INCREASED IRRITABILITY IN NITELLA DUE TO GUANIDINE

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(Received for publication, May 21, 1942)

Guanidine[1] produces striking effects on animals which find a parallel in *Nitella*. This is the more interesting since these effects in animals have been attributed to nervous activity involving the action of acetylcholine.

The experiments on *Nitella* were undertaken because it appeared that loss of excitability and of the potassium effect (ability to distinguish electrically between K^+ and Na^+) in distilled water was due to the washing out of a substance which was called for convenience *R*. In some cases excitability remained after the potassium effect was lost and it was then found that the action current could restore the potassium effect.[2] Since the action current presumably carried cations from the sap into the surface it appeared possible that *R* contained organic cations. Accordingly experiments were made with guanidine since it can supply such cations, even at high pH, as it is a strong organic base.

The parallel phenomena may be considered under two heads.

1. Restoration of Excitability.—In cases of myasthenia gravis the muscles may lose their excitability so that the patient is unable to move even an eyelid. Dosage with guanidine may then produce great improvement of the excitability and use of the muscles.[3]

When cells of *Nitella* have lost their excitability as the result of exposure to distilled water it may be restored by guanidine: this is also true of the potassium effect.[4]

2. Hyperexcitability.—Guanidine may cause fibrillary tremors and tonic contractions of skeletal muscles.[3,5] The threshold for electrical stimulation necessary to cause muscular contraction when the stimulus is applied to the nerve is lowered.[5,6] It has been suggested as the result of experiments on dogs that guanidine sensitizes the muscle to the action of acetylcholine.[7] A similar suggestion comes from studies on myasthenia gravis.[3]

[1] For convenience this term will be used to include guanidine, methyl guanidine, and di-methyl guanidine.

[2] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1934–35, **18**, 681.

[3] Minot, A. S., Dodd, K., and Riven, S. S., *Science*, 1938, **87**, 348, and the literature there given.

[4] Osterhout, W. J. V., *J. Gen. Physiol.*, 1940–41, **24**, 7.

[5] Paton, D. N., and Findlay, L., *Quart. J. Exp. Physiol.*, 1916, **10**, 315.

[6] Frank, E., Stern, R., and Nothmann, M., *Z. ges. exp. Med.*, 1921, **24**, 341.

[7] Frank, E., Nothmann, M., and Guttmann, E., *Arch. ges. Physiol.*, 1923, **201**, 569.

Guanidine also produces hyperexcitability in *Nitella*. This is evident since (*a*) the threshold of electrical excitability[8] may be lowered and (*b*) a single electrical stimulus may produce a long series of quick action currents,[9] which is perhaps analogous to the fibrillary tremors of muscle under the action of guanidine.

There is no reason to suspect that acetylcholine plays a rôle in *Nitella* since the application of acetylcholine produces no change in P.D. and does not act as a stimulus. It would therefore seem that in *Nitella* guanidine sensitizes the protoplasm directly to the electrical stimulus.

A typical response to a single stimulus in an untreated cell is seen in Fig. 1.[10] But when cells are treated with guanidine a stimulus instead of producing a single response may give the result seen in Fig. 2. To explain such curves let us consider the role of K^+ in producing P.D.[11] The normal outwardly directed (positive) P.D. is presumably due chiefly to the outwardly directed concentration gradient[12] of K^+ across the inner protoplasmic surface, called for convenience Y (the aqueous part of the protoplasm may be called W and the outer non-aqueous surface X).

This gradient presumably disappears[13] when K moves outward into W as the result of the stimulus.

[8] The electrical stimulus consisted of 100 to 500 mv. D.C. applied at a distance of 1 cm. or more from the spot recorded, as described in previous papers.

[9] Such series of action currents are frequently propagated along the cell. They are more apt to occur in cells that have been kept a long time in the laboratory.

[10] The cells, after being freed from neighboring cells, stood in the laboratory at 15° ± 1°C. in Solution A (*cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17,** 87) for several days. They belonged to Lot B (*cf.* Hill, S. E., and Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1938, **24,** 312).

