THE MODE OF ACTION OF POTASSIUM ON UNSTRIATED MUSCLE

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The action of potassium appears to depend upon a difference in its concentration within and without the fibres, both in the unstriated muscle (Singh, 1939) and in the striated muscle (Wright, 1942). Yet it must have other actions also, dependent upon its action on the cell membrance, permeability, etc.; it may have calcium-like effects. In this paper an attempt has been made to elucidate some of its actions on the excitability of unstriated muscle.

Results

Potassium inside the fibres.—According to Straub, muscarine acts on the heart only as it penetrates into the muscle cells, and once having arrived in the interior, has no further action. In this respect, potassium acts similarly. The concentration of potassium inside the fibres is greater than that outside, and it does not cause diminution in excitability. This may be related to greater concentration of calcium inside the fibres, which antagonises the action of potassium. The same concentration of potassium outside causes inexcitability, which is antagonised by increase in the concentration of calcium.

As calcium and potassium mutually antagonise the depressant action of each other within certain limits with resulting increase in excitability, it follows that the excitability to alternating current will increase, if the osmotic pressure of the saline is increased upto a certain limit. This limit is found to be 1·4 times normal, after which increase in the concentration of the ions inside the fibres begins to exert a depressant effect. The effect of electrolyte-free medium (Singh, 1944 *a*) shows that potassium inside the fibres becomes toxic, if the concentration of the ions outside the fibre is decreased, thus increasing its relative concentrations inside the fibres. The depressant effect of increase in osmotic pressure of the saline would presumably be due to a similar cause.

The depression of excitability if the osmotic pressure of the saline is increased is partly due to increase in the concentration of the ions inside the fibres, and partly due to the substance used in increasing the tonicity of the saline. The latter action is shown by the fact that the various sugars sodium salts (chloride, bromide, nitrate, idoide and thiocynate) and chlorides (lithium, sodium, ammonium and potassium), do not produce identical results, if used in increasing the tonicity of the saline. They produce osmotic results as well as their own individual actions; the former action is shown by the fact that the sodium chloride and sucrose, which are chemically unrelated produce identical results, when used in increasing the tonicity of the saline.

The relative concentration of ions inside the fibres is increased if the external medium is made sodium deficient by replacement with sucrose. The excitability to alternating current in the frog stomach is at first decreased and then increased. The same results are produced if the osmotic pressure of the saline is increased by adding sucrose or sodium chloride at pH 7. The similarity of the action of sodium deficient and hpyertonic solutions, is probably due to the fact, that in both instances, the concentrations of the ions is relatively increased in the interior of the muscle fibres.

When sodium chloride is added, then the relative concentration of all ions is the same outside and inside, so that the effect of increase of osmotic pressure are not due to increase in the concentration of all the ions inside the fibres, but of those more potent than sodium or chloride, that is, potassium or calcium. As increase in osmotic pressure of the saline causes contraction, the effects of such increase must be due to potassium, as calcium has an inhibitory effect.

When the osmotic pressure of the saline is incresed, both in *Mytilus* and frog stomach muscles, the excitability to alternating current may at first be decreased and then increased. This suggests that potassium inside the fibres, above its normal concentrations, either causes contraction or depression in excitability, just as it does outside the fibres. The subsequent increase in excitability shows an increase in the concentration of the antagonistic factor, probably calcium. The inhibitory effect of increase in osmotic pressure would therefore be due to increase in the concentration of potassium, as well as calcium inside the fibres; the increase in concentration of the former will cause a secondary increase in the concentration of the latter, due to adaptation (Singh, 1944 *b*). It is so interesting, that increase in the concentration of potassium inside and outside the fibres causes some similar results. Thus when the concentration of potassium is increased outside, the excitability to alternating current at first decreases and then increases, and finally decreases. These are also the effects of increase in osmotic pressure of the saline.

