a 5 msec ramp pulse the exponential terms occur as transients during the early portion of the response and the only other distortion is a DC level which can be removed.

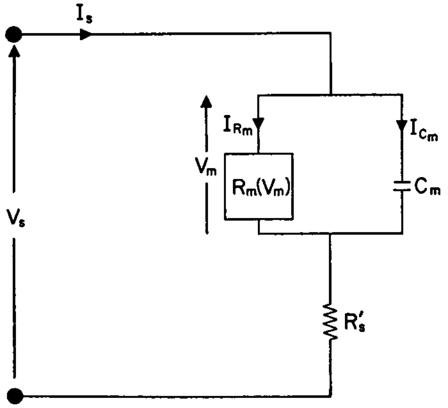

FIGURE 6 Equivalent circuit for frog skin. The element $R_m(V_m)$ represents the resistance of the excitable membrane.

The hysteresis data of Fig. 1 show that a ramp slope of 100 v/sec does obtain the negative slope in a quasi-steady state. Furthermore, the comparison between the quasi-steady-state I-V relation obtained with step clamp and ramp clamp (Fig. 4) correlate well. Thus the ramp-clamp measurements reported in this paper appear to meet the desirable criterion described above.

Breakdown at High Potential

The low resistance state of the skin at high potentials appears to be a breakdown of the excitable membrane within the skin. This conclusion is based upon the observation that this state is invariant from skin to skin. The skin resistance in this state is also approximately equal to the series resistance R'_s (25–100 $\Omega \cdot cm^2$) in the skin up to the excitable membrane (Fishman and Macey, 1968 *b*). An extrapolation of the membrane resistance R_m gives values $<10\ \Omega \cdot cm^2$. A similar phenomenon was observed and analyzed by Coster (1965) in *Nitella*. There are many other examples of membrane breakdown for membrane potentials exceeding 200 mv (Rudolph and Stämpfli, 1958; Cole and Moore, 1960; Julian et al., 1962).

Reconstruction of Current-Clamp Excitation From Voltage Ramp Data

The motion in the I-V plane for a constant current applied to a fully recovered skin can now be described qualitatively. Assuming the quasi-steady state I-V characteristic in Fig. 7, a step of current equal to threshold results in a rise in skin potential to point 1, determined by the time constant of the skin. The skin potential rises slowly to point 2 on the I-V curve, where a fast transition across the unstable region to point 3 occurs. The potential excursion during this transition corresponds to the rising phase of the spike. Since point 3 is in the low resistance state, the skin I-V curve collapses and a transition occurs to the collapsed curve at point 4. This event corresponds to the falling phase of the spike. If the current remains applied to the skin, it remains in the collapsed state. Thus only one spike occurs for one

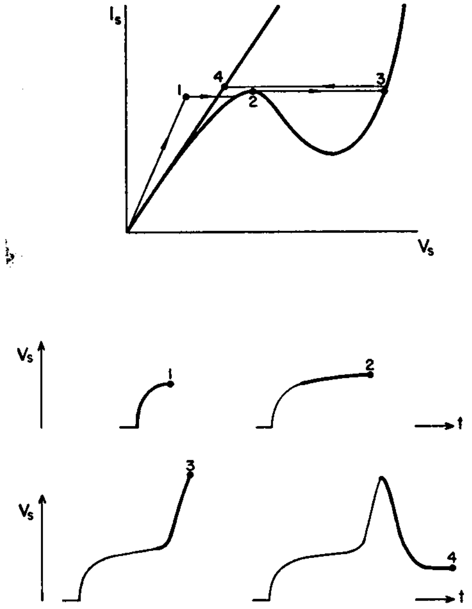

FIGURE 7 Hypothetical motion in the I-V plane for the application of a threshold step of current through the skin under current clamp. The potential excursion for movement in the I-V plane is shown below. Point 4 is on the collapsed I-V characteristic which results when the skin potential is allowed to progress beyond halfway between points 2 and 3. (See text for details.)

continuously applied step. It is the collapsed state (no negative slope) which is responsible for the absolute refractory condition and the lower-than-subthreshold skin resistance measured by Finkelstein (1964) during the falling phase of the spike. It is also interesting to recall that refractoriness in frog skin only occurs if the spike is allowed to continue into its falling phase. Thus if the current stimulus is removed at any time before the transition from point 2 to 3 (Fig. 7) reaches at least the halfway point, the skin does not enter the low resistance state and no collapse of the negative slope occurs.

