CURRENT-VOLTAGE RELATIONS OF SINGLE NODES OF RANVIER AS EXAMINED BY VOLTAGE-CLAMP TECHNIQUE

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INTRODUCTION

THE RELATIONSHIP between the potential difference across an excitable membrane and the current that flows through the membrane has been investigated on the squid giant axon by the use of the so-called voltage-clamp technique (7, 17). This technique involves the sudden elevation of the membrane potential and, by an automatic control of the membrane current, the maintenance for a certain period of time of this new potential level. The analysis of the experimental data obtained by this voltage-clamp technique led Hodgkin and Huxley (6) to many interesting conclusions regarding the mechanism of action potential production.

The present investigation was undertaken with a view to securing new information about the electrophysiological properties of the excitable membrane at the node of Ranvier of the toad nerve fiber using the voltage-clamp technique. Since the capacity of the nodal membrane does not seem to play any significant role in the process of action potential production (13, p. 649), the significance of voltage-clamping in the nodal membrane is somewhat different from that in the squid giant axon. Nevertheless, since membrane potential and the membrane current are the two main variables that serve to describe the state of the membrane, it is worth while to investigate the time course of the membrane current when the membrane potential is held constant at various new levels. In the present investigation the voltage-clamp technique was applied to the normal nodal membrane, and the nodal membrane during the refractory period, under low sodium and under partial narcosis.

METHODS

Single motor nerve fibers isolated from sciatic-semitendinosus preparations of the toad (*Bufo marinus*) were used. The method of dissecting and mounting the fiber on a bridge-insulator is essentially the same as that employed in a previous investigation (14). The fiber was mounted, as shown in Fig. 1, across three pools separated by two air gaps. The middle pool of the bridge-insulator, in which the node to be studied (N_1) was immersed, was approximately 1.4 mm. wide in the middle and about 70 mm. long. The large lateral pool (where node N_0 is) was roughly 5 ×5 cm. in size and had a circular edge facing the middle pool. The air-gap between the large and the middle pool was generally 0.3 mm. wide in the present experiments. The grid electrode for mounting a portion of the single

fiber preparation (including N_2) was approximately 5 mm. in diameter; the air-gap between the middle pool and the grid electrode was 0.7–0.9 mm. All the electrodes were of the Ag-AgCl-agar (Ringer) type and the potential difference among them did not exceed a few millivolts. Both node N_0 and N_2 were rendered inexcitable with a 0.1% cocaine-Ringer solution.

Designing and constructing the high input impedance preamplifier (A_1 in Fig. 1) was one of the major difficulties in the present investigation. We constructed two different

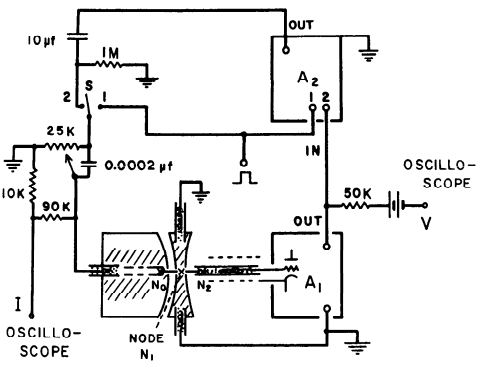

FIG. 1. Experimental setup used for voltage-clamp experiments on single node preparations of toad. Node to be studied, N_1, is kept in normal Ringer; the two lateral nodes, N_0 and N_2, are rendered inexcitable with cocaine. A_1 represents high input impedance preamplifier of which circuit diagram is shown in Fig. 2. A_2 is Tektronix preamplifier with a gain of 1000; polarity of output signal is same as that of input marked "1." Terminals marked I and V are connected respectively to current and potential channels of a dual-beam oscillograph. For further detail, see text.

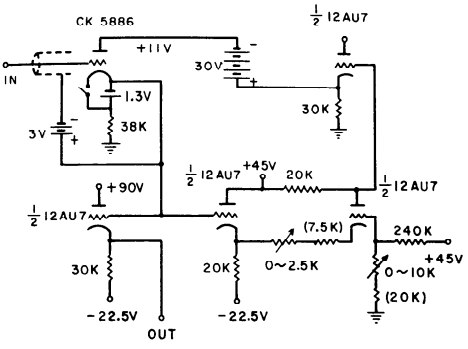

FIG. 2. Circuit diagram of high-input impedance preamplifier used for experiments of Figs. 3–5. Input tube (CK5886) is a miniature electrometer tube. Twin triode tubes (12AU7) were used in other stages. Except for stage at right-hand lower corner which is a grounded-grid amplifier, all tubes are used as cathode-followers. Variable resistor of 2.5 K is used to raise gain of input (electrometer tube) stage close to unity. Other variable resistor (up to 10 K) is used for adjustment of D.C. level of driven shield around input lead to bring it close to ground potential. All batteries shown in diagram are of miniature type.