The frog stomach and the heart can be stimulated electrically in the electrolyte-free medium (Singh, Sehra and Mrs. Singh, 1945 *a*, *b*), as well as by increase in osmotic pressure of the saline to 1·4 times normal. This shows that this contraction as well as spontaneous contractions are due to potassium inside the fibres. The occurence of spontaneous contractions suggests, therefore, that potassium is periodically liberated from some labile combination in the cells.

As the excitability to potassium outside the fibres depends upon the concentration of potassium inside, and as the latter can be varied by changes in osmotic pressure, this suggests that potassium exists in two forms within the cells, one free and the other combined, because the changes in the concentration of the active potassium only would affect the excitability to potassium outside the fibres, if this excitability is dependent upon a difference in concentration of potassium on two sides of the muscle membrane. The free potassium inside the fibres, therefore, determines, what may be termed as "internal excitatory state", analogous to the central excitatory state in the neurone. The internal excitatory state will increase the excitability to the excitation produced by ions inside the muscle fibres, but decrease that to ions outside.

In the frog stomach, the adaptation occurs to alternating current in the electrolyte-free medium. This shows that the factor of adaptation occurs inside the fibres, that is due to liberation of calcium. Calcium, therefore, also probably exists in a combined and free form inside the cells (Singh, 1944), and the free or labile calcium inside the fibres, therefore determines the "internal inhibitory state", analogous to the central inhibitory state in the neurone.

Adaptation to excitation occurs, by increase in the inhibitory state, while adaptation to inhibition occurs by increase in the excitatory state (Singh, 1944 *b*). The increase in the excitatory state after inhibition is responsible for the withdrawl contractions, which are the same phenomenon as rebound in the spinal reflexes. The above phenomenon are similar to those in the neurone. In the latter when the P effect predominates, a further P effect is less easily produced than the N effect; the production of the P is said to be occluded and that of N facilitated and *vice versa.* Just as with knee jerk a succession of inhibitions and rebounds after adrenaline may cause dog stomach to enter into a tonic contraction. The above explains a very common phenomenon in plain muscle. If a substance depresses the excitability, its removal causes an increase in excitability before return to the normal. Similarly if a substance increases the excitability, there may be a depression of excitability before return to the normal; this latter phenomenon is not always found. The above also accounts for the supernormal states in the plain muscle.

The refractory period of the muscle is partly due to this inhibitory state. This is shown by the fact that in the frog heart complete tetanus is not produced if the concentration of calcium is increased. It has been explained that the length of the refractory period prevents the frog heart from tetanising, so that if the concentration of calcium is decreased, then the refractory period must get shorter. The reason why then acetylcholine favours tetanus in the frog heart is that adaptation to inhibition is produced by increase in the excitatory state. Spontaneous contractions are then due to the interaction between the excitatory and inhibitory states, and also accounts for the fact that a relaxed muscle may contract and a contracted muscle relax on stimulation.

When *Mytilus* muscle, which is comparatively less sensitive to potassium, is stimulated with the latter or adrenaline, it may become inexcitable after two or three stimulations, owing to the development of an inhibitory state. The above experiment shows that the inhibitory state persists longer, and rises more slowly than the excitatory state (Singh, 1944 *b*), as happens in the neurone. Fatigue is therefore due to accumulation or "facillitation" of the inhibitory state; it is therefore identical with adaptation and refractory period. As the refractory period varies in different tissues, it is not due to exhaustion of "food energy".

It is generally found that whatever diminishes the excitability of unstriated muscle, also increases the latent period, and diminishes the rate of rise of tension. But the opposite holds at high temperatures, when the excitability diminishes, but the refractory period also deminishes. This diminution in the refractory period at high temperatures is presumably due to increase in the rate of development of the excitatory state and the rapid adaption or fatigue to increase in the rate of the inhibitory state. The refractory period would therefore be due to the resting or threshold inhibitory state. In *Mytilus* muscle excess of magnesium may increase the latent period several hundredfolds, without appreciably altering the rate of rise of tension of the contraction produced by alternating current.

