ADAPTATION OF CUTANEOUS TACTILE RECEPTORS

VI. INHIBITORY EFFECTS OF POTASSIUM AND CALCIUM*

By HUDSON HOAGLAND

(*From the Physiological Laboratory, Clark University, Worcester*)

I

In previous papers in this journal evidence has been presented indicating that potassium released from cutaneous cells of frog's skin, when the skin is stimulated by pressure, produces a reversible failure of responsiveness of the free nerve endings serving as tactile receptors anastomosing among the cutaneous cells. Fig. 1 shows schematically an antidromic single axon (*cf.* Adrian, Cattell, and Hoagland, 1931). In brief, the evidence for the potassium hypothesis of adaptation is based principally on the following findings.

The cutaneous cells are rich in potassium (Rubin, 1936). Isotonic KCl solutions applied to the underside of the skin containing a single axon ending greatly hastens the rate of failure of the ending to respond to an interrupted jet of air impinging on the outer side of the skin. Washing with Ringer's solution results in recovery from adaptation, both when this is produced by the localized repetitive air jet stimulation and when the adaptation had been hastened by washing with a solution of isotonic KCl (Hoagland, 1934).

Adaptation is not produced by electrically initiated antidromic impulses back-fired over the axon branches and, therefore, is not the result of the activity of the axon branches *per se*. The time curves showing production of and recovery from adaptation are inconsistent with any known properties of peripheral nerve. Intermittent air jet stimulation of a region of skin several millimeters distant from a responsive single ending produces failure of response of the ending to a similar direct intermittent stimulus applied to the skin area containing the ending immediately afterward.

This indicates the passage of an inhibitory humor from the first region not

943

supplied by the particular axon under investigation to the second stimulated region containing the axon ending (Hoagland, 1935 a). When freshly removed pieces of skin are mounted in front of the interrupted air jet and stimulated, and at the same time are washed with Ringer's solution, it is found that these washings are always richer in potassium than are similar washings made without the air jet stimulation. Potassium is thus shown to come out of the stimulated and washed skin but does not do so when the skin is merely washed but not stimulated. Calcium is not found to be washed out of the "beaten" skin (Hoagland and Rubin, 1936).

These experiments indicate that the failure of the endings is due to raising the concentration of potassium, K_o, *outside* of the axon branches, which thus reduces their excitability by lowering the ratio of potassium inside the nerve to that outside, (K_i/K_o). These ideas have been extended to account for adaptation in other mechano-receptors (*cf.* Hoagland, 1935 b).
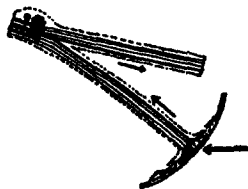

FIG. 1. Arrangement of nerve fibers giving the antidromic discharge. Leads are placed on the dorsal cutaneous nerve cut at its entrance to the skin. The antidromic touch spot is usually located ventrally. The bifurcating fiber branches in the dorsal root ganglion.

II

In the present experiments an attempt has been made to determine the effects of applied Ringer solutions rich in K or Ca on the responsiveness of single axon endings. The amount of K in frog's nerve has been shown to be of the order of 188 ± mg. per cent (Fenn, Cobb, Hegnauer, and Marsh, 1934). This is about fifteen to twenty times greater than the K content of frog plasma. Accordingly a solution was made up consisting of Ringer's solution plus fifteen times the normal K content. If this solution could be applied directly to the endings it should reduce the K_i/K_o ratio substantially, and hence inhibit the excitability. Even if the cutaneous cells surrounding the axon endings prevented the K from reaching them at this concentration some decline in responsiveness of the endings might be expected. Conceivably, pressure on the skin could release enough potassium from

cells in intimate proximity to the axon branches (Hoagland and Rubin, 1936) to reduce the concentration ratio, K_i/K_o, to unity.