During current clamp, the rising phase of the voltage spike occurs very rapidly, and is probably complete before the quasi-steady state I-V characteristic shows any substantial change. Assuming this to be the case, a quantitative description of the rising phase of the spike can be made by use of the equivalent circuit shown in Fig. 6 and the empirical I-V relation obtained by ramp voltage-clamp measurement. If the skin is driven by a constant current I_0, then using the notation of Fig. 6 we have

$$I_0 = I_{C_m} + I_{R_m} = C_m \frac{dV_m}{dt} + I(V_m) \tag{3}$$

where $I(V_m)$ is the steady-state characteristic of the element R_m. During current clamp, the voltage drop, V_s, across the entire skin is given by

$$V_s = V_m + I_0 R'_s \tag{4}$$

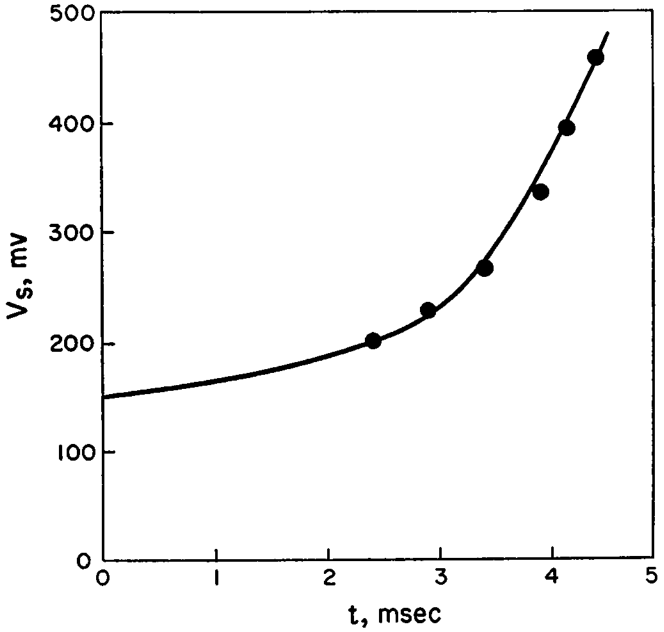

Figure 8 A comparison of the observed time course of the rising phase of the spike under current clamp (smooth curve) with the predicted rise (dots) from equation 8 and the I-V relation obtained by ramp voltage clamp in the same skin.

so that

$$\frac{dV_s}{dt} = \frac{dV_m}{dt}. \tag{5}$$

Our primary assumption is that the ramp clamp I-V characteristic of R_m is a reasonable approximation to the quasi-steady state I-V relation during current clamp. During ramp clamp $I_{R_m}(V_m)$ for $t \gg \tau$, is related to the *observed* total skin current $I_s(V_s)$ and the ramp slope V_0 by

$$I_s(V_s) = I_{R_m}(V_m) + V_0 C_m. \tag{6}$$

Rearranging equation 6

$$I_{R_m}(V_m) = I_s(V_s) - V_0 C_m \equiv I(V_s) \tag{7}$$

where $I(V_s)$ is defined as the observed ramp-clamp current minus the DC component $V_0 C_m$. Combining equations 3, 5, and 7 we have

$$\frac{dV_s}{dt} = \frac{I_0 - I(V_s)}{C_m}. \tag{8}$$

Equation 8 describes several properties of the current-clamp spike.

The maximum rate of rise of the spike will occur at the valley of $I_s(V_s)$. Further, the rate of rise of the spike should increase with increasing clamp currents (i.e., for increasing values of I_0 above threshold). This prediction is verified in spike data (Fig. 2 *C*, Fishman and Macey, 1968 *b*). In addition, the voltage peak of the spike should correspond to the voltage coordinate of point 3 (Fig. 7) on the low resistance slope of the N-shaped curve. This was verified by comparing the corresponding ramp voltage-clamp and current-clamp data on 18 different skins. The mean ± SE of the spike voltage peaks was 463 ± 11.5 mv and the mean difference (measured-predicted) ± SE was 16 ± 5 mv. Finally, reconstruction of the spike rising phase (transition from point 2 to 3 in Fig. 7) can be obtained graphically using equation 8. This has been done with spike and ramp voltage-clamp data from the same skin (Fig. 8). The computed points of rising phase of the spike (beginning at voltage threshold) from ramp clamp I-V data show excellent agreement with the experimentally observed spike during current clamp. This correspondence indicates that the I-V relation obtained by ramp clamp in these experiments is a very useful tool in observing and predicting many of the properties of excitability in frog skin. It can be used to determine the ionic dependence of the relation and the results interpreted with respect to spike generation.

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