In unstriated muscle there may be both summation of excitatory and inhibitory states, and also subliminal excitation (Singh, 1939) or inhibition.

The action of distilled water on the frog stomach (Singh, 1944) suggests that normal tone and viscosity of the muscle are due to combination of ions inside the fibres with myosin. In the electrolyte-free medium relaxation is slow, so that ions inside the fibres also produce tonic contraction. In sucrose solution, the muscle contracts, so that the factor causing tonic contraction resides inside the fibres.

The greater tone and viscosity of the unstriated muscle appears to be related to its greater sodium content (Singh, 1938). It therefore appears that sodium chloride inside the fibres antagonises the action of calcium, as it does outside the fibres. Like potassium and calcium, sodium probably exists in the bound and free form. Tonic contractions such as slow relaxation are then probably due to mobilisation of sodium, twitch, to mobilisation of potassium, and adaptation to mobilisation of calcium inside the fibres. Tonic contraction may also be due to ions outside the fibres; the sodium inside the fibres, may then be said to determine the "tonic and viscous state".

Potassium outside the fibres.—The contracture which is produced by increasing the concentration of potassium in the saline is diminished by increase in the osmotic pressure of the saline, which increases the concentration of ions within the muscle fibres. The action of potassium thus appears to be antagonistic on two sides of the muscle membrane. This veiw is supported by the further observation, that tone which is produced in the frog stomach by increase of osmotic pressure, is diminished by increase in the concentration of potassium in the saline within certain limits. Thus tone produced by increase in the osmotic pressure of the saline to twice the normal, is diminished if the concentration of potassium is increased to five times the normal the excitability to alternating current increasing at the same time (Fig. 1).

The maximum excitability of the muscle, thus depends upon a certain optimum ratio of potassium without and within the fibres. If this ratio is disturbed then the excitability diminishes and a contracture develops. This ratio can be disturbed in four ways:

1. Increase in the concentration of potassium within the muscle fibres; thus occurs when the osmotic pressure of the saline is increased.

2. Decrease in the concentration of the ions inside the fibres; this occurs when the osmotic pressure of the saline is decreased.

3. An increase in the concentration of potassium outside the fibres.

4. A decrease in the concentration of potassium outside the fibres; this also occurs in familial periodic paralysis (Aitken, Allot, Caslteden, and Walker, 1937).

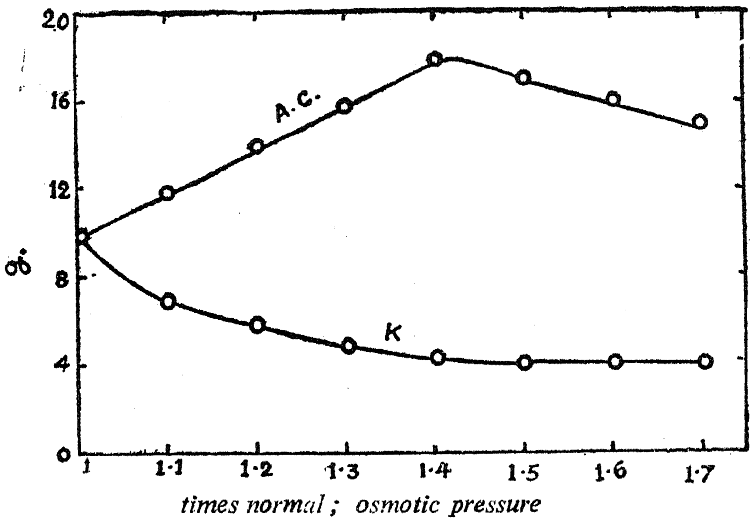

FIG. 1 A. Frog stomach : The effect of osmotic pressure on tone produced by replacement of 20% of the sodium of the saline by potassium.

AC = tension produced by alternating current ; K = tone produced by potassium.