To compare the effects of Ca on the responsiveness of the axon branches, solutions of Ringer plus fifteen times the normal Ca content were also used. It seemed desirable to test the effect of excess of Ca on adaptation directly, despite the finding that Ca is not washed out of the skin when it is "beaten" with the air jet (Hoagland and Rubin, 1936). We were not interested in sodium effects since excessive amounts of Na in Ringer have not been found to produce appreciable effects on adaptation. Frog plasma contains 239 mg. per cent of Na (Fenn, Cobb, Hegnauer, and Marsh, 1934) and frog skin 91 mg. per cent of Na (Rubin, 1936). Therefore, we should not expect that the high local plasma content of Na surrounding a free-ending axon would be appreciably augmented by excretion of Na from epithelial cells under deforming pressure.[1]

The antidromic preparation was similar to that used in previous experiments (Hoagland, 1934). The air jet was arranged to impinge upon a single "antidromic spot" in the skin (cf. Fig. 1) of a completely pithed frog. Short slits were cut in the skin above and below the spot, about a centimeter apart, and a bulbed pipet was used for passing the solutions across the underside of the skin. The single axon impulses set up in response to the interrupted air jet stimulus were recorded optically and audibly by means of a Matthews oscillograph and loud speaker.

The procedure consisted of passing normal Ringer's solution across the underside of the skin and then stimulating with the air jet at a pressure of approximately 400 mm. of Hg, with the nozzle clamped rigidly 5 to 10 mm. above the spot. The frequency of stimulation, 140 per second, was regulated by a notched disc rotating between nozzle and skin. Owing to the large number of adaptations measured it was not expedient to photograph the impulses. Instead, the time to failure of impulses was taken with a stop-watch by listening to the loud speaker and checking the audible recording by observing the impulses on a standing wave screen. Occasionally photographic verifications were made of the reliability of this method.

---

[1] Talaat (1933) studied the effect of ions on the excitability of the axon endings in frog's skin. He found adaptation to be greatly retarded if Ca were removed from the Ringer's solution. Citrate and oxalate in Ringer's bathing the skin prolong the discharge by removing Ca. These experiments have little bearing on the present studies since they involved removing ions from the bathing fluids and testing the effects on axon excitabilities. In the present experiments bathing solutions were made with ionic properties similar to those called for in conformity with the hypothesis that adaptation may be due to the release of ions from cutaneous cells to plasma under pressure. The solutions, therefore, contained certain ions in excess of those in Ringer's solution. In Talaat's experiments the removal of Ca might be expected to alter profoundly the excitabilities of nerve membranes quite independently of the K_i/K_o effect.

When adaptation had occurred the air was turned off and exactly 75 seconds were allowed for recovery (Hoagland, 1934). During this time the underside of the skin containing the spot was again washed with Ringer's solution. In each case about 8 cc. of solution were used at each washing. At the conclusion of the rest period the stimulus was again applied and the adaptation was measured again. In this way it was possible, by obtaining successive readings, to determine the time to adaptation for a single axon in a region of skin bathed in Ringer's solution.

Using this procedure the solutions containing excess K or excess Ca were applied from time to time and the effects of these solutions on the time to adaptation were measured. Regardless of which of the three solutions was used, 75 second rest intervals were always allowed to elapse between successive stimulations.

Figs. 2 and 3 show the results of typical experiments. The total time intervening from the beginning of the experiment to a given adaptation determination is plotted as abscissa against the time to adaptation of each successive stimulation as ordinate. The figures are labeled to show which of the three solutions—Ringer's, Ringer's with (15 × K), or Ringer's with (15 × Ca)—were used to wash the skin in the 75 second rest interval prior to the succeeding adaptation determination.

Only about 20 per cent of some hundred single ending preparations isolated in these experiments showed sufficient durability for our purposes. It is necessary for an ending and its nerve to survive for at least an hour and to give, when the skin is bathed in Ringer's solution, approximately a constant adaptation time. In all of the experiments it was necessary to place the dorsal cutaneous nerve used for recording in a moist chamber. This was done by packing Ringer-soaked cotton around the nerve as it emerged from the frog's back and passed to a glass tube containing the lead electrodes. The reasons for failure of the endings are unknown. Usually the failure is abrupt, all responsiveness suddenly disappearing. In some cases the nerve fiber in the region of the electrodes clearly failed since the impulses gradually decreased in amplitude, although the ending gave normal responses to the intermittent stimulus. Some of the tested endings showed considerably more variation in their normal adaptation times than those shown in the published figures. Qualitatively, however, they bore out the results depicted in the figures.