preamplifiers, both of which yielded satisfactory results. The detail of the preamplifier designed and constructed by one of us (A.B.) will be published elsewhere; this preamplifier was used in the experiments of Figs. 6–8. The circuit diagram of the preamplifier used in the experiments presented in other figures is illustrated in Fig. 6; this preamplifier had less grid current than the other. The problem in the construction of these preamplifiers was that there was in the A.C. output of these preamplifiers an appreciable phase shift when the input was driven by a source of A. C. through a resistance of 40–50 megohms. This phase shift was reduced by proper shielding of the grid lead. The preamplifier was constructed in such a way that both D.C. and A.C. potentials of the driven shield around the grid lead followed the potential of the input.

The other preamplifier (A_2 in Fig. 1) was a Tektronix preamplifier (#122) with a gain of 1000 and the high filter setting either at 10 kc. or 40 kc./sec. The phase shift in the A.C. caused by this amplifier did not exceed about 20° at 20 kc./sec. Under the voltage-clamp conditions, *i.e.*, with switch S in Fig. 1 connected to position 2, an oscillation often started when the potential divider at the output of A_2 (25 K in Fig. 1) exceeded about 40%. The frequency of this oscillation was around 50 kc./sec. The experiments were carried out with the maximum amount of (negative) feed-back limited by this oscillation. The potential

divider connected to the current channel of the oscillograph (90 and 10 K in Fig. 1), the small condenser (0.0002 μf) as well as the series resistance (50 K) at the output of A_1 were designed to reduce the phase shift by decreasing the effect of the capacity of the relatively long lead wires to the oscillograph.

With the arrangement of Fig. 1, in which switch S was in position 1, the source of rectangular voltage pulses (Tektronix pulse generator Type 161) was connected directly to the electrode in the large lateral pool in which N_0 was immersed. Under these experimental conditions, the voltage source is connected to the nodal membrane at N_1 through a high, nearly ohmic resistance between the large lateral pool and the small middle pool. Therefore the node under study is subjected to a nearly constant membrane current. (During the first 0.1 msec. the capacity of the myelin sheath between N_0 and N_1 is expected to distort the time course of the membrane current.) When switch S was at position 2, the membrane potential across N_1 (recorded by channel V of the oscillograph) roughly followed the time course of the potential variation arising from the source of rectangular pulses. (Note that A_2 is a high-gain differential amplifier; for greater detail, cf. 17.) It was not easy under the present experimental conditions to determine the resistance (R) existing between the nodal membrane under study and the electrode in the large lateral pool. Therefore the membrane current through N_1 was expressed in terms of $I \cdot R$, instead of I, which corresponded to the potential of the electrode in the large lateral pool. Since the resistance (R) is in the range between 50 and 100 megohms (depending upon the fiber diameter and the internodal length), a rough measure of the membrane current can be obtained from the data presented in this paper.

There is one more factor to be taken into consideration in the discussion of our experimental setup. That is the effect of variation in the membrane potential at N_1 upon the measurement of the membrane current, $I \cdot R$. When the nodal membrane at N_1 undergoes a profound potential variation, the voltage of the electrode in the large lateral pool cannot serve as a measure of the membrane current through N_1. Under the voltage-clamp conditions, however, the variation in the potential of the large electrode is in the range of 1–2 V. (cf. Fig. 3). Since the potential variation at N_1 is of the order of 0.1 V., the potential of the electrode in the lateral pool can serve as a relatively faithful measure of the membrane current at N_1.

All the experiments were carried out in a special refrigerated chamber, the temperature of which was maintained at 14–15°C.

Results

Voltage-current relationship of a normal node. In Fig. 3 is presented a typical example of records taken from approximately 20 single node preparations. In most of the preparations the action potential of the node reached a magnitude of 90 110 mV. when the external shunting resistance (between N_1 and N_2 in Fig. 1) was carefully raised by washing the myelinated portion of the fiber in the air gap with an isotonic glucose solution. When the clamping voltage was negative, *i.e.*, when the nodal membrane was hyperpolarized along a rectangular time course, the membrane current observed was nearly rectangular (record A in Fig. 3); the nodal membrane behaved like an ohmic resistor in this voltage range. When the clamping pulse was positive and was in the range of the ordinary threshold (15–25 mV.), the membrane current required to maintain the new constant level was inward (represented by a downward deflection in records B and C). In this voltage range, the time course of the membrane current was extremely variable; this variability will be discussed in greater detail later.

