THE MODE OF ACTION OF NERVES ON UNSTRIATED MUSCLE

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The views regarding the mode of action of nerves at their endings are well known. In the present research, an attempt has been made to determine the mode of action of nerves on unstriated muscle. Singh (1938 *a*) has shown that unstriated muscle shows two kinds of contractions. One kind is produced by alternating current and spontaneous activity (Singh, 1939), and the other by addition of substances to smooth muscle from without. If nerves act by producing a chemical substance outside the muscle fibres, then the resulting contraction would be of the second kind. Narayana and Singh (1944) found that in the dog's stomach, the calcium required for contractions produced by acetylcholine or by vagus stimulation, was more (0·005–0·01 *M* $CaCl_2$) than that required for the contraction produced by alternating current (0·002 *M* $CaCl_2$). The significance of this finding was not then realised; later on it was found to be a feature when the contraction belonged to the potassium group (Singh, 1945), thus suggesting that the contraction of the dog's stomach produced by stimulation of the vagus nerve was due to the secretion of a substance outside the muscle fibres, presumably acetylcholine.

Experimental

The muscle used in these experiments was from the stomach of the frog *Rana Tigrina*. Two kinds of muscle nerve preparations were made. In one, the entire stomach tied at the two ends, was suspended in a bath and the mesentery placed on a pair of electrodes; this recorded the contractions of longitudinal fibres. In the other, the two nerves supplying the anterior and posterior surfaces of the stomach were dissected down to a common segment which was then cut out transversely and then bisected longitudinally at the greater curvature; the mucous membrane was subsequently removed. This provides an ideal nerve-smooth muscle preparation for recording the contractions of the powerful circular muscle fibres. During stimulation of the nerves, the solution was lowered in the chamber, and the preparation suspended in the air. The nerves were stimulated by maximal induction shocks for 30 seconds every 15 or 20 minutes. As the responses of frog's

stomach are so variable, three pieces were taken from another or the same frog and the responses to alternating current, potassium and acetylcholine (I in 5000–2500) were obtained for comparison. This high concentration of acetylcholine was used as the frog's stomach is relatively insensitive, though responses obtained by nervous stimulation are usually powerful; inexcitability to nervous stimulation has not yet been found. During the same season the responses from the same batch of frogs are similar. The contractions by all kinds of stimulation are quite regular and can be obtained for several hours.

Results

The contractions produced by the longitudinal fibres are feeble, while those produced by the circular fibres are powerful when stimulated through the nerves; indeed the contractions are as powerful as those produced by any other form of stimulation. Spontaneous contractions may interfere, but often they are quite small. The latent period of the contraction produced by nervous stimulation is 5–20 seconds; adaptation may be rapid so that the response may begin to decline before the period of stimulation is over, or it may continue to increase for about 15 to 20 seconds after stoppage of the stimulus. The response is a twitch, but sometimes the relaxation is slow.

Nervous stimulation of circular fibres produces a contraction similar to that produced by alternating current and spontaneous activity while stimulation of longitudinal fibres produces a contraction which is similar to that produced by potassium and acetylcholine.

Action of Drugs

Effect of atropine.—1 in 10^{7}–10^{6} atropine sulphate inhibits the response to potassium, acetylcholine and of longitudinal muscle to nervous stimulation. 1 in 10^{5} then improves the response to nervous stimulation and potassium. The response to alternating current may be similarly affected, but usually 1 in 10^{7} improves the response to alternating current (Singh and Mrs. Singh, 1946), as well as the response to nervous stimulation of circular fibres. Higher concentrations are depressant to both. 1 in 10^{4} is depressant to all forms of stimulation (Fig. 1).

Acetylcholine action is sometimes very susceptible to the inhibitory action of atropine, 1 in 10^{7} and higher concentrations of atropine completely abolishing the response produced by 1 in 5000–2500 acetylcholine. At other times the muscle is very resistant, and the response persists even with 1 in 10^{4} atropine. When the response is a tonic contraction, then it is very

