EXCITATION OF UNSTRIATED MUSCLE WITHOUT ANY IONIC GRADIENT ACROSS THE MEMBRANE.

By

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Singh and Singh (1947); Singh and Bhatt (1957) showed that the frog's stomach muscle from *Rana tigrina* had the remarkable property that it could be stimulated by direct current and acetylcholine, if the sodium of the saline was partially or completely replaced with potassium. The concentration of sodium was gradually decreased, replacing the sodium of the saline. At one point, therefore, the concentration of sodium and potassium in the saline would be the same as in the muscle, and there would be no gradient of sodium and potassium across the muscle membrane. The muscle still responds to electric current, acetylcholine and adrenaline.

In the present research these experiments have been extended and the action of potassium-rich solutions further investigated.

These experiments were performed on transverse pieces of the stomach muscle of the frog, *Rana tigrina*; the muscle was stimulated as decribed in previous papers. The experiments were performed at 30°C.

RESULTS

Acclimatisation to potassium. As with sucrose, the muscle has to accommodate itself to potassium. If the sodium of the saline is completely replaced with potassium, all at once, there may be great increase in tone, and the muscle becomes inexcitable to alternating and direct currents (12 volts for 10 sec.). It may remain inexcitable for 2 to 3 hours, after which it recovers, as the tone decreases due to accommodation, and responds for about 20 to 24 hours.

Sometimes, sudden large increase in the concentration of potassium, appears to damage the muscle, which recovers very little or not at all. Under such circumstances, the concentration of potassium should be gradually increased, such as by replacing 10 to 25 per cent of the soduim every 15 minutes. This gradual increase in the concentration of potassium prevents increase in tone due to accommodation of the muscle to potassium.

Effect of calcium.—In the absence of calcium, recovery does not occur if the sodium of the saline is replaced with potassium. Potassium thus exerts a toxic action on the muscle membrane. The optimum concentration of calcium is 0·007 M, though the response differs very little if the concentration of calcium is varied from 0·0056 M to 0·014 M (Fig. 1).

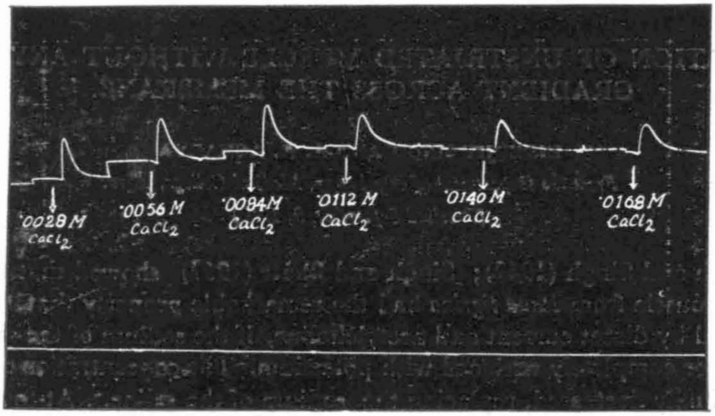

Fig. 1. Frog's stomach muscle. The effect of variations of calcium concentration of the saline on muscle which had been acclimatised to 0.112M potassium chloride containing 0.0028M $CaCl_2$. Muscle stimulated with D.C. 12 Volts/10 Sec. Muscle immersed for 15 min: in each solution.

Effect of sodium.—In hypotonic sucrose solution, the mechanical response is several times bigger than in saline (Singh and Bhatt, 1957), but if all the sodium of the saline is replaced with potassium, the mechanical response in only about 30 to 40 per cent that in saline; this is presumably due to the toxic effect of potassium on the membrane, as it is partly neutralised by excess of calcium in the saline.

The concentration of potassium in the muscle is 0·06 m.eq per gram of wet weight (Gokhale and Singh, 1945; Singh and Bhatt, 1957). If the concentration of potassium in the saline is increased to this value, the muscle responds to electric current, acetylcholine and adrenaline (Fig. 2). The concentration of potassium can be varied to any desired value on either side of 0·06 m.eq./ml and the muscle responds to electrical and chemical stimuli as mentioned. When the sodium is so replaced by potassium, at one point, the concentration of these two ions would be the same within and without the cells. The muscle thus responds in the absence of any ionic gradient of sodium and potassium; similarly it responds in the absence of any gradient of calcium magnesium, bicarbonate and phospate ions.