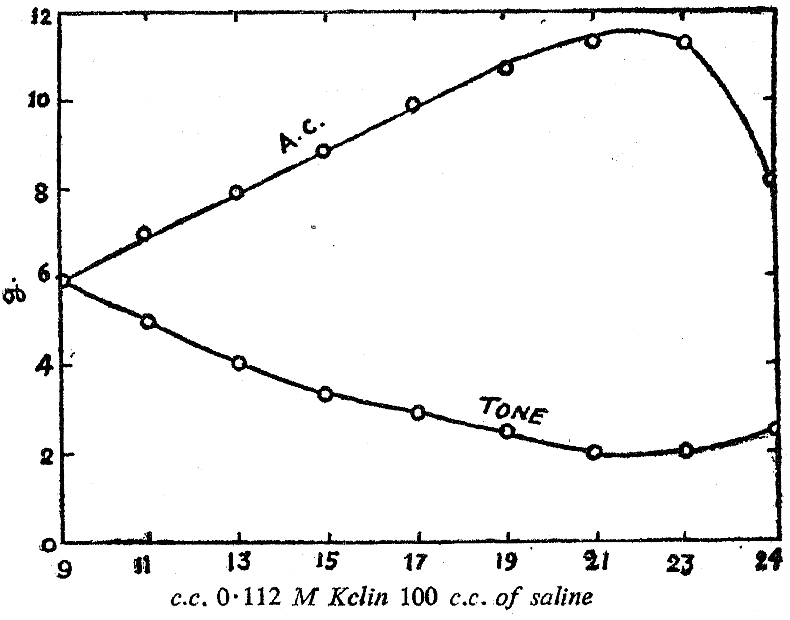

FIG. 1 B. Frog stomach : effect of potassium on tone produced by doubling the osmotic pressure of the saline by sucrose.

If the above view is correct, then the optimum concentration of potassium in the saline for excitability should vary with the osmotic pressure of the saline. It has been found (6 experiments), that with the decrease of osmotic pressure, the optimum concentration of potassium required for excitability to electric current decreases; the opposite happens with increase in osmotic pressure (Table I). These results suggest

TABLE I

Frog stomach. The effect of change in osmotic pressure of the saline on the excitability to alternatining current 8*V*/10 *Seconds*

Osmotic pressure of the saline Molar strength	Optimum concentration of potassium, M KCl
0·0672	0·00560
0·0896	0·00784
0·1120	0·01008
0·1344	0·01232
0·1568	0·01456
0·1792	0·01780
0·2016	0·02240
0·2240	0·02520

that one of the actions of potassium is dependent upon its difference in concentration on two sides of the muscle membrane.

Under normal circumstances, a change in the optimum concentration of potassium for excitability probably signifies a change in the internal excitatory state. Since the concentration of potassium in unstriated muscle fibres is less than the striated muscle fibre, the internal excitatory state is greater in the former than in the latter, so that the former is more excitable to electrical stimulation than the latter.

The depressant action of potassium.—Potassium has three kinds of depressant actions. Small concentrations of potassium, diminish tone and increase the excitability to alternating current in *Mytilus* muscle. This is clearly a calcium-like effect. This is probably attended with decrease in permeability.

When the concentration of potassium is suddenly increased in the *Mytilus* saline, there is at first a depression of excitability to alternating current, but increase in that to ions outside. This probably produced by difference in the concentration of potassium on two sides of the muscle membrane.

The increase in excitability is followed by a lasting depression to all forms of excitability. This kind of depression is produced by all abnormal substances when present in excess in the saline. Tone also decreases though tonic contractions by potassium also depresses all forms of excitability. This is antagonised by calcium.

If potassium is in great excess in *Mytilus* saline, then all forms of excitability and tone are abolished; the impedance is greatly decreased, This is probably due to increase in permeability so that for normal excitability an intermediate permeability is necessary. From the above, it appears that potassium may produce its effect (1) by virtue of a difference of concentration on two sides of the muscle membrane, (2) by action on the muscle membrane, and (3) by action on the muscle colloids.