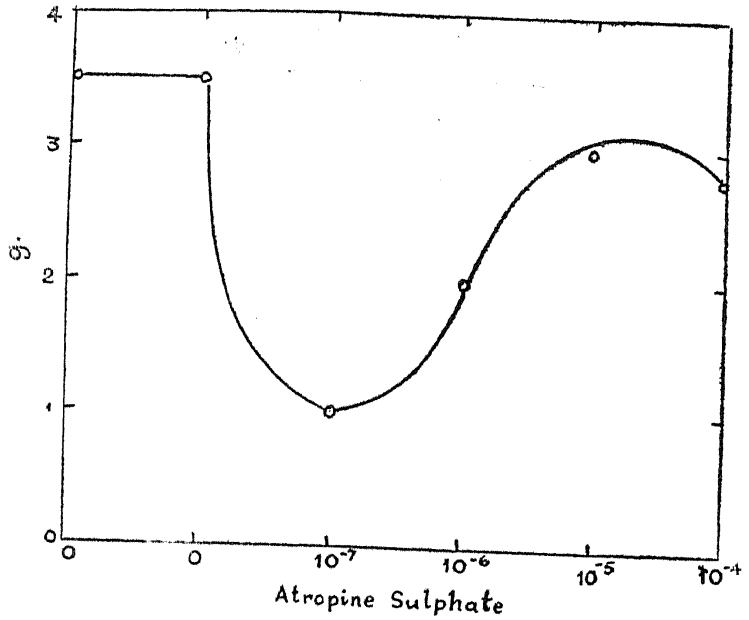

FIG. 1. Frog stomach; action of atropine on the response to nervous stimulation of longitudinal fibres.

susceptible; when it is a series of twitches, then it is more resistant. Calcium converts the tonic contractions of unstriated muscle into twitches (Singh, 1938 *b*). It would therefore appear that the membranes of muscles which respond by twitches contain more calcium and hence are less permeable than those which respond by tonic contraction. *Mytilus* muscles, which give twitches, swell less in various solutions, and so are less permeable than others (Singh, 1938 *c*; 1944 *a*). This is in agreement with Dale's view that the chemical transmitter in the case of vagal stimulation is secreted at a place at which atropine cannot penetrate. This is best explained if it is assumed that the chemical transmitter is liberated in the outer zone (Singh, 1944 *b*). The twitches produced by acetylcholine may be even increased by atropine (1 in 10^6).

Effect of eserine.—Previously it was found that small concentrations of eserine sulphate have an inhibitory effect on the response to acetylcholine (Singh, 1939). This was confirmed in the present series of investigations. It was found that tonic contraction is very susceptible to inhibition, 1 in 10^7 completely abolishing the response (Fig. 2). Thus with eserine also, tonic contraction is more susceptible than twitches. The response of longitudinal muscle to nervous stimulation is affected similarly; 1 in 10^7 causing inhibition, and 1 in 10^6 and 1 in 10^5, causing increase, the response being more resistant than that produced by acetylcholine. The response to alternating current and nervous stimulation of circular muscle is increased by 1 in

10^{7}–10^{6}, and depressed by 1 in 10^{5}. The response to potassium may be affected either way. The response to nervous stimulation of circular fibres may be affected also as in the case of longitudinal fibres.

The response to nervous stimulation is characterised by long latent period, and by the fact that it may continue long after cessation of the stimulus. The question arises whether it is a composite or a single response. In one experiment only it was found that, to begin with, there was a single response; as the concentration of eserine was increased from 1 in 10^{7} to 1 in 10^{4}, the response was gradually split into two, one occurring during and the other on cessation of stimulation. The one occurring during stimulation was suppressed and the other occurring on cessation was augmented by 1 in 10^{5}–10^{4} eserine, the former resembling the response produced on stimulation of circular fibres by nerves. Thus nerves produce responses during and after cessation of stimulation, and these responses are affected differently by eserine. Other forms of stimulation also produce similar responses (Singh, 1938 *a*; 1939; 1942).

Effect of adrenaline.—1 in 10^{7} improves the response to alternating current, and to nervous stimulation of circular fibres, or this action may be produced by smaller concentrations if 1 in 10^{7} is inhibitory. Higher concentrations are depressant. 1 in 10^{7} depresses the response to acetylcholine, potassium and nervous stimulation of longitudinal fibres. 1 in 10^{6} has also a depressant action. 1 in 10^{5} may potentiate the response to potassium as in *Mytilus* muscle (Singh, 1938 *a*) and nervous stimulation. Here again the tonic contraction by acetylcholine is more susceptible than twitches, 1 in 10^{7} completely abolishing the response, and if 1 in 10^{5} is depressant, then the response to nervous stimulation is more resistant, as with atropine and eserine.

Effect of acetylcholine.—The action of acetylcholine resembles that of eserine. It has two kinds of effects. First, it has an inhibitory action on the response to alternating current, potassium, acetylcholine and nervous stimulation of longitudinal fibres in small concentrations (1 in 10^{6}–10^{7}); in larger concentrations (1 in 10^{5}), it has a potentiating effect. Secondly, it has a potentiating action on the response to alternating current and nervous stimulation of circular fibres in small concentrations, and an opposite action in larger ones.