When the positive rectangular clamping pulse was in the range between 35 and 90 mV. (records D, E, F), there was a surge of inward membrane current following the onset of the pulse. Then this inward current showed a

gradual decrease and, when the new potential level was maintained long enough, the direction of the membrane current reversed. The maximum intensity of the inward membrane current decreased with increasing clamping voltage. When the size of the clamping pulse was close to the amplitude of the action potential (records G, H), the current intensity at the peak of the inward surge was close to zero. With a further increase in the level of the clamping voltage, the current at the peak remained above the zero line;

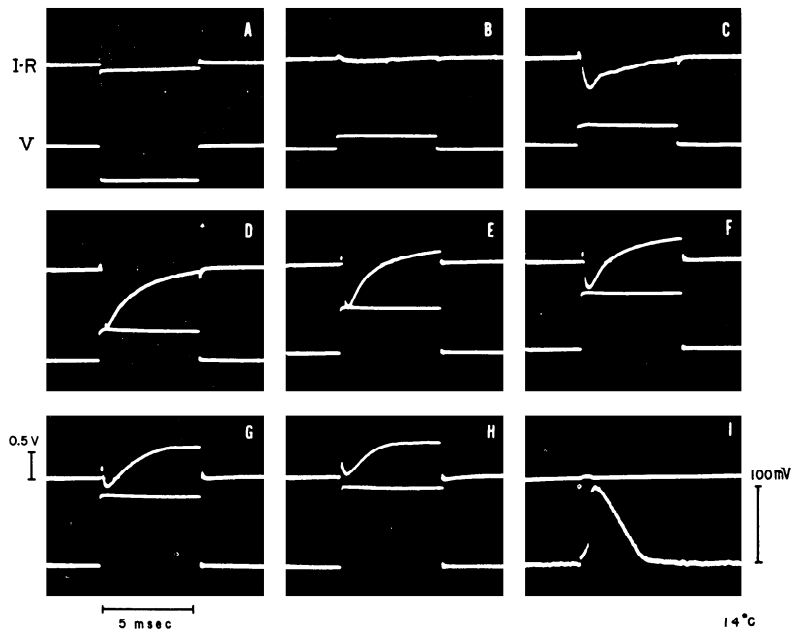

FIG. 3. Records showing relationship between membrane current, I, and change in membrane potential, V, of single node of Ranvier as examined by voltage-clamp technique (records A–H). Record I on right-hand lower corner is an action potential of node obtained with switch S in Fig. 1 at position "1." Recording was made at usual interval of approximately 15 sec. R signifies resistance of nerve fiber (of order of 70 megohms) which is in series with nodal membrane under study and electrode in large lateral pool; trace I · R displays time course of potential of electrode in large lateral pool required to maintain membrane potential at given levels. Note that capacitative surge of membrane current is small.

namely, the membrane current was outward-directed during the entire period of voltage clamp.

The relationship between the peak value of the membrane current and the level of the clamping voltage (determined on a different preparation) is shown in Fig. 4. For negative values of clamping voltage V, *i.e.*, for hyperpolarizing rectangular pulses, the level of the early part of the steady inward current was plotted against the size of the voltage pulse. The slope of the straight line representing the I-V relationship in this range can be regarded as the measure of the membrane resistance of the resting node. The mem-

brane resistance determined from this slope did not conflict with the value obtained by a previous method (13), *i.e.*, approximately 35 megohms for a 1.4 mm. long portion of a nerve fiber including a node.

The relationship between the membrane current at the peak of the inward surge and the rectangular clamping voltage was expressed also by a straight line in the range of voltage more positive than about 40 mV. In some single node preparations there was a small (but systematic) divergence