The measurements were made on *Nitella flexilis*, Ag., using the technique described in former papers (Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937–38, **21,** 541). Temperature about 20–26°C. Regarding the amplifier see the reference just cited.

There was no indication of injury in these experiments.

[11] Strictly speaking they may be said to depend on the movement of ions in general but the effect of K^+ is so predominant that we may, for convenience, confine the discussion to it.

[12] Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18,** 215.

[13] Cole and Curtis (Cole, K. S., and Curtis, H. J., *J. Gen. Physiol.*, 1938–39, **22,** 37) have shown that the outwardly directed P.D. begins to fall off before the increase in the conductivity of the protoplasm begins. This might be brought about in a variety of ways, *e.g.* by a decrease in the value of $u_K \div v_{Cl}$, as occurs, for example, in *Valonia* under the influence of various organic substances involving no loss of resistance: such substances might be produced as the result of stimulation. *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1936–37, **20,** 13; *J. Cell. and Comp. Physiol.*, 1941, **18,** 129.

Under normal conditions K^+ presumably moves out through Y, producing the initial rise in the curve (spike) seen in Fig. 1. When the outwardly moving

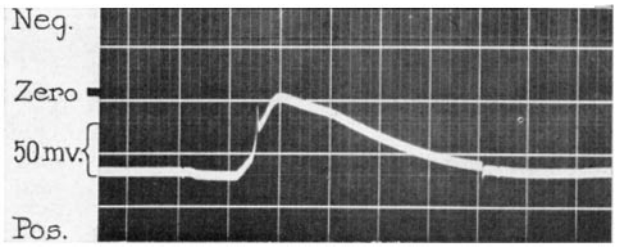

FIG. 1. Action curve in an untreated cell, recorded between two points, D and F, both in contact with Solution A (owing to a block the curve is monophasic).

The cell was freed from neighboring cells and kept in Solution A at 15 ± 1°C. for 50 days. The record was made at 22°C.

Heavy time marks 5 seconds apart.

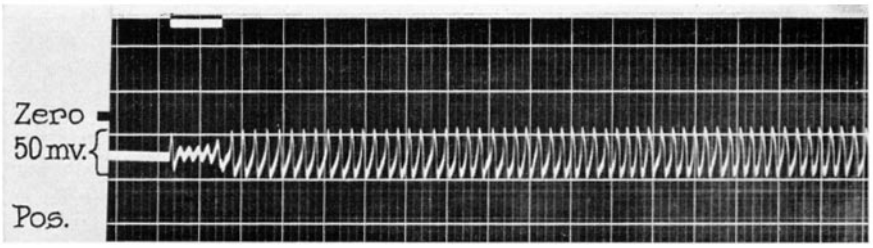

FIG. 2. Result of a stimulation after an exposure of 3 hours to 0.01 M guanidine hydrochloride. During the passage of the stimulating current (shown by the white signal line above) the amplitude of the responses remained small but it subsequently increased and became steady (for about 7 minutes) and then the responses ceased abruptly. These quick action currents were propagated along the cell.

The cell was freed from neighboring cells and kept in Solution A for 50 days at 15 ± 1°C. It was then kept at 22°C. for a short time and exposed to the reagent at this temperature.

The spot recorded was in contact with Solution A and was connected through the galvanometer to a spot at the end of the cell in contact with Solution A saturated with chloroform which kept its P.D. constant approximately at zero and made the record monophasic.

Heavy time marks 5 seconds apart.

K^+ reaches the outer non-aqueous surface layer X it sets up an outwardly directed concentration gradient of K^+ and an outwardly directed P.D., causing a fall of the curve. This is reversed as K^+ reaches the outside of X and thus destroys the outwardly directed concentration gradient of K^+ across X.

Hence the curve rises. Then recovery sets in: this consists in restoring to the sap the K^+ which has moved outward during the action current.