The question arises, why, if the response to nervous stimulation is due to liberation of acetylcholine, the presence of acetylcholine in the saline then should not enhance the action of the former. The depressant action of acetylcholine is due to contracture or adaptation. In frog's stomach

contracture does not occur with small doses (1 in 10^5) as it is relatively insensitive, and adaptation to chemical stimulation is slow. It is thus found experimentally that 1 in 10^5 acetylcholine potentiates the response to nervous stimulation.

Action of Divalent Cations

Effect of calcium.—The optimum concentration of calcium for the response to alternating current is 0·0028–0·0042 *M* $CaCl_2$ and for acetylcholine, 0·0028 *M* $CaCl_2$; for potassium it is 0·0014 *M* $CaCl_2$ and for nervous stimulation, 0·0014–0·0028 M $CaCl_2$. In the absence of calcium, or if the concentration of calcium is reduced, there is a temporary state of hyper-excitability to potassium, acetylcholine and nervous stimulation.

Excess of calcium is depressant, but excitability again increases in about 0·007 *M* $CaCl_2$ as in avian and rabbit's gut muscle. The potentiating action of calcium to potassium as found in mammalian muscle is not found in frog's muscle, though this action is produced by excess of strontium.

Effect of strontium.—Strontium increases the response to all forms of stimulation in concentration of 0·0014–0·0028 *M* $SrCl_2$. The action of calcium on mammalian muscle is similar to that of strontium on frog's muscle. In frog's muscle strontium can replace calcium; as a matter of fact it may have a stronger effect. The stronger action of strontium appears to be due to diminished adaptation. This is probably due to diminished ionisation of calcium (Singh, 1944 *c*).

Effect of barium.—Barium has two kinds of action, one resembling that of calcium and the other that of potassium in causing contracture. Owing to its latter action, it is usually depressant. The calcium effect can be shown by the fact that it can produce persistent contracture in the absence of the Ca ion, and shows the calcium effect on the response to potassium, if contracture is not caused. The calcium effect is more evident in heart muscle; this is probably because the calcium in it is more mobile thus reducing its sensitivity to chemical stimulation (Singh, 1946).

Effect of magnesium.—Small concentrations of magnesium (0·0014 *M* $MgCl_2$) increase the excitability to alternating current nervous stimulation, potassium and acetylcholine. The response to potassium can withstand larger concentrations (0·0028 *M*), as in *Mytilus* muscle. The favourable action of magnesium is probably due to de-ionisation of calcium.

Action of Monovalent Cations

Effect of hydrogen ions.—The optimum pH for excitability depends on the buffer used. In borate it is about 9–8·5 and in phosphate, 8–7·5. In

phosphate the excitability remains unaffected while in borate the response to nervous stimulation is likely to fail. As the pH is decreased to pH 7, the response decreases and thereafter the response increases up to pH 6·5. If the pH is further decreased, the response to potassium, acetylcholine and nervous stimulation of longitudinal fibres increases up to pH 5·4–5·2 and the response to alternating current and nervous stimulation of circular fibres decreases. This is an important point in support of the view that response to nervous stimulation is due to the secretion of a chemical substance, and resembles the potentiating action of hydrogen ions on the response to potassium in mammalian muscle. Gessel and his associates (1944) have presented evidence that acetylcholine liberated by vagal stimulation may be potentiated by acids which retard the breakdown of acetylcholine by choline esterase. As acids also potentiate the response to potassium it appears that this potentiation is not due to an action on cholinestearase only.

Effect of lithium.—The antagonism between the response to nervous stimulation of longitudinal fibres and that to alternating current is well shown by the action of lithium (Fig. 3). Lithium up to 0·04 *M* LiCl decreases the response to nervous stimulation of longitudinal fibres, acetylcholine and potassium, the responses to alternating current being increased. With

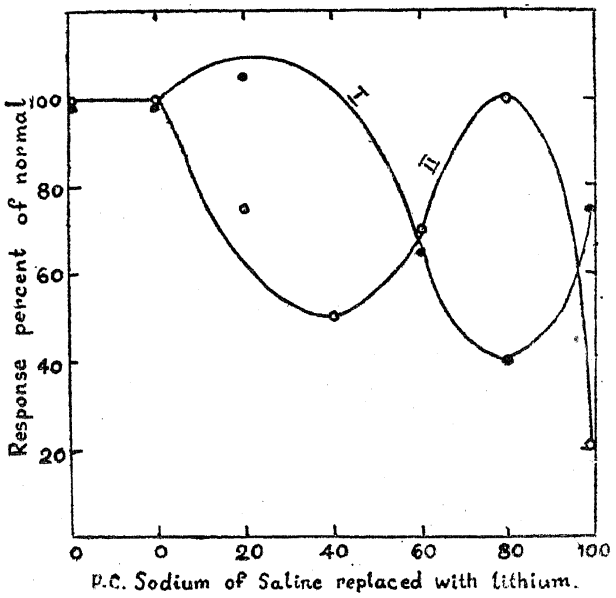

FIG. 3. Frog stomach. Action of lithium on the response to alternating current (Curve I) and nervous stimulation of longitudinal fibres (Curve 2).

