

THE EFFECT OF AMMONIUM ON THE POTASSIUM CONTENT OF UNSTRIATED MUSCLE AND ITS RELATION TO THE CONTRACTION PRODUCED ON WITHDRAWAL OF CHEMICAL SUBSTANCES FROM AROUND THE MUSCLE

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Received December 18, 1944

AMMONIUM is known to penetrate cells and replace potassium. Ammonium has some interesting effects on unstriated muscle; it produces a contraction on withdrawal (Singh, 1939b). It was assumed that ammonium penetrated the cells, and that the contraction produced by its withdrawal was due to the presence of ammonium ions within the cells. The present experiments were performed to test the validity of this assumption, and to correlate the effect of ammonium on the responses of unstriated muscle with its effect on the potassium content of the latter.

The potassium content of the muscle was determined by the method described by Cummings (1939). The frog stomach muscle (from *Rana tigrina*) was divided into two nearly equal parts; one piece was soaked in the experimental and the other in the control solution. The values were recorded as mg/g of dry as well as wet weight of muscle.

RESULTS

The potassium content of the unstriated muscle from six frog stomachs varied from 2.25 to 3.5 mg/g wet, there being a considerable variation from animal to animal. The potassium content of 6 pieces from dog stomach varied from 2.15 to 3.12 mg/g. There is thus no significant difference between the potassium contents of unstriated muscle from dog and frog stomachs, though the ionic concentration of their media differs. This is in agreement with results of some previous workers. Thus, the potassium content of ox stomach is 3.65 mg/g and that of ox retractor penis 2.67 mg/g. (Constantino, 1911), that of frog stomach muscle from 3.6 to 3.43 mg/g. (Meigs and Ryan, 1912). The potassium content of the striated muscle of pig is 2.5 mg/g, that of ox 3.6 mg/g. (Katz, 1896), that of cat about 3.5 mg/g. (Fenn, 1938) that of rat about 3.5 mg/g. (Fenn and Cobb, 1936), that of frog

striated muscle, about 3.5 mg/g. (Katz, 1896; Meigs and Ryan, 1912, Fenn and Cobb, 1936).

The dog stomach muscle is therefore relatively richer in sodium than frog muscle; the former also exhibits greater tone than the latter (Singh, 1940). This is in agreement with the view previously mentioned that the greater sodium content of unstriated muscle, compared to that of striated muscle is related to greater tone of the former (Singh, 1938a). The dog retractor penis shows greater tone than dog stomach and it is significant to note that the potassium content of ox retractor penis is less than that of ox stomach and the sodium content correspondingly greater (Constantino, 1911).

The effect of ammonium on the potassium content of the unstriated muscle of dog and frog stomachs is shown in Table I. In these experiments

TABLE I

No. of experiments	Nature of Muscle	No. of pairs	Nature of solution	K, mg./g. wet weight	Percentage change in experimental solution
1	Dog Stomach	6	{ Unsoaked	2.67 ± 0.04	- 84
2	Do.	6	{ Saline	0.41 ± 0.03	- 0.03
3	Frog Stomach	5	{ 0.123 M NH ₄ Cl	0.28 ± 0.02	- 39
4	Do.	8	{ Unsoaked	0.27 ± 0.02	- 44
5	Do.	7	{ Saline	2.41 ± 0.16	+162
6	Do.	6	{ 0.03 M NH ₄ Br	1.65 ± 0.04	+435
7	Do.	7	{ 0.03 M N (C ₂ H ₅) ₄ Br	1.12 ± 0.22	- 44
			{ 0.03 M N (CH ₃) ₄ Br	0.62 ± 0.24	- 44
			{ 0.03 M NH ₄ Cl, pH 7	0.29 ± 0.04	- 44
			{ 0.03 M NH ₄ Cl, pH 8	0.76 ± 0.03	- 44
			{ K-free Saline, pH 8	1.55 ± 0.47	- 44
			{ K-free Saline, pH 6	1.09 ± 0.38	- 44
				0.61 ± 0.38	- 44
				1.14 ± 0.02	- 44
				0.39 ± 0.15	- 44

only physiological concentrations of various ions have been employed; these concentrations have been used to study their effect on the responses of unstriated muscle (Singh, 1939, 1940).