TONUS

In unstriated muscle, tonus can be produced in the following ways:—

1. *Intrinsic tone.*—An isolated muscle shows tonus, which does not appear to use oxygen, as it appears to be unaffected by cessation of oxygen supply; in fact the muscle dies in that state. Plain muscle always dies in a state of tonus. This tone appears to be due to the action of ions in the muscle fibres on the muscle protein, as shown by the action of distilled water. In water, the muscle though alive, actively elongates; this can be ascribed to diminution of concentration of ions inside the muscle fibres, as the muscle gains water. An isolated muscle preserves the property of elongation after death if the latter is caused to occur naturally in saline; it appears that in this way death causes the least disturbance in protoplasm, and myosin preserves some of its properties.

The above tone is characterised by absence of spontaneous contractions; the viscosity may be high or low, and is not much affected by increase in osmotic pressure of the saline; inhibition is not prominent.

2. *Tonus due to saline.*—An isolated unstriated muscle may show considerable tone, dependent upon oxygen supply; the tone diminishes in the absence of oxygen. The tone is marked with spontaneous contractions and low viscosity. Excitability to alternating current is low; ***inhibition is facilitated.*** It is due to the constituents of the saline, such as sodium chloride, calcium, potassium and hydrogen ions. Thus the tone may diminish if the sodium chloride content of the saline is reduced, showing that the latter salt is one of the stimulating factors. Again reduction in the sodium chloride content may only be effective if the potassium is absent, showing that the latter is also stimulating agent; the effect of potassium may be reverse. The reduction in the sodium chloride content of the saline may be effective only in alkaline solution, showing that the hydrogen ions are also stimulating agents; their action may be reverse of the above. Calcium has both stimulating and inhibitory properties.

3. *Tonus due to stimulating substances.*—This is due to the action of stimulants. The oxygen consumption may or may not increase; the viscosity

is high. Spontaneous contractions may be absent. The excitability is depressed; *inhibition is antagonised.*

4. *Tonus in sucrose solution.*—This is due to unbalanced action of ions inside the fibres. In frog stomach it is reduced by reducing the osmotic pressure. Spontaneous contractions are absent, excitability is depressed and excitation and inhibition are abolished.

5. *Tonus due to hypertonic saline.*—This is due to increase in the concentration of potassium inside the fibres; spontaneous contractions are increased. Inhibition is antagonised.

6. *Tonus due to hypotonic saline.*—This is due to the increased action of ions outside; inhibition is increased and viscosity is high.

Effect of potassium on tone.—Small concentrations of potassium (normally present in saline) have an inhibitory influence. In isotonic glucose in *Mytilus* muscle, and isotonic sucrose in frog stomach, they have a stimulating action. Larger concentration of potassium have an inhibitory action. This latter action would be due to their antagonising the ions inside by restoring somewhat the ionic in balance within and without the fibres. With increase in the concentration of potassium, the excitability to alternating current is not increased, as it does when potassium antagonises the tone produced by increase in osmotic pressure. It would appear that ions in the saline have a protective action against the depressant action of potassium. In *Mytilus* muscle, if the osmotic pressure is increased by sodium chloride then increase of excitability may result (Fig. 2); the latter action is more