The following generalizations may be made from Figs. 2 and 3:

1. Bathing the skin in solutions of Ringer plus fifteen times the normal K content greatly hastens subsequent adaptation. The depression of excitability is usually immediate. Subsequent applica-
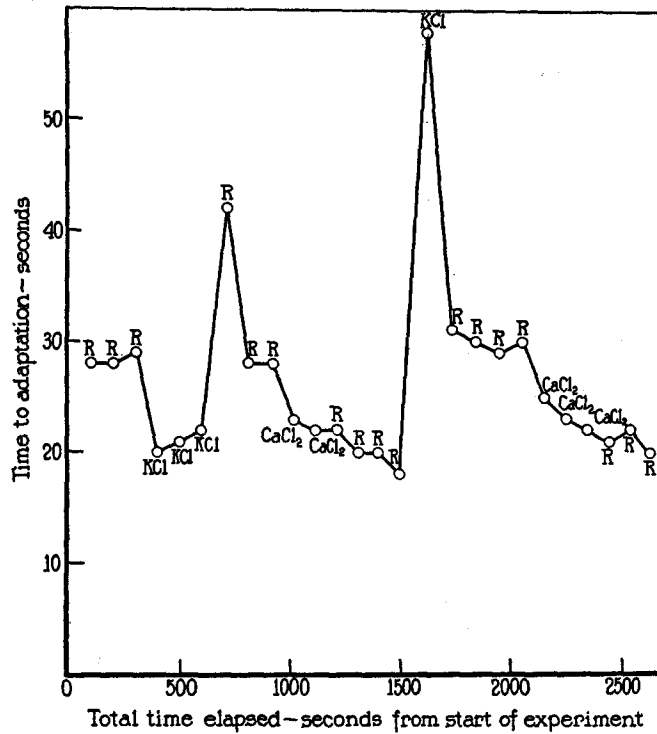

FIG. 2. Plot showing the effect on the excitability of a single axon ending to solutions of Ringer, of Ringer plus fifteen times the normal K content, and of Ringer plus fifteen times the normal Ca content. The points on the figure are accordingly labeled R, KCl, and CaCl₂ to indicate which solution was used to bathe the skin prior to that particular adaptation determination.

For further discussion see text.

tions of the K rich solution do not cause appreciable decrements in the time of adaptation.

2. Washing with Ringer's solution not only removes the inexcitability of the ending produced by K but usually produces a compensatory rebound in the ending's responsiveness; the ending reacts for a

longer time to the same stimulus after K depression and recovery produced by washing with Ringer's than it normally does.

3. Application of solutions containing fifteen times the amount of Ca in Ringer's solution also produces a depression of responsiveness corresponding in magnitude roughly to the K depression. The Ca depression, however, is usually not produced so rapidly, several suc-
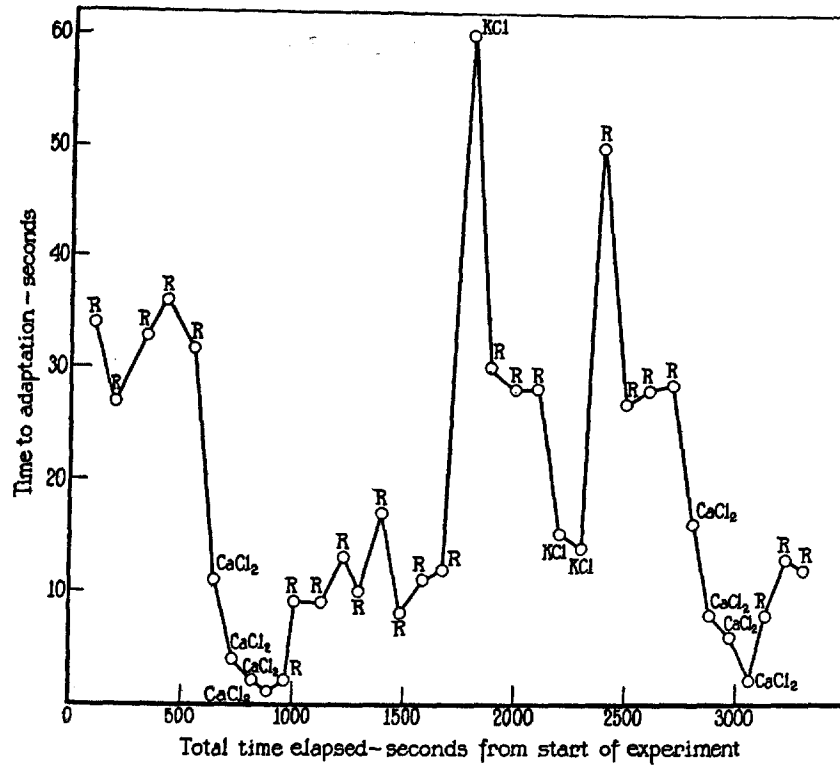

FIG. 3. See legend to Fig. 2.

cessive applications showing progressive increments in the decline of the response.