In frog muscle there is a significant difference between the potassium contents of muscle soaked in normal and ammonium salines, so that ammonium enters the cells and replaces potassium; in frog muscle such a concentration of ammonium produces a withdrawal contraction, having properties of the A.C. contraction, which appears to be produced by ions within the muscle fibres (Singh, 1938b, 1939b).

In dog stomach withdrawal of ammonium produces no such contraction and the remarkable fact was observed, that there was no significant

difference between the potassium content of dog muscles soaked in normal and ammonium salines; besides the concentration of ammonium employed to soak the dog muscle was 0.123 M NH_4Cl , and for frog muscle it was only 0.03 NH_4Cl . Withdrawal of the latter, concentration of ammonium produces no response in dog muscle.

More ammonium replaces the potassium in alkaline than in acid solutions; this is comparable to the effect of pH on the potassium content of frog gastrocnemius (Fenn and Cobb, 1935). The loss of potassium from the muscle in a potassium-free solution is greater in acid than in alkaline solutions (Table I) as is found with the frog gastrocnemius. The ammonium withdrawal contraction is greater in acid than in alkaline solutions, suggesting that the contraction is due to the outward passage of ammonium ions.

Withdrawal of tetra-ammonium salts $\text{N}(\text{CH}_3)_4 \text{Br}$, $\text{N}(\text{C}_2\text{H}_5)_4 \text{Br}$ produces no contraction, which has the properties of the A.C. contraction; the permeability of the muscle to these salts is also less than that to ammonium (*cf.* Ing and Wright, 1931). Withdrawal of these salts, however appears to produce a tonic contraction, as the relaxation of the muscle on withdrawal of the tetramethyl ammonium bromide is very slow and is practically devoid of intermittent contractions.

Though no actual contraction has been observed on withdrawal of these tetra-ammonium salts, this slow relaxation is in reality a tonic contraction, as suggested by the following observations. (1) The contraction produced on addition of tetramethyl ammonium bromide is intermittent so that the contraction on withdrawal of the salt is not merely due to its diminishing concentration when normal saline is added, resulting in a diminution of the stimulus. (2) Tone is antagonistic to spontaneous contraction. Withdrawal of these salts immediately suppresses the spontaneous contractions in frog stomach, guinea-pig uteri; subsequently the spontaneous contractions are enhanced before return to normal. Withdrawal of thiocyanate which may result in a tonic contraction, also suppresses the spontaneous contractions and the response to A.C. in frog muscle, these latter contractions having similar properties and being antagonistic to tonic contractions (*cf.* effect of withdrawal of caffeine on the response to A.C. in *Mytilus* muscle Singh, 1938b). (3) The stimulating power of these salts varies in the order $\text{N}(\text{CH}_3)_4 \text{Br} < \text{N}(\text{C}_2\text{H}_5)_4 \text{Br} < \text{N H}_4 \text{Br}$; the rate of relaxation of the contractions produced by these substances also varies in the same order. It has been shown previously that contractions produced by withdrawal of thiocyanate, nitrate, ammonium in dog muscle are tonic and their magnitude varies similarly as the initial contractions produced by these substances.

That such a slow relaxation on withdrawal of a substance is a tonic contraction is shown by the following experiments. Withdrawal of bromide does not produce a tonic contraction; withdrawal of nitrate, thiocyanate and ammonium produces such a contraction, the potency of these substances being in the order $\text{NO}_3 < \text{SCN} < \text{NH}_4$. The rate of relaxation of the contractions produced by these substances also varies in the same order; further these substances may either produce a contraction on withdrawal or may render the relaxation slow, and it is possible to pass from a stage of slow relaxation to that of contraction on withdrawal. The slow relaxation on withdrawal of thiocyanate is thus a less powerful contraction than that produced on withdrawal of ammonium. The above phenomena are very interesting, as the cessation of electrical stimulation may also either result in slow relaxation or in tonic contraction, the slow relaxation being an intermediate stage between rapid relaxation and a tonic contraction (Singh, 1938c, 1940).