further increase of concentration of lithium, 0·08 *M* LiCl, the response to the former three is increased and to the latter decreased. With complete replacement of sodium of the saline with lithium, the response to all forms

of stimulation is decreased, though that to alternating current may increases; the response to nervous stimulation of circular fibres is similar to that to alternating current.

Effect of sodium.—Replacement of 20% of the sodium chloride of the saline by sucrose decreases the response to alternating current and nervous stimulation of circular fibres and increases that to potassium, acetylcholine and nervous stimulation of longitudinal fibres. The action is however variable and may be just the opposite. In electrolyte-free medium (Singh, 1944 *d*), the response to nervous stimulation lasts for about an hour.

Effect of ammonium.—The replacement of about 60 to 80% of the sodium chloride of the saline with ammonium increases the response to acetylcholine and potassium but decreases that to alternating current, nervous stimulation of longitudinal as well as circular fibres. In these muscles, ammonium may cause contraction.

Effect of potassium.—The optimum concentration of potassium for the response to alternating current, nervous stimulation of circular fibres, acetylcholine and potassium is 0·01 *M* KCl; that for nervous stimulation of longitudinal fibres is one-half to one-third of the above. Potassium and ammonium are depressant to nerve. A higher concentration of potassium would be antagonistic to leakage of potassium from the fibres.

Action of Anions

Effect of bromide.—Low concentration, 0·02 *M*, may inhibit or increase the excitability; higher concentration, 0·04–0·06 *M*, has an inhibitory action. This action of bromide is probably related to its inhibitory action in the central nervous system. Higher concentrations, 0·08 *M*, potentiate the response to acetylcholine, potassium and nervous stimulation of longitudinal fibres; the response to alternating current and nervous stimulation of circular fibres is depressed. The replacement of all the chloride by bromide is depressant.

The action of nitrate, iodide and thiocyanate is similar, but is obtainable with smaller variable concentrations; they are much more depressant.

Effect of cyanide.—Small concentrations, 1 in 10^7, are inhibitory; 1 in 10^5 may then increase the excitability. 1 in 10^4 may increase the response to potassium and acetylcholine and depress others. The concentration of anions required to produce the above effects varies. 1 in 10^5 may potentiate the response to nervous stimulation of longitudinal of fibres, but usually cyanide depresses the response to nervous stimulation.

Effect of Osmotic Pressure

The effect of increasing the osmotic pressure of the saline is variable. Increase of osmotic pressure of the saline by adding sucrose to 1·4–2 times normal may increase the excitability to all forms of stimulation; at other times it may cause decrease. These variable results are probably due to the fact that increase in the concentration of potassium inside the fibres may antagonise either the excitatory or inhibitory action of ions outside the fibres; it will decrease the excitatory action of substances in the former case and increase in the latter.

Effect of Temperature

The optimum temperature for response to potassium and nervous stimulation of longitudinal fibres is 20° C. This suggests that in the case of latter stimulation, the stimulating ion is the potassium. The optimum temperature for nervous stimulation of circular fibres is 20–25° C., that for acetylcholine, 30° C., and for alternating current, 20–25° C. The optimum temperature may vary with that of the saline, thus exhibiting adaptation (Fig. 4).