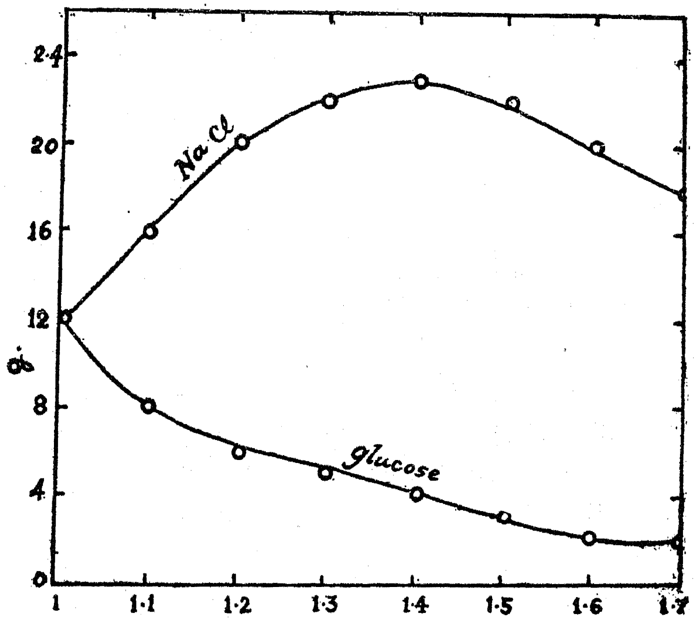

FIG. 2. *Mytilus* muscle : Effect of increase in osmotic pressure by NaCl and glucose on tension produced by alternating current. Results are very variable, however,

marked in muscles that have been obtained from animals stored in cold, and thus contain more sodium. It thus appears that sodium chloride protects the muscle membrane from the action of potassium. In frog stomach also, sodium chloride is more efficacious than sucrose at pH 8.

Both frog heart as well as frog stomach remain irritable in the electrolyte free medium, but skeletal muscle does not. This is probably due to the fact that the former two contain more sodium than the latter. In the guinea pig uterus, removal of potassium from the saline may cause increase or decrease of tone. The former action shows that as in *Mytilus* muscle it has an inhibitory action. The former action occurs in alkaline and the latter in acid solutions. After 10 minutes in both instances, reintroduction of potassium causes marked inhibition. These results can be explained as previously (Singh, 1944 *b*) that potassium suppresses the liberation of calcium or the inhibitory state. In alkaline solutions, the liberation of calcium is not sufficient to overcome the increase in tone but in acid solutions, the hydrogen ions reinforce the action of liberated calcium.

In the frog stomach, small concentrations of potassium cause contraction by antagonising the action of sodium chloride, but their stimulating action in isotonic sucrose solution is not understood.

Effect of calcium.—Calcium in small concentrations decreases tone produced by (1) ions outside, (2) isotonic sucrose or increase in osmotic pressure of the saline, that is ions inside. This is in agreement with the suggestion that it produces adaptation. In isotonic sucrose solution, small concentrations of potassium, cause contraction, but calcium causes relaxation. In frog saline, both have a stimulatory effect, calcium and potassium, in this instance antagonising the inhibitory effect of the sodium chloride. By same action in *Mytilus* muscle, they produce relaxation. The inhibitory effect of sodium chloride is like that of calcium.

In guinea pig uterus, withdrawl of calcium causes decrease of tone, both in alkaline (pH 8, borate buffer), and in acid (phosphate buffer) solutions. Reintroduction of calcium causes contraction in alkaline solutions, but in acid solutions may cause a preliminary inhibition. The cause of this is probably the same as that of potassium inhibition, that is liberation of calcium on withdrawal of calcium (Singh, 1944 *b*); for the same reason the diminution of tone on withdrawl of calcium is less in acid solutions than in alkaline soultions. The liberated calcium in acid solutions would partly compensate for the withdrawal of calcium.

SUMMARY AND CONCLUSIONS

(1) The action of potassium, calcium and sodium suggest that they exist in the combined and free form in the unstriated muscle, they respectively determine the excitatory, inhibitory and tonic and viscous states in the muscle.

(2) One of the actions of potassium is due to its difference in concentration on the two sides of the muscle membrane. This is shown by the fact that the optimum concentration of potassium in the saline for excitability depends upon its concentration inside the fibres. For maximum excitability there is an optimum ratio of potassium on two sides of the muscle membrane.

(3) The second action of potassium is probably on the cell membrane as it is antagonised by calcium.

(4) A third action of potassium is probably on the muscle colloid as it affects the viscosity of the muscle.

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