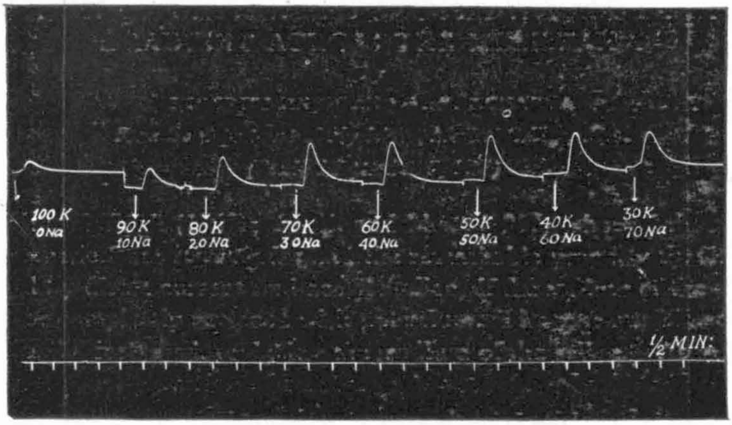

Fig. 2. Frog's stomach. The effect of variations of sodium concentration of the saline on muscle which had been acclimatised to 0.112 M potassium chloride containing 0.0028 M $CaCl_2$. Figures refer to percentage replacement of the potassium of the saline with sodium. Muscle stimulated with D. C. 12 Volts/10 Sec. Muscle immersed for 15 min: in each solution.

Effects of osmotic pressure. Increase in osmotic pressure of the saline to 1·4 times normal by addition of sucrose to the saline increases the concentration of potassium within the fibres. This increases the phasic response and one kind of tone, and decreases another kind of tone (Singh, 1939). This shows that one kind of tone and the phasic response is related to the concentration of potassium. Potassium, presumably causes contraction of the polypeptide chains responsible for this tonic and phasic responses. Relaxation of the muscle by increase in osmotic pressure of the saline shows that this kind of tone is subserved by a different set of polypeptide chains, which are relaxed by potassium (Singh, 1957). If the sodium of the saline is completely replaced with potassium, the phasic response increases with osmotic pressure when the latter is increased to 1·4 times normal by addition of sucrose to the saline, one kind of tone also increases (Fig. 3). These osmotic effects show that large concentrations of potassium do not destroy the muscle membrane, though it is somewhat damaged, as larger concentrations of calcium are required for excitability.

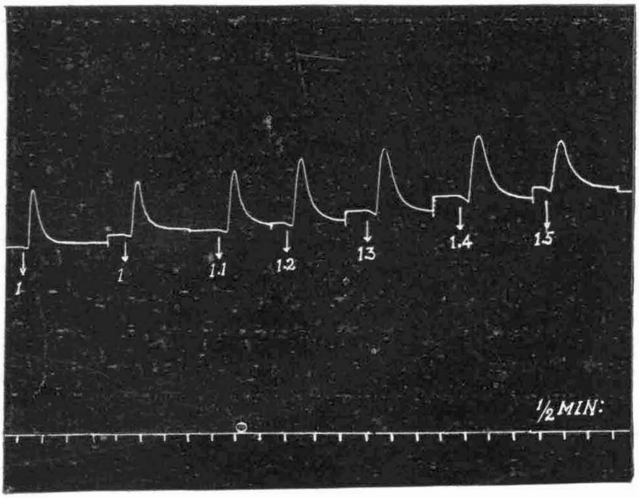

Fig. 3. Frog's stomach muscle. The effect of variations of osmotic pressure of the potassium chloride solution. The muscle had been acclimatised to 0.112 M. potassium chloride containing 0.0028 M $CaCl_2$. The osmotic pressure of the potassium solution is increased by adding sucrose. The figures refer to the osmotic pressure of the solution X times normal (which is 0.112 M KCl). Muscle immersed for 15 min. in each solution.

Effect of hydrogen ions. When the sodium of the saline is completely replaced with potassium, the muscle acts best in unbuffered solutions. Variations of pH by addition of biocarbonate or phosphate depress the response.

Effect of temperature. The response in potassium saline is best obtained at 30°C. Recovery is incomplete at higher or lower temperatures.

DISCUSSION

The facts that the muscle can be excited in hypotonic sucrose solution and in potassium rich solutions, shows that ionic gradients of sodium or potassium across the membrane are not necessary for excitability. The ion that is necessary for excitability is intracellular potassium (Singh and Acharya, 1957). Sudden increase in the concentration of intracellular potassium causes the muscle to be excited (Singh, 1942). The effects of electric current are similar to those produced by increase in osmotic pressure of the saline, so that electric current in some way increases the concentration of potassium within the fibres. Potassium may then leak from the muscle producing accommodation, and accumulate outside the fibres, so that on cessation of electric current, the increase in the potassium concentration outside, again excites the muscle (Singh, 1944).

SUMMARY

1. Frog's stomach muscle can be excited if all the sodium of the saline is replaced with potassium. At first the excitability diminishes and then the muscle recovers and responds to direct current, acetylcholine and adrenaline for several hours.

2. If all the sodium of the saline is replaced with potassium in one step, then the muscle may permanently become inexcitable. This loss of excitability can be avoided by gradual increase in the concentration of potassium. Recovery is promoted by calcium and a temperature of 30°C. is optimum.

3. The optimum concentration of calcium for the mechanical response in potassium saline is 0·007 M $CaCl_2$.

4. The muscle responds to electrical and chemical stimulation in concentrations of sodium and potassium, which abolish the concentration gradients of these ions across the muscle membrane.

5. The muscle acts best in unbuffered solutions, if the sodium of the saline is replaced with potassium.

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