Experimental evidence favoring this explanation is found in cases where the sensitivity of *X* to K^+ can be altered. Thus in *Nitella* we can make *X* insensitive to K^+ by leaching with distilled water. We then find, as expected, only one peak in the action curve.[14] Conversely in *Chara* where *X* is normally insensitive to K^+ and the action curve has but one peak we find, as expected, a double peak when *X* is made sensitive to K^+ by means of guanidine.[15]

It may be added that in *Valonia*[12] the movement of K^+ appears to produce effects resembling those seen in the action curve of *Nitella*.

This discussion applies to the normal procedure in which K^+ is supposed to move outward into the cellulose wall or the external solution. Recovery is then relatively slow since it involves the return to the sap of the K^+ which has moved out.[16] But if K^+ moves outward only a very short distance, *i.e.* just outside *Y*, it is evident that recovery could be much quicker. In that case the curve would have but one peak since K^+ would not reach[17] *X*. We might then get such curves as are seen in Fig. 2. Whether the penetration of guanidine could accomplish this is an interesting question.

The action curves occurring under the influence of guanidine show interesting features.[18] Among these are the following.

1. In many cases bursts of action currents alternated with periods of rest (Figs. 3 and 4) (this is also seen in some untreated cells[19]). Similar phenomena have been observed in untreated nerve, *e.g.* by Adrian[20] and by Hoagland.[21]

2. The action curve may seem to go below the base line which records the

[14] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1939–40, **23,** 743.

[15] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1940–41, **24,** 9.

[16] This movement of K^+ is presumably due to the forces which in the resting state of the cell cause K^+ to move from the external solution to the sap.

[17] If the curve did not drop abruptly after reaching the apex of the spike we should not have a double peak. This abrupt fall of the curve can occur only if the outwardly moving K^+ reaches *X* in the form of a fairly sharp moving boundary. This might be interfered with by protoplasmic motion which is usually present in *Nitella*.

If *W* already contains much K or guanidine ion the movement of K into *W* may not greatly increase the outwardly directed P.D. across *X* and hence may not cause much drop in the curve after the apex of the spike. In that case the first peak will be inconspicuous or lacking.

[18] Some of these have also been observed under the influence of NaCl or in cells which have stood for a long time in the laboratory in Solution A. Regarding these see Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18,** 377; 1938–39, **22,** 91. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1934–35, **18,** 499.

[19] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1934–35, **18,** 512 (Fig. 15).

[20] Adrian, E. D., The basis of sensation, London, Christophers, 1928.

[21] Hoagland, H., *J. Gen. Physiol.*, 1932–33, **16,** 695, 715.

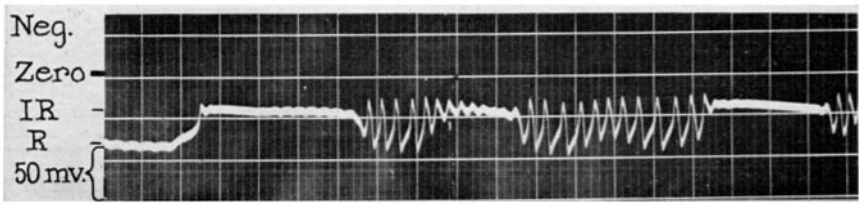

FIG. 3. At the start the curve (*R*) shows the usual (complete) resting value of the P.D.; then a spontaneous action curve is seen. No recovery occurs for about 20 seconds and during this interval the level of the curve may be called the "incomplete resting stage" (*IR*). It might be mistaken for the complete resting stage if we had not seen the rise of the curve: the subsequent quick action curves might then be erroneously regarded as carrying the curve below the complete resting stage (*i.e.* making the P.D. more positive than in the normal resting condition).

The record was made between two points, *C* and *F*, both in contact with Solution A (owing to a block the record is monophasic). The activity was spontaneous; *i.e.*, no stimulus was applied.

The cell was exposed for 117 minutes to 0.01 M guanidine hydrochloride at pH 5.

