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THE INCREASE IN THE RATE OF HEAT PRODUCTION OF FROG'S SKELETAL MUSCLE CAUSED BY HYPERTONIC SOLUTIONS

BY K. YAMADA*

From the Department of Physiology, University College London, W.C. 1

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SUMMARY

1. The rate of heat production of resting muscle is increased by hypertonic solutions.

2. The threshold osmolality required to produce the increased heat rate is less than 2 times normal; at 2·5–3 times normal the heat production rises to 20–50 mcal.g^{-1}.min^{-1}, which is 10–20 times the basal rate.

3. In anaerobic conditions, the effect of hypertonic solutions on heat rate is only one tenth of that in aerobic conditions.

4. A glycerol-treated muscle, with damaged tubular system, still gives a normal response to hypertonic solutions, though it does not respond to raised K^+ concentration.

5. The metabolic response to hypertonic solutions is considerably suppressed by procaine.

6. Ouabain, 10^{-5}–10^{-4} M, has no effect.

7. The response remains substantial in a muscle which has been depolarized in isotonic K_2SO_4.

8. The membrane potential is slightly reduced by hypertonic solutions, but this cannot account for the increase of the resting metabolism.

9. It is suggested that the effect may be due to the release of calcium ions, which produce an increase in myosin ATPase activity.

INTRODUCTION

It is well known that *hypotonic* solutions within a certain range enhance the twitch of frog muscle, whereas *hypertonic* solutions diminish, and in sufficient concentration abolish, the twitch (Overton, 1902; Fenn, 1936; Howarth, 1957, 1958) even though the muscle in hypertonic solutions can produce a normal action potential (Hodgkin & Horowicz, 1957).

* Present address: Department of Physiology, Juntendo University School of Medicine, Hongo, Tokyo, Japan.

Hill (1957, 1958) showed that much of the initial heat production still remains in response to a single shock in hypertonic muscle, and more recently Caputo (1966) has shown that the hypertonic muscle produces a normal contracture in response to the application of caffeine.

In the present experiments it will be shown that hypertonic solutions give rise to an increase in the rate of heat production at rest in the skeletal muscle of the frog. An increase in metabolic rate caused by hypertonic solutions has already been noticed by several authors, as measured by oxygen consumption (Sekine, Iijima, Genba, Tanaka & Kanai, 1957); by both oxygen consumption and lactic acid formation (Muller, 1962); and by both break-down of phosphocreatine and lactic acid formation (Daemers-Lambert, Debrun, Dethier & Manil, 1966).

A similar increase in heat rate and oxygen consumption has long been known to occur (*a*) when the K^+ concentration in the bathing medium is raised beyond a certain threshold but not so far as the mechanical threshold ('Solandt effect'; Hegnauer, Fenn & Cobb, 1934; Solandt, 1936; Keynes & Maisel, 1954; Hill & Howarth, 1957; Novotný, Vyskočil, Vyklický & Beránek, 1962; Muller & Simon, 1960; Novotný & Vyskočil, 1966) and (*b*) when the muscle is stretched beyond a certain length ('Feng effect'; Feng, 1932; Euler, 1935; Clinch, 1968; see Table 3).

Subcontracture concentrations of caffeine (Hartree & Hill, 1924; Saslow, 1936) and 10^{-5} M-2, 4-dinitrophenol (Ronzoni & Ehrenfest, 1936) are also known to stimulate the metabolism of the muscle.

A preliminary account of this work has already appeared (Yamada, 1968).

METHODS

Measurement of heat production

Hill–Downing thermopile. In the early experiments (Figs. 1–4) pairs of sartorii from *Rana temporaria* were used with a thermopile which was made by Downing and has been described by Hill (1932). The thermopile has 110 constantan-iron couples, giving 5760 μV/deg.

The upper (tibial) end of the muscles was attached by a chain to an isometric lever. The length of the muscle was adjusted to its resting length *in situ* (measured with the frog's legs in line). The output from the thermopile was recorded directly by a Devices M2 recorder (Devices Ltd., Welwyn Garden City) through an all-copper reversing switch to eliminate the effect of stray potentials generated outside the thermopile. The usual sensitivity of the system required for the present purpose was around 29 μV or $5{\cdot}0 \times 10^{-3}$ deg/cm deflexion on the chart. The thermopile and its glass container were submerged in a large Dewar vessel containing water at 17–20° C. The water was stirred by blowing in air.