Slow relaxation of a tonic contraction is also due to persistence of the contraction though the stimulating substance is withdrawn; the tonic contraction probably results in increased viscosity, as the rate change of length is diminished, and this increase in viscosity persists. The excitability of the muscle during such a contraction is greatly diminished; adrenaline antagonises such a state of muscle. In *Mytilus* muscle barium chloride produces such a contraction; all substances that produce tonic contraction, produce such a state of the muscle to some degree so that the contraction produced on withdrawal of the thiocyanate or ammonium is not very marked, if the muscle already enters into a tonic contraction, resulting in diminution of excitability. Such a tonic contraction may be prevented by previous treatment with adrenaline. Tonic contraction produced by A.C. in *Mytilus* muscle has similar properties (Singh, 1940).

Thus both with electrical and chemical stimulation, slow relaxation of a contraction is due (1) either to after-stimulation or (2) to structural changes, probably attended with increase in viscosity.

Increase in osmotic pressure of the medium increases the concentration of potassium within the fibres. Thus the concentration of potassium in 4 frog muscles immersed for 18h. in saline of the same osmotic pressure as that of 0.120 M NaCl was $1.01 \pm$ mg/g, and in 4 muscles immersed in saline, the osmotic pressure of which was that of 0.154 M NaCl, the concentration of potassium was $1.36 \pm$ mg/g. The ammonium withdrawal contraction is augmented if the osmotic pressure is increased to 1.3-1.4 times normal; this is in agreement with the view that the ammonium withdrawal contraction is due to ions within the cells, as increase in osmotic pressure would increase the concentration of ammonium within the muscle fibres.

With frog muscle, if the concentration of ammonium in the saline is increased beyond 0.03 M NH_4Cl then the ammonium withdrawal contraction is depressed. With dog muscle, which is acclimatised to a higher concentration of sodium chloride, the ammonium withdrawal contraction can be produced if all the sodium is replaced with ammonium, the optimum concentration being 0.123 M NH_4Cl . This shows that the diminution of the withdrawal contraction in frog muscle with high concentrations of ammonium is due to a secondary depressant effect of the latter (Singh, 1939 *b*).

DISCUSSION

Facts which suggest that the contraction of frog muscle on withdrawal of ammonium is due to the outward passage of ammonium ions are (1) soaking of frog muscle in ammonium-rich saline results in a greater loss of potassium than soaking in normal saline. (2) With dog muscle, which shows no contraction on withdrawal of ammonium having the properties of the A.C. contraction, there is no significant difference between the action of ammonium-rich and normal salines. (3) There is greater loss of potassium from the muscle in a potassium-free saline at pH 6 than at pH 8, but in the presence of ammonium, there is greater loss at pH 8 than at pH 7. The ammonium withdrawal contraction is greater in acid solutions than in alkaline solutions, suggesting that it is due to the outward passage of the ions. (4) There is greater loss of potassium in ammonium-rich saline, than in saline containing tetra-ammonium salts.

It has been mentioned previously (Singh, 1939 *b*), that one kind of adaptation is associated with penetration of the ions into the cells; this agrees with the present analysis, as adaptation to ammonium is more rapid than to the tetra-ammonium salts.

SUMMARY

(1) In frog stomach muscle, ammonium enters the cells and replaces potassium; this is associated with the ammonium withdrawal contraction having the properties of the A.C. contraction.

(2) In dog muscle there is no significant difference between the potassium contents of muscles soaked in normal saline, and in ammonium-rich saline respectively; with this is probably associated the absence in dog muscle of the ammonium withdrawal contraction having the properties of the A.C. contraction.

(3) Ammonium causes greater replacement of potassium than the tetra-ammonium salts; with this is probably associated the fact that the latter do not produce a contraction on withdrawal, similar to that of ammonium in frog

muscle. Withdrawal of the latter salt produces a tonic contraction similar to that produced by withdrawal of ammonium in dog muscle.

(4) There is greater loss of potassium produced by ammonium in alkaline than in acid solutions; but there is greater loss of potassium in a potassium-free saline in acid solutions. As the ammonium withdrawal contraction in frog muscle is more marked in acid than in alkaline solutions, it suggests that the contraction is due to the outward passage of the ammonium ion.

(5) Slow relaxation of a contraction produced by a chemical substance may be due to tonic contraction on withdrawal of the substance or to persistence of the previous contraction, probably due to increase in viscosity of the muscle.

We wish to thank the late Professor A. J. Clark, F.R.S., for suggesting these analyses and Lt.-Col. S. S. Sokhey, I.M.S., Director, Haffkine Institute, for providing the necessary facilities.

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