The cell was freed from neighboring cells and kept in Solution A for 30 days at 15 ± 1°C. The record was made at 22°C.

Heavy time marks 5 seconds apart.

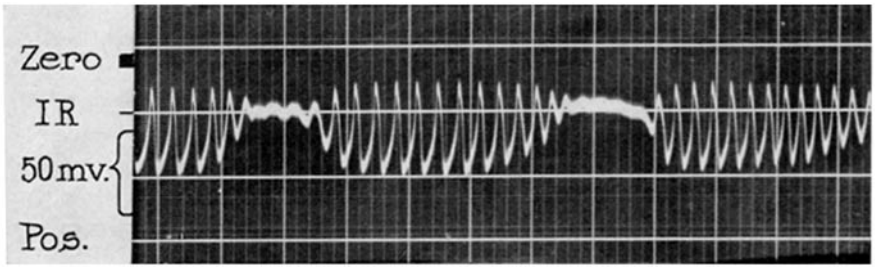

FIG. 4. Shows a regular wax and wane of the amplitude of the action curve in a cell exposed for 4 hours to 0.01 M guanidine hydrochloride at pH 7. The record was made between two points, *D* and *F*: *D* was in contact with Solution A and *F* was in contact with 0.01 M KCl which kept the P.D. constant approximately at zero and made the record monophasic.

Before and after the "wax and wane" phase the curve is at an incomplete resting stage (*IR*) as in Fig. 3. The activity was spontaneous; *i.e.*, no stimulus was applied.

The cell was freed from neighboring cells and kept in Solution A for 50 days at 15 ± 1°C. The record was made at 22°C.

Heavy time marks 5 seconds apart.

P.D. of the cell at rest but it is probable that in such cases the base line does not represent the true resting potential. We may distinguish between the resting potential *R* found after complete recovery and the incomplete resting potential,

IR, found after incomplete recovery (*i.e.* when some of the K^+ which moves from the sap into *W* during the action fails to go back into the sap during recovery and remains in *W* to set up an outwardly directed P.D. across *X*). For

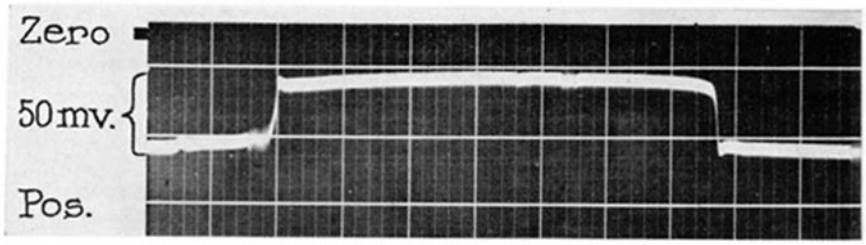

FIG. 5. Shows a "square topped" action curve in a cell exposed for 140 minutes to 0.01 M guanidine hydrochloride at pH 7. The action curve is recorded between two points, *E* and *F*, both in contact with Solution A (owing to a block the action curve is monophasic). The activity was "spontaneous;" *i.e.*, no stimulus was applied.

The cell was freed from neighboring cells and kept in Solution A for 30 days at 15 ± 1°C. The record was made at 22°C.

Heavy time marks 5 seconds apart.