Analysis of temperature record. When heat is produced at a constant rate by muscles on a thermopile, the deflexion observed becomes constant in a few minutes. Heat production is then balanced by heat loss. The rate of heat production is given by

$$h = 60\, yck/\mu \text{ cal.g}^{-1}.\text{min}^{-1},$$

where y is the output from the thermopile (μV), c is the specific heat of muscle (cal.g^{-1}.deg^{-1}), k is the cooling constant (sec^{-1}), and μ is the temperature sensitivity of the thermopile (μV deg^{-1}) (see, for example, Hill, 1965). The specific heat c, has been taken to be 0·88 cal.g^{-1}.deg^{-1} at all temperatures (Hill, 1931). k was determined, in the usual way, from the time course of fall of temperature following brief warming of the muscle by 50 kHz sinusoidal current.

'*Integrating*' *thermopile.* In the later experiments, the integrating thermopile developed by Wilkie (1963, 1968) was employed. With a muscle on the thermopile the absolute calibration is obtained by passing a current through the calibrating wire and adjusting it until a steady deflexion is observed. Measuring the current I A, the rate of heat production in the calibrating wire of resistance $R\Omega$, is 0·239 I^2R cal/sec. If the steady deflexions were d_0 scale divisions, the calibration number *for steady deflexions* would be $0{\cdot}239 \times I^2R/d_0$ cal/sec for each scale division. This value can be converted into the units cal.g^{-1}.min^{-1} if the weight of the muscle is known. The output from the thermopile was led, via an all-copper reversing switch and a shift and a calibrating circuit, to a Downing galvanometer with photo-electric amplification (Wilkie, 1968). The sensitivity needed for a single sartorius was generally about 2 μV/cm deflexion.

Solutions

The composition of the normal Ringer was (all concentrations in mM): NaCl, 115; KCl, 2·0; $CaCl_2$, 1·8; NaH_2PO_4, 0·68; Na_2HPO_4. 1·3. The compositions of the hypertonic solutions, to give an increase in osmolality of approximately x times, were:

(A) (Sodium chloride increased), NaCl, x times 115; other components similar to normal Ringer (denoted as xR NaCl).

(B) (Sodium and potassium chlorides increased), NaCl, x times 115; KCl, x times 2·0; other components similar to normal Ringer (xR NaCl–KCl).

(C) (Sucrose added), $(x-1)$ times 7·5 g sucrose added to 100 ml. normal Ringer (xR sucrose; see Dydyńska & Wilkie, 1963).

(D) (Glycerol Ringer) 200 (x = 1·9) or 400 mM (x = 2·8) glycerol added to normal Ringer.

In some experiments (E) (Isotonic K_2SO_4), (K_2SO_4 95 mM, saturated with solid $CaSO_4$ (Hill & Howarth, 1957) was used instead of normal Ringer.

(F) Solutions for testing the Solandt effect were made by the addition of KCl to normal Ringer.

The solutions were stirred with oxygen unless otherwise stated.

Measurements of membrane potentials

Membrane potentials of the muscle in various hypertonic solutions were measured using conventional micropipette technique.

RESULTS

The relation between tonicity of medium and rate of heat production. The pair of sartorii mounted on the Hill–Downing thermopile was allowed to rest in the normal oxygenated Ringer solution for about 1 hr. From time to time the thermopile was raised from the medium and the temperature of the muscle was recorded. The rate of heat production of a muscle in normal Ringer, before the application of any modified solution, was $0{\cdot}83 \pm 0{\cdot}14$ mcal.g^{-1}.min^{-1} at 17–20° C (mean of eight measurements in

eight muscles, ± S.E. of mean). This is considerably smaller than the value of 2·4 mcal.g^{-1}.min^{-1} obtained by Hill & Howarth (1957). Normal Ringer was then replaced by the solution under study. After 5–10 min the thermopile was raised from the new solution and the temperature of the muscle was recorded for the following 5 min. The muscle was then re-immersed in the solution for 5 min. This procedure was repeated three or four times (total 30–40 min) and the solution was then replaced by normal Ringer.