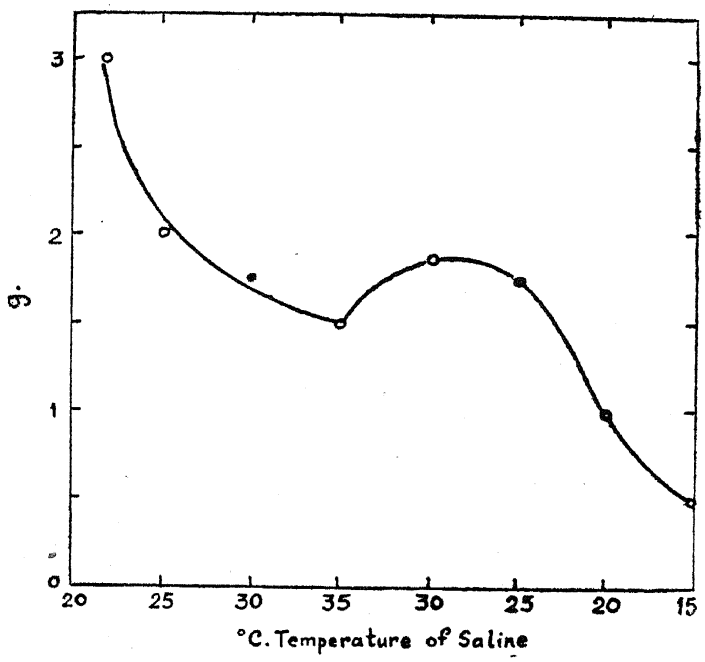

FIG. 4. Frog stomach. Effect of temperature on the response to nervous stimulation of circular fibres; note adaptation.

DISCUSSION

Dale and Feldberg have shown that acetylcholine is liberated from the stomach during vagal stimulation. This liberation of acetylcholine is of

functional importance as the contraction produced by vagal stimulation is similar to that produced by acetylcholine (Narayana and Singh, 1944). During nervous stimulation of frog's muscle the contraction produced resembles that produced by potassium or acetylcholine. It thus appears that acetylcholine is liberated, though the stimulating ion may be potassium. The potassium probably comes from within the cells, as increase in potassium concentration in the saline does not augment the response; acetylcholine probably causes depolarisation of the membrane, increasing its permeability, and causing leakage of potassium ions from within the fibres. These then cause stimulation as has been explained in connection with the occurrence of contraction caused by alternating current.

The action of atropine suggests that acetylcholine is liberated not around the fibres, but in the adjacent zone (Singh, 1944 *b*).

The occurrence of a contraction which is similar to that produced by alternating current, when nerves are stimulated, suggests the possibility of electrical transmission, which will precede chemical transmission, as found by Lorent de No in the central nervous system (McDowell, 1944). The function of chemical transmission would be to impart tonic properties to the phasic contraction produced by electrical transmission. The function of cholinestearase in certain situations may be to prevent this action where it is not desired; eserine by inhibiting its action would bring out the tonic function. It is possible that in some places in the body, the transmission is electrical, in others only chemical or electro-chemical; one or the other being suppressed as required.

Denervated structures become more sensitive to neurohormones. This suggests that these neurohormones are continuously or very frequently secreted. In unstriated muscle increased sensitivity to an ion follows when the muscle is deprived of that particular ion, such as calcium or potassium (Singh, 1942; 1946).

Summary and Conclusions

1. The nature of response of frog's stomach muscle to nervous stimulation is described. The contraction is similar to that produced by acetylcholine and potassium, and is not of the same type as that produced by alternating current, suggesting that acetylcholine is liberated during nervous stimulation of frog's stomach. Excitation by nervous stimulation appears to involve the potassium ion.

2. Nervous stimulation also produces a contraction similar to that produced by alternating current, thus suggesting that electrical transmission

precedes chemical. It is suggested that chemical transmission imparts tonic properties to the effects of electrical transmission.

3. On nervous stimulation, circular fibres of the stomach give the second kind of contraction, and longitudinal the first kind or tonic contraction. It is probable that the function of the longitudinal fibres is to maintain a tonic pressure on its contents and prevent the sagging of the stomach, and that of the circular fibres is to mix the contents by rhythmic contractions, as well as to exert a tonic pressure.

References

Gessel, *et al.* .. *Am. Jour. Physiol.*, **141**, 312; quoted from C. J. Wiggers, *Physiology in Health and Disease*, London, 1944.

McDowell, R. J. S. .. *Handbook of Physiology and Biochemistry*, London, 1944, p. 593.

Narayana, B., and Singh, I. .. *Proc. Ind. Acad. Sci.*, 1944, **20**, 192.

Singh, I. .. *J. Physiol.*, 1938 *a*, **92**, 62.

———— .. *Ibid.*, 1938 *b*, **94**, 322.

———— .. *Ibid.*, 1938 *c*, **91**, 398.

———— .. *Ibid.*, 1939, **96**, 367.

———— .. *Ind. Jour. Med. Res.*, 1942, **30**, 629.

———— .. *Proc. Ind. Acad. Sci.*, 1944 *a*, **20**, 209.

———— .. *Ibid.*, 1944 *b*, **20**, 195.

———— .. *Ibid.*, 1944 *c*, **19**, 91.

———— .. *Curr. Sci.*, 1944 *d*, October.

———— .. *Proc. Ind. Acad. Sci.*, 1945, **22**, 123.

———— .. *Ibid.*, 1946, **23**, 58.

——— and Mrs. Singh, I. .. *Ibid.*, 1946, **23**, 301.