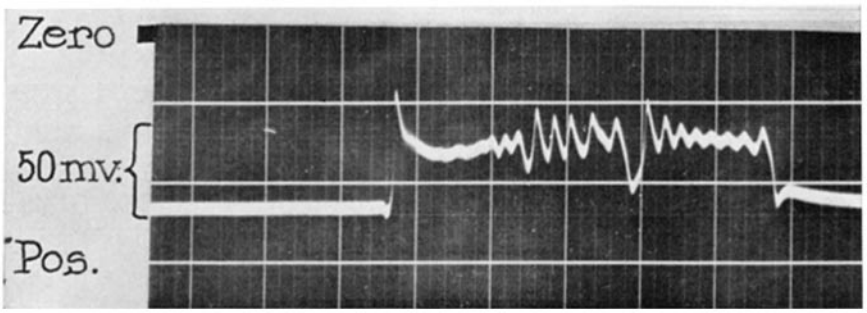

FIG. 6. Transition to "square topped" action curve in a cell exposed for 61 minutes to 0.01 M guanidine hydrochloride at pH 7. The record was made between a spot *D* in contact with Solution *A* and another spot *F* in contact with 0.01 M KCl which kept the P.D. constant approximately at zero and made the record monophasic. The activity was spontaneous; *i.e.*, no stimulus was applied.

The cell was freed from neighboring cells and kept for 30 days in Solution A at 15 ± 1°C. The record was made at 22°C.

Heavy time marks 5 seconds apart.

example, in Fig. 3 we see at the start a base line which may be regarded as representing the complete resting potential *R*. But later we see an incomplete resting potential, *IR*. When recovery becomes more complete the curve goes below the *IR* level.[22]

[22] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1934–35, **18,** 499; Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938–39, **22,** 91.

3. Action curves not going to zero. The observed P.D. in the resting state is presumably due chiefly to the concentration gradient of K^+ across V and when this disappears during the action the P.D. vanishes and the curve goes to

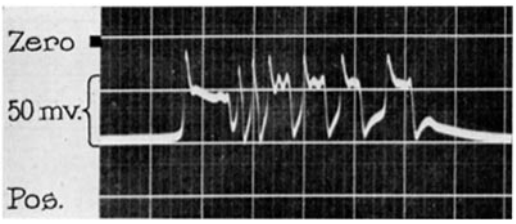

FIG. 7. Transition to "square topped" action curves in a cell exposed for 61 minutes to 0.01 M guanidine hydrochloride at pH 7. The record was made between two points, D and F: D was in contact with Solution A and F with Solution A saturated with chloroform which kept the P.D. at F constant approximately at zero and made the record monophasic. The activity was "spontaneous;" *i.e.*, no stimulus was applied.

The cell was freed from neighboring cells and kept in Solution A for 30 days at 15 ± 1°C. The record was made at 22°C.

Heavy time marks 5 seconds apart.

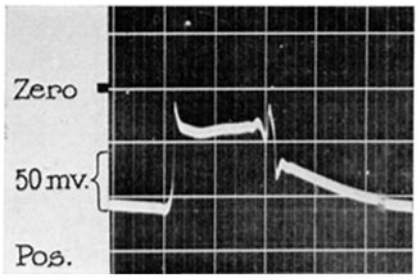

FIG. 8. Transition to "square topped" action curve in a cell exposed for 53 minutes to 0.01 M guanidine hydrochloride at pH 7.0. The record was made between two points, D and F, with Solution A at D and Solution A saturated with chloroform at F which kept the P.D. at F constant approximately at zero and made the record monophasic. The activity was spontaneous; *i.e.*, no stimulus was applied.

The cell was freed from neighboring cells and kept in Solution A for 30 days at 15 ± 1°C. The record was made at 22°C.

Heavy time marks 5 seconds apart.

zero unless at the end of the upward movement (spike) of the curve some effective cations (K^+, Na^+, or guanidine ions[23]) remain in W to set up an out-

[23] The apparent mobility of the guanidine ion in the outer protoplasmic surface exceeds that of Na^+ and this may be true of the non-myelinated nerve of the spider crab since, according to Wilbrandt (Wilbrandt, W., *J. Gen. Physiol.*, 1936–37, **20,** 519), the guanidine ion acts somewhat like K^+.

wardly directed concentration gradient across X. Then there will be an outwardly directed P.D. across X and the curve will not go to zero. Several of the figures show this condition.[22]

4. Wax and wane in the amplitude of the action curves (Fig. 4). From what has just been said we may infer that a rise in the level of the apices of successive spikes indicates a decrease[22] in the concentration of effective ions (K^+, Na^+, and guanidine ions) in W at the end of the spike. When the apices fall to lower and lower levels the opposite is indicated.