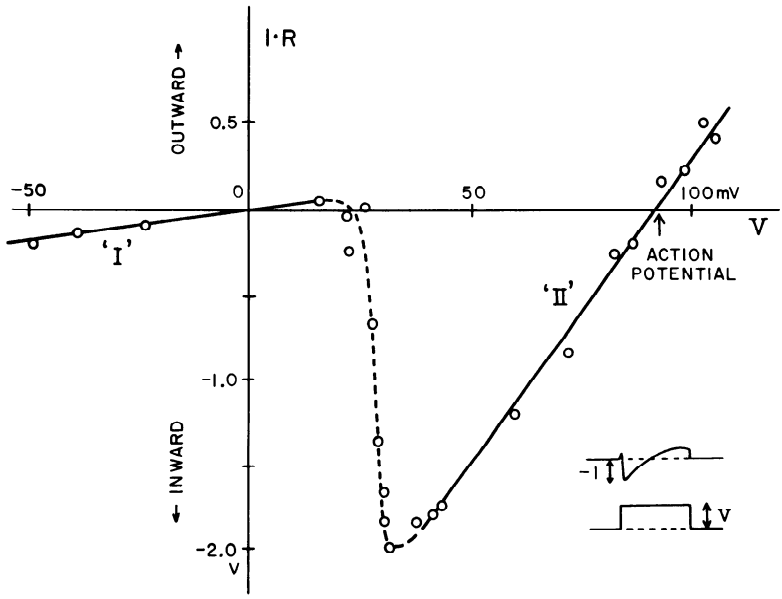

FIG. 4. Relation between peak value of inward surge of membrane current, I, and size of rectangular clamping voltage, V. Straight line 'I' was determined from steady level of membrane current. Signs of V and I were chosen in such a way that Ohm's law can be represented by straight line with positive slope. Arrow indicates amplitude of action potential of node.

of the observed membrane current from the straight line when the voltage exceeded the peak amplitude of the action potential (see Fig. 8, left). Since, however, voltage-clamping with such strong voltage pulses was found to bring about a depression in the spike-amplitude for a period of about 1 minute or more, no systematic attempt was made to explore the divergence of the results from linearity. The linearity just mentioned between V and I in the range of V above 40 mV. indicates that both the membrane resistance and the "membrane e.m.f." reach the same, constant value soon after the onset of these depolarizing pulses. In other words, the node emerges into a fully excited state following the onset of these pulses.[1]

[1] As in the argument developed in a previous paper (17), the nodal membrane is represented by a battery with a finite internal resistance. Both this resistance and the e.m.f. are assumed to change relatively slowly, *i.e.*, only at the rate comparable to the evolution of the action potential.

In the present series of experiments, it was found that the slope of the I·R-V relation in the excited state ("II" in Fig. 4) was 7–11 times as large as that in the resting state. The ratio between the two slopes represents, to a rough approximation, the ratio of the membrane conductance in the excited state to that in the resting state. When one takes the whole (ohmic) network connected to the source (I R) and the variability of the membrane resistance of N_1 into consideration, it is found that the ratio between the membrane conductances should be slightly (5–10%) greater than the ratio of the slopes mentioned above. The result that the conductance of the nodal membrane is approximately 10 times as large as that of the resting membrane is consistent with the results obtained previously by the pulse method of measuring the membrane resistance (16).

Following the peak of the inward surge of the membrane current, the current was found to decrease first rapidly and then more slowly (records D, E, F in Fig. 3). This behavior of the membrane is understandable when one recalls that the "membrane e.m.f." varies in parallel with the resistance during activity (11, 16). Let $E_{II}(t)$ denote the "e.m.f." of the active membrane and $R_{II}(t)$ its resistance. Then, the membrane current, $I(t)$, under the voltage-clamp conditions will be given by

$$I(t) = \frac{V - E_{II}(t)}{R_{II}(t)},$$

where V denotes the size of the clamping voltage. When $R_{II}(t)$ varies roughly with $E_{II}(t)$, the change in $I(t)$ should be more rapid than the nearly linear decay (with time) in $E_{II}(t)$. In the present argument, $I(t)$ is nothing more than a kind of action current observed when V is held constant.

There is a small complicating factor in this simple argument. That is the fact that a strong inward membrane current applied to the node during activity tends to prolong the time course of $E_{II}(t)$ (cf. Fig. 5 in reference 14; record D of Fig. 3 of present paper). Evidently this effect of an inward current upon $E_{II}(t)$ is related to the prolongation of the nodal action potential by anelectrotonus (11, p. 108).

The steady level of the membrane current observed toward the end of a rectangular clamping voltage pulse of about 10 msec. duration is a measure of the delayed rectification (2) in the nodal membrane. In the present investigation, the I·R-V relation at this stage could be approximated in the range of V greater than about 40 mV. by a straight line passing through the point defined by $I = 0$ and $V \doteqdot 25$ mV. with a slope two to three times as steep as that for the resting state. Since the phenomenon of delayed rectification has been investigated previously by using linearly and exponentially rising current pulses (12), no detailed analysis was made on this phenomenon.

Rapid variation of membrane current under voltage-clamp conditions. In the range of rectangular clamping voltage pulses between 15 and 30 mV., it was frequently observed that the membrane current underwent very rapid

variations. Examples of such oscillation of the membrane current from three different nodes are presented in Fig. 5. There were also some preparations in which such oscillatory behavior of the membrane current could not be demonstrated.

The time course of the oscillatory membrane current was not sinusoidal; it was more-or-less rectangular and its amplitude gradually decayed with time. The frequency of such oscillation was between 1.5 and 3 kc./sec. at 14°C., at which temperature the duration of the action potential was approximately 3 msec.; it varied from preparation to preparation and from time to time in one preparation. The mode of oscillation was very sensitive to the

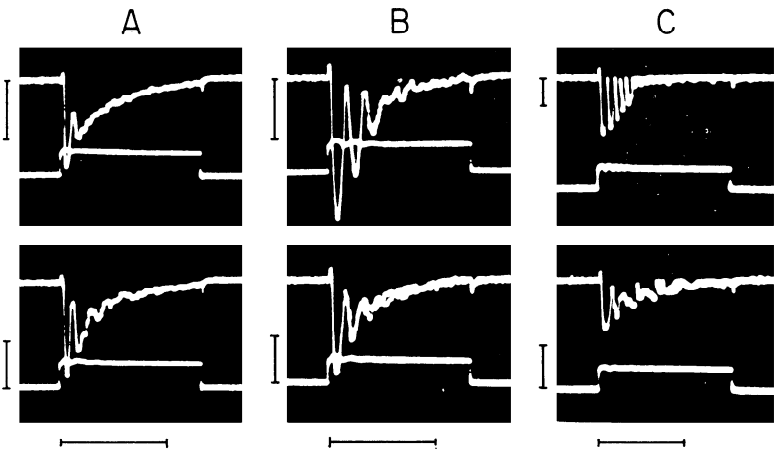

FIG. 5. Records showing rapid variation of membrane current observed when membrance potential was clamped at a level slightly above ordinary threshold. Records were obtained from three different single node preparations. Note that a slight change in level of clamping voltage brings about a large change in time course of membrane current. Calibration for potential trace is 50 mV., and that for current trace (with a dimension of I·R) is 1 V. Time mark, 5 msec. Temperature, 14°C.

level of the clamping voltage. The envelope of the oscillatory membrane current was always below the base line of the current trace and generally above the course of the non-oscillatory membrane current for a pulse of about 40 mV. (record D in Fig. 3).

At first sight the rapid variation of the membrane current under voltage clamp appeared to be an artefact. The oscillation in the positive feed-back amplifier (i), the thermal noise arising from the high input resistance amplified by the high-gain differential amplifier (ii) and the slight imperfection of the voltage clamp due to the high resistance between N_0 and N_1 in the feed-back circuit (iii) were considered as possible causes of artefacts. However, based on the following arguments, we came to the conclusion that this is a physiologically significant phenomenon.

(i) The frequency of variation in the membrane current is far slower than that of an

oscillation caused by an excessive feed-back in the preamplifier. The oscillatory membrane current is always inward, *i.e.*, below the base line of the current trace. The oscillation ceases when the clamping voltage is increased by about 10 mV. The time course of the oscillation is non-sinusoidal; it is sometimes a repetition of rectangular pulses. These facts cannot be explained on the assumption that the oscillation in the membrane current is caused by the feed-back amplifier.

(ii) The transitional range in the I–V relationship (shown by broken line in Fig. 4) is extremely steep; if the thermal noise varies the voltage V in an irregular fashion, the membrane current is expected to show a large variation. However, the actual variation in V is too small to account for the observed large change in I(t). Two successive sweeps under constant experimental conditions frequently reveal that the phase relation between the onset of V and the oscillation is more or less fixed (records B, bottom, in Fig. 5); this fact excludes the possibility that the random thermal noise is the cause of the oscillation.

(iii) In practice voltage clamping is always imperfect. This imperfection manifests itself as an elevation in the level of V when there is a strong membrane current (see record D in Fig. 3). In the present investigation, no attempt was made to exclude the possibility that this imperfection was related to the oscillation. Our present attitude is to analyze, admitting the existence of a slight imperfection of voltage clamping, the mechanism of production of this oscillatory membrane current as observed.

The rapid variation of the membrane current at such high frequencies has never been reported in previous experiments without voltage clamp. On the basis of the two-stable state hypothesis (17), we interpret the oscillatory behavior of the membrane as deriving from the property of the nodal membrane to undergo transitions repeatedly between the excited and the resulting states when the automatic control of the current tends to maintain the membrane potential at a constant level. The previous experiments on the abolition and re-establishment of the action potential (14, Fig. 9) has revealed that the change in the "membrane e.m.f." during activity is far slower than the change in the membrane current in the present experiment. Therefore, we interpret this oscillatory change in the membrane current as due to the rapid change in the percentage of the "active" area of the membrane. In a previous paper (17) the concept has been introduced that in threshold excitation only a small portion of the membrane is activated. The present interpretation of the voltage-clamp oscillation of the membrane current is to ascribe it to a rapid change in the active fraction, a, of the membrane under these particular experimental conditions.

It has been pointed out (3, 15) that the iron or cobalt wire model of the nerve membrane can exhibit almost all the known electrical properties of the excitable membrane. It is interesting to note that a high-frequency oscillation of the current under the voltage-clamp conditions has already been observed by Franck and Meunier (4). The interpretation of the oscillatory current in the nodal membrane described above is analogous to that proposed by the electrochemists for the corresponding phenomenon in the model. Both in the model and in the actual nerve membrane, the voltage-clamp oscillation of the membrane current is labile and progresses far more rapidly than the ordinary repetitive firing of action potentials.

Comparison of current-voltage relation of refractory or partially narcotized node with that of node in low-sodium Ringer. It is well known that the spike amplitude of a single node can be reduced by refractoriness or by weak narco-

sis (cf. 11). Under the influence of these agents the loss of the membrane impedance during activity is less marked than in a normal node (18). The resistance of the nodal membrane in the relatively refractory period or the membrane resistance of the weakly narcotized node is not different from that of a normal, resting node (18, 13). In the present investigation, an attempt was made to compare the current-voltage relation of a refractory or weakly narcotized node with that of a node immersed in Ringer with a low-Na concentration.

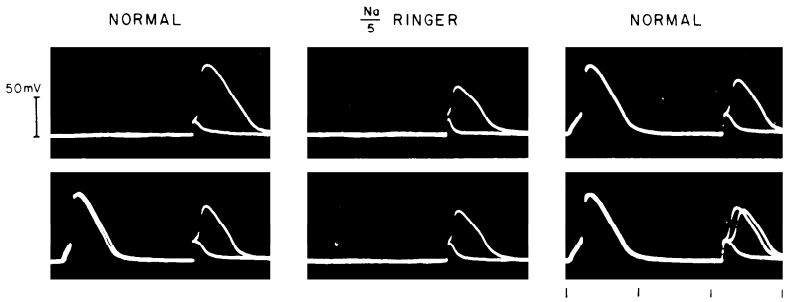

FIG. 6. Comparison of action potential of node in low-sodium Ringer (middle column) with action potentials of a node in relatively refractory period (two lateral columns). Large action potentials are normal responses of node in normal Ringer. Note that threshold membrane potential, spike-amplitude and spike-duration are very similar in refractory node and in node under low sodium. Time marks 5 msec. apart. 14°C.

In Fig. 6 is presented an example of the experiments designed to show the similarity between subnormal action potentials of a refractory node and those of a node in a low-Na Ringer. These records were obtained with the experimental set-up of Fig. 1 in which switch S was kept at position 1 and two independent sources of short rectangular pulses were used to drive the electrode in the large lateral pool. As in the preceding experiments, both node N_0 and N_2 were inexcitable. The middle pool was filled first with normal Ringer and then with a Ringer in which the Na concentration was reduced to 20% of the normal value by replacing Na with choline. These subnormal action potentials have a shorter duration than the action potential of a normal node. Neither the relative refractoriness nor a treatment of the node with a low-Na Ringer changed the resting potential of the node by any measurable amount. The effect of lowering the Na concentration of the medium was almost perfectly reversible.

Figure 7 shows the voltage-clamp behavior of a node in low-Na Ringer (solid lines) compared with that of a node in a normal Ringer (broken lines). The amount of reduction in the amplitude of the action potential resulting from lowering the external sodium concentration agreed roughly with the data obtained by Huxley and Stämpfli (8); the reduction in the spike-amplitude in Na/5 Ringer was 30–40 mV. As in the normal node, the membrane current at the peak of the inward surge was close to zero when the rectangular clamping voltage reached the spike amplitude of the node.

As in the similar observation on the squid axon by Hodgkin and Huxley (5), a reduction in the Na-concentration of the medium strongly modified the amplitude of the inward surge of the membrane current without affecting the later, steady portion of the membrane current appreciably. The upper tracings in Fig. 7, left, show that the steady level was also affected to some extent by substitution of Na with choline; Hodgkin and Huxley (5) reported a similar observation in the squid axon. In Fig. 7, right, the membrane current at the peak of the inward surge was plotted against the size of the

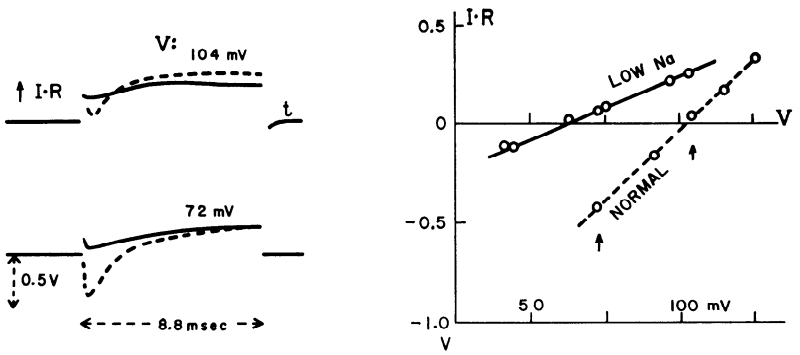

FIG. 7. *Right:* comparison of current-voltage relation at peak of activity of normal node (broken line) with that of same node immersed in Ringer with low (approximately 20%) sodium concentration (continuous line). *Left:* time courses of membrane currents under voltage clamp in node in normal Ringer (broken line) compared with those of same node after reducing sodium in Ringer. Clamping voltages are given. Duration of action potential in normal Ringer was approximately 2.8 msec. 14°C.

rectangular clamping voltage. It is seen that the slope of the I-V straight line for the excited state was strongly affected by reduction of Na in the medium. This finding is consistent with the observation by Grundfest *et al.* (9), demonstrating that the impedance loss during activity is less in an axon in a low-Na medium than in a normal axon. In a Na/5 Ringer the membrane conductance at the peak of activity determined from the slope of the I-V straight line was 40–50 per cent of the conductance of a normal node. A reduction in Na in the medium was found to decrease also the steepness of the intermediate portion of the I-V relation (shown by the broken line in Fig. 4). When the external Na concentration was reduced below about one-tenth of the normal value, the whole I-V curve remained above the abscissa ($I = O$) for $V > O$.

The effect of the refractoriness and that of weak narcosis upon the I-V relation of a node was essentially the same as that of lowering the external Na concentration. When the spike amplitude was decreased during the relatively refractory period or by a dilute urethane-Ringer solution, there was always a distinct reduction in the membrane conductance at the peak of activity. For a given decrease in the spike amplitude, the membrane con-

ductance at the peak of activity suffered roughly the same amount of reduction in the three cases, *i.e.*, during the relatively refractory period, under a low-Na Ringer and under narcosis. These findings are illustrated in Fig. 8.

The small photograph in Fig. 8, top left, shows how the I-V relation for a refractory node was investigated. First, a triangular voltage pulse which had approximately the same amplitude and duration as a normal action potential was applied to the node. When the membrane potential was clamped

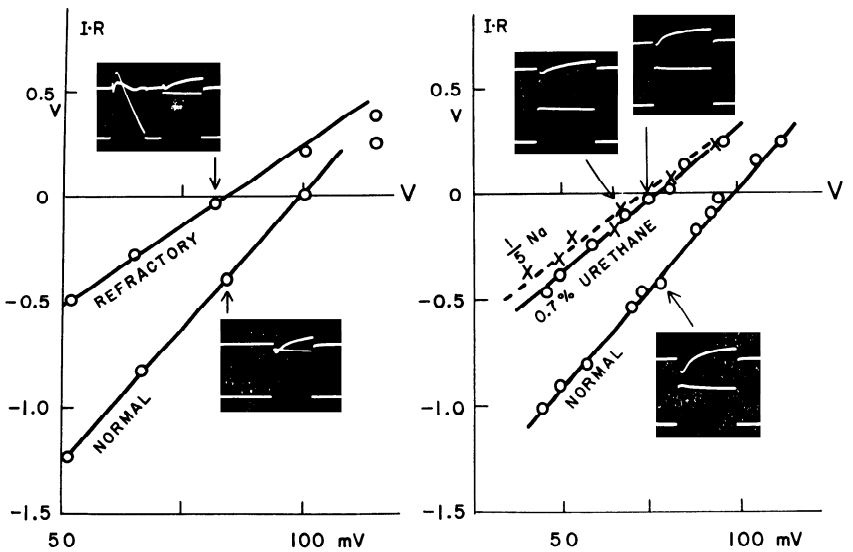

FIG. 8. *Right:* current-voltage relations of node determined first in normal Ringer, then in 0.7% urethane-Ringer solution and finally in Na/5 Ringer. Following measurement in urethane-Ringer, node was washed with normal Ringer; recovery was almost perfect. *Left:* current-voltage relation of normal node compared with that of same node during relatively refractory period. Node was made refractory by a triangular voltage pulse that preceded rectangular pulse. 14°C.

with this "artificial action potential," the membrane current reduced, as might be expected, to a very small value except during its early phase. The artificial action potential was followed by rectangular voltage pulses of varying amplitudes. The time courses of the membrane currents associated with these rectangular clamping voltages were compared with the currents observed when the rectangular pulses were delivered alone, *i.e.*, without being preceded by the artificial action potential. It was found by this method that the time courses of the membrane currents of a node in the refractory period were practically the same as those of a node in a low-Na medium. (For such a comparison, it is necessary, as in the experiment of Fig. 6, to chose a proper moment in the relatively refractory period depending on the concentration of Na in which the node to be compared with was immersed.) This finding is consistent with the fact that the time course

of the action potential of a refractory node is similar to that of a node in low-Na Ringer (Fig. 6).

Under the conditions of the present experiments, the action potential of a weakly narcotized node was slightly shorter in duration than the action potential of the same amplitude produced by a node treated with low-Na Ringer. This is not surprising, since the spike duration is known to be extremely sensitive to various physiological and biochemical conditions of the fiber (10, 12). The time course of the inward surge of the membrane current under the voltage-clamp conditions was slightly shorter in the narcotized node than in the node in low-Na Ringer. Nevertheless, the membrane conductance at the peak of activity was found to behave in essentially the same manner in a weakly narcotized node as in the node treated with low sodium (Fig. 8, right).

Discussion

Some of the implications of the experimental results reported in the present paper have already been discussed under Results. The finding that the effects of refractoriness, weak narcosis and low Na are very similar suggests that these three agents exert their effects upon the same mechanism in the process of action potential production. It has been postulated that the gradual fall in the "membrane e.m.f." during activity is due to some chemical product generated in the membrane (14). It is not difficult to understand the similarity between the effects of these three agents from this point of view. In the sodium theory (6), the peak potential of the response is determined primarily by the concentration of Na in the medium. Although the present observation on the effect of low Na agrees well with the observation by the British investigators, it seems to us very difficult to reconcile our results on the refractoriness and on narcosis with their theory.

The oscillation of the membrane current under the voltage-clamp conditions seems to present further difficulty to the sodium theory. The mathematical term called the "sodium inactivation" evidently varies too slowly to account for the oscillatory membrane current. According to this theory, the oscillatory behavior has to be attributed to the imperfection of our voltage clamp. Admitting such imperfection, it still seems hard to us to explain the time courses of these rapidly changing membrane currents in terms of this theory. Our interpretation based on the two-stable state hypothesis has been presented under Results. We attribute this phenomenon to the variation in the active area, or in the number of active patches, of the membrane. The concept of active patches has been postulated also by del Castillo and Suckling (1) to explain their observation on the subthreshold response.

Another observation deserves further discussion—that is about the intensity of the membrane current under the voltage clamp conditions. In the examples shown in Figs. 3, 4 and 5, it is seen that, when the membrane potential, V, is maintained at a level of about 30 mV., a strong current flows

through the node. Estimating the resistance in series with node N_1 under study to be about 70 megohms, the membrane current corresponding to the I·R value of 2 V. is found to be about 3×10^{-8} ampere or slightly more. This current is approximately 10 times as strong as the action current through the nodal membrane under ordinary experimental conditions. The maximum value of the inward membrane current is increased considerably by a preliminary anodal polarization of the node or, in the case of a break excitation, under the voltage-clamp conditions.

When the intensity of the membrane current mentioned above is divided by the estimated area of the nodal membrane (roughly 3×10^{-7} cm.^{2}), one is surprised by the high density of the membrane current which a node can take without bringing about any irreversible change in the state of the node. It is the order of 100 mA./cm.^{2}. It has been pointed out under Results that, in a node subjected to such a strong inward current (*i.e.*, in a node under anodal polarization or in a node clamped at about 30 mV.), the "membrane e.m.f." decays slower than in a node traversed by a weaker membrane current.

Summary

1. So-called voltage-clamp experiments were carried out on single node preparations of the toad. The current-voltage relationship (Fig. 4) was similar to that determined on the squid giant axon, except for the greater steepness of the intermediate, labile portion of the current-voltage curve (shown by dotted line in Fig. 4).

2. The membrane conductance at the peak of activity determined by this technique was approximately 10 times as great as that of the resting nodal membrane.

3. When the membrane potential was maintained at a level slightly above the ordinary threshold, the membrane current underwent rapid vibratory changes.

4. The similarity between the effect of reducing the sodium concentration in the medium and the effect of refractoriness and of weak narcosis was stressed. When the action potential was reduced in size to the same extent by these three different agents, the membrane current under voltage-clamp behaved in a similar manner regardless of the agent used.

Addendum. Since submission of the present paper, three short reports which are relevant to the present subject have appeared. On the voltage clamp of the node, see Frankenhaeuser and Person, *Acta physiol. scand.*, 1957, *42*, Suppl. 145, and del Castillo *et al.*, *Nature, Lond.*, 1957 (Dec. 7), *180:* 1290. On the oscillatory membrane current in the squid axon under voltage clamp, see Tasaki and Bak, *Science*, 1957 (Oct. 11), *126:* 696. The synopsis of the work reported in this paper was presented in Symposium on Myelin held on June 15, 1957, sponsored by Korey (see *Progress in Neurobiology*, III).

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