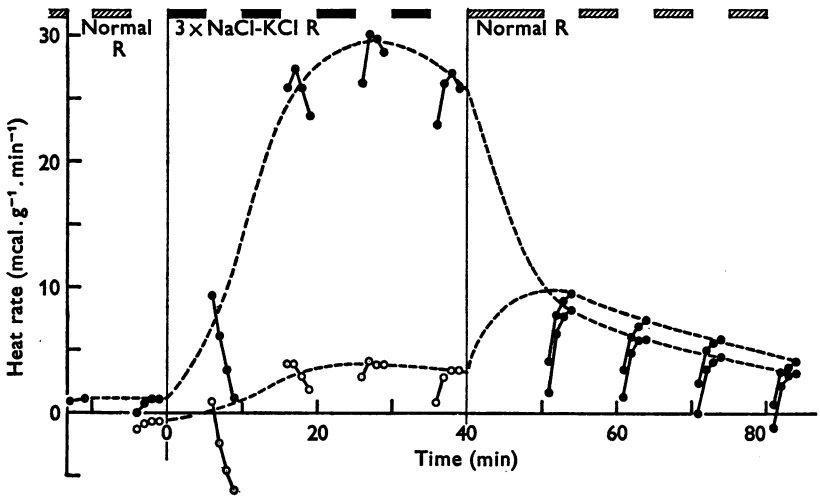

Fig. 1. The hypertonicity response produced by 3R (NaCl–KCl, B) in oxygen (filled circles) and its suppression (open circles) in the absence of oxygen (i.e. in N_2). The normal Ringer was replaced by the hypertonic solution at time zero. After 40 min of the treatment the muscle was returned to normal Ringer. The heat rate can only be measured while the muscles are *not* immersed: hence the breaks in the blocks at the top of the Figure. Dashed lines have been drawn so as to connect one group of circles to the next.

The filled circles in Fig. 1 show the effect when using solution 3R NaCl–KCl (B). The periods when the muscle was in the solution and out of it are shown by blocks and the gaps along the top respectively. The readings were made at 1 min intervals. After the first withdrawal from the hypertonic solution, the temperature of the muscle falls. This effect arises because, until it has come to equilibrium, the vapour pressure of the muscle is higher than that of its surroundings and water tends to evaporate, leading to cooling. The reverse effect, when normal Ringer is replaced, is visible but less striking. After this early period was over (in about 20 min) the deflexion reached an almost steady level 3–4 min after the muscle had been raised from the solution, and this level was taken as indicating the resting heat rate at that moment. The 3R (NaCl–KCl, B) hypertonic solution (filled circles) produces a very large increase in resting heat rate, which

reaches a maximum in 20–30 min. Following the return to normal Ringer, the resting heat rate returned rapidly at first, and then gradually, towards the original level.

Figure 2 shows the maximal heat rate attained in 20–30 min in various solutions of osmolality between 0·5 and 3R. The rate approaches 30 mcal. $g^{-1}.min^{-1}$ in 3R NaCl–KCl (B); hypotonic solutions have no effect. The threshold hypertonicity required to produce an increased resting heat rate has not been determined accurately, but from this graph it is quite certain that it lies at less than 2R.

Curare (10^{-5} g/ml.) does not change the effect of hypertonic solutions. Some decrease in heat production might have been expected, because it has been reported (Furshpan, 1956) that hypertonic solutions cause an increase in the spontaneous activity at motor nerve endings in frog's skeletal muscle.

The effect of anaerobic conditions on the hypertonic response. Since the Solandt effect is greatly reduced by anaerobic conditions (Hill & Howarth, 1957), while the stretch response is reported not to be (Feng, 1932; Euler, 1935), the effect of anaerobic conditions on the hypertonicity response has been studied.

The experiments with NaCl–KCl (B) Ringer were repeated, employing the technique already described save that all the solutions had been vigorously bubbled with O_2-free nitrogen ($O_2 < 0{\cdot}001\,\%$). Hypertonic solutions then produce only a small effect (see Fig. 1 open circles). For example in 2·5 R NaCl–KCl (B) the rate was 2·1 mcal.$g^{-1}.min^{-1}$ (mean of three measurements in three muscles) as compared with a value of 21·5 in the presence of oxygen (mean of four measurements in four muscles). The rise in metabolic rate when the previously anaerobic muscle is exposed to oxygen probably shows that an oxygen debt had been incurred.

The effect of 'glycerol treatment'. The solutions (D) made hypertonic by adding glycerol to normal Ringer (approx. 1·9x with 200 mM glycerol or 2·8x with 400 mM glycerol) had almost no effect on the resting heat rate of the muscle. This is a curious discrepancy from the other hypertonic solutions, since it has been shown that the glycerol hypertonic solution produces a shrinkage of the fibre similar to the other NaCl or sucrose hypertonic solutions, at least during the earlier stage (say the first 10 min) of the soaking (Krolenko & Adamjan, 1967; Caputo, 1968).

On returning the muscle to normal Ringer (which causes marked structural change in the endoplasmic reticulum according to Eisenberg & Gage, 1967; Howell & Jenden, 1967; Krolenko, Adamjan & Shwinka, 1967) the rate of heat production increased almost as much as during the normal aerobic response (Figs. 3 and 4). This was associated with weak spontaneous contractions and spikes could be recorded from surface

electrodes. The spikes were found to be suppressed in sodium-free choline Ringer as had been reported by Eisenberg & Eisenberg (1968*b*). Howell (1969) reported that after the removal of glycerol the majority of fibres were depolarized considerably, in some muscles well beyond the threshold even for the mechanical response; and this seems, at least partly, to be the cause of the increase in heat rate. This high heat rate observed after

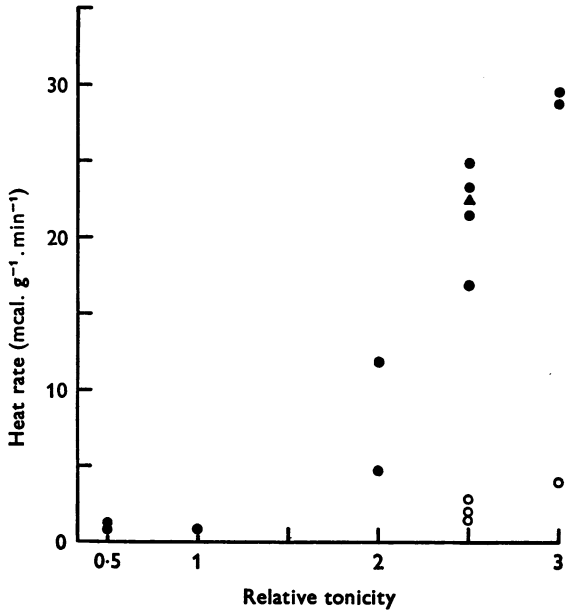

Fig. 2. The relation between the tonicity of the solution and the maximal increase in heat rate that it produced in eight pairs of sartorii at 19–20·5° C. Filled circles (●) represent the response to hypertonic solutions in which both NaCl and KCl concentrations were raised (solution B) except for one encircled dot (⊙), where only NaCl was increased (solution A). One filled triangle (▲) shows the response to 2·5R NaCl-KCl (B) after the muscle had been removed from glycerol Ringer. The open circles (○) show the response to 2·5 and 3R NaCl–KCl (B) under anaerobic conditions.

returning muscles from glycerol Ringer to normal Ringer largely disappeared over the following two hours. When hypertonic solutions were applied to a muscle in this condition, the usual increase in heat rate was observed (Figs. 3, 4; see also Fig. 2). A solution with high KCl content was virtually without effect (Fig. 4). The same result has recently been reported by Van der Kloot (1969). The failure to elicit a Solandt effect could be due either to the depolarization of the majority of the fibres (Howell, 1969) or to damage to the T-tubule or its disconnexion from the surface membrane following the removal of glycerol (Eisenberg & Gage, 1