The presence of such ions in W at the end of the downward movement of the curve would prevent complete recovery and a decrease in such ions would make recovery more complete. Hence the increasing vertical amplitude in Fig. 4 might be explained as indicating a decrease and the wane as indicating an increase in the concentration of such ions in W.

5. Recovery is sometimes sudden, giving "square topped"[24] action curves as in Fig. 5.

It has been suggested[25] that recovery involves two operations, (*a*) the return from W to the sap of the K^+ which comes out of the sap during the action and (*b*) the healing of breaks in Y (such breaks may account for some of the increase in permeability which accompanies the action). If (*a*) occurs before (*b*) no recovery will occur until (*b*) is complete: the latter (healing of breaks)[26] might occur suddenly which can hardly be the case with (*a*). Various "transitions" to "square topped" action curves are seen in Figs. 6, 7, and 8.

It may be added that great variation in the response to the action of guanidine was observed and in some cases no response was obtained.

DISCUSSION

In animals guanidine lowers the E.M.F. necessary to produce stimulation and sets up trains of action currents. These effects are also seen in *Nitella* but here there appears to be no reason for involving the action of acetylcholine as has been done with animals. When we apply acetylcholine to *Nitella* there is little or no effect and there is no evidence that it plays any part in stimulation. Even in animals it is doubtful whether guanidine acts by affecting sensitivity

[24] See Osterhout, W. J. V., and Hill, S. E., Some ways to control bioelectrical behavior, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1936, **4,** 47 (Fig. 3).

[25] Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938–39, **22,** 91. Osterhout, W. J. V., *J. Gen. Physiol.*, 1938–39, **22,** 417. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1938–39, **22,** 115.

[26] The production and healing of "breaks" due to surface tension may be sudden, as indicated by experiments with oily films. *Cf.* Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927–28, **11,** 684; Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18,** 221.

to acetylcholine. It has been suggested that guanidine affects potassium metabolism.[27]

Guanidine may produce such effects to some extent by increasing the conductivity of the aqueous layer of the protoplasm[28] (*W*) as previously suggested in connection with the action of NaCl[29] in producing long trains of quick action currents. If we may judge by experiments on *Valonia*[30] it penetrates readily.

The shapes of the action curves might also be accounted for to some extent by the increased conductivity of *W* as discussed in a previous paper in connection with NaCl.[29] It may be noted that according to Fühner[31] guanidine acts somewhat like NaCl on frog muscle.

The increased conductivity of the aqueous protoplasmic layer *W* would permit the same current density to be attained with a lowered value of the applied E.M.F.

In addition guanidine may have specific effects as when it restores irritability and the potassium effect in *Nitella*.[32]

SUMMARY

Guanidine applied to *Nitella* may lower the threshold of E.M.F. required to produce electrical stimulation and may give rise to trains of action currents. Its effect thus appears to be somewhat similar to that observed in animals.

Rapid action currents are produced as well as "square topped" action curves and transitional forms.

These effects may be due in part to increased protoplasmic conductivity produced by the penetration of guanidine.

[27] Thompson, V., and Tice, A., *J. Pharm. and Exp. Therap.*, 1941, **73,** 455.

[28] The resistance of the protoplasmic layer as measured is chiefly due to *X* and *Y* and hence might not be much affected by guanidine.

[29] Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938–39, **22,** 91.

[30] Osterhout, W. J. V., Damon, E. B., and Jacques, A. G., *J. Gen Physiol.*, 1927–28, **11,** 193.

[31] Fühner, H., *Arch. exp. Path. u. Pharm.*, 1908, **58,** 1.

[32] Guanidine is able to denature proteins. *cf.* Greenstein, J. P., *J. Biol. Chem.*, 1939, **130,** 519. Mirsky, A. E., *J. Gen. Physiol.*, 1940–41, **24,** 709.