4. Washing with Ringer's solution produces either very little (Fig. 3) or no recovery (Fig. 2) of endings made less responsive with solutions containing an excess of Ca. This irreversibility in Ringer's of Ca depression is sharply in contrast with the depression produced by

K which is reversible in Ringer's. It suggests that different mechanisms are involved in the depressive actions of the cations. Talaat (1933), it should be recalled, found that absence of Ca greatly prolongs the time to adaptation.

5. When solutions of 15 × K are applied after depression produced by solutions containing 15 × Ca, an immediate recovery occurs from the Ca depression. In this case there is also a compensatory rebound, the response lasting considerably longer than normal. This antagonistic recovery is especially interesting owing to the inhibitory action of solutions of 15 × K when applied to the endings without being preceded by the Ca solution.

6. When depression of responsiveness of an ending is produced by a solution of Ringer's plus 15 × K, the application of a solution of Ringer's plus 15 × Ca does not produce recovery as described in the foregoing paragraph. This effect is not shown in the published figures but was clearly seen in several of the preparations yielding the above typical results.

III

The inhibitory K effect illustrated by the foregoing experiments is quite consistent with what one would expect in connection with the potassium mechanism of adaptation reviewed at the beginning of this paper. The fact that Ca also inhibits the responsiveness of the endings suggests the possibility that this substance may normally be involved in adaptation since cutaneous cells not only store K (132 mg. per cent) but also Ca (302 mg. per cent) (Rubin, 1936), and conceivably Ca might be released when pressure is applied to the skin and thus inhibit the endings.

There are, however, several reasons which make it seem highly improbable that Ca is normally involved in adaptation. Hoagland and Rubin (1936) showed that Ca is not washed out of the skin in detectable amounts when the frog's skin is beaten with the air jet. They found, however, appreciable quantities of K released under these conditions. The K inhibitory effect occurs rapidly and apparently completely after one application of the K rich solution, while the Ca inhibition is usually slower in developing, taking distinctly longer to produce adaptation than does a comparable amount of K. Probably

the most interesting fact is that the Ca inhibition is essentially irreversible in Ringer's solution. This irreversibility is sharply in contrast with the abrupt reversibility of both the normal recovery from adaptation and recovery from adaptation hastened by the addition of K rich solutions. In view of these facts it would seem justifiable to conclude that Ca is not normally involved in the mechanism of adaptation of the free ending receptors. At present there seems to be no satisfactory explanation of the rebound of recovery above the normal level shown in the figures.

SUMMARY

1. Both solutions of Ringer plus fifteen times the normal K content, and solutions of Ringer plus fifteen times the normal Ca content markedly hasten the adaptation of single freely branching axon endings in frog's skin to repetitive air puff stimuli.

2. The K effect is produced more rapidly than is that of Ca. The K effect is reversible by washing with Ringer's solution, while the Ca effect is not. The Ca inhibition can, however, be reversed and recovery effected by washing with K rich solutions.

3. Evidence is discussed which indicates that Ca probably plays no rôle in normal adaptation, and the experiments are interpreted as substantiating the hypothesis of adaptation due to K.

CITATIONS

Adrian, E. D. Cattell, M., and Hoagland, H., *J. Physiol.*, 1931, **72**, 376.

Fenn, W. L., Cobb, D. M., Hegnauer, A. H., and Marsh, B. S., *Am. J. Physiol.*, 1934, **110**, 74.

Hoagland, H., *J. Gen. Physiol.*, 1934, **18**, 255.

Hoagland, H., *J. Gen. Physiol.*, 1935 *a*, **19**, 221.

Hoagland, H., Pacemakers in relation to aspects of behavior, New York, The Macmillan Company, 1935 *b*.

Hoagland, H., and Rubin, M. A., *J. Gen. Physiol.*, 1936, **19**, 939.

Rubin, M. A., *J. Gen. Physiol.*, 1936, **19**, 935.

Talaat, M., *J. Physiol.*, 1933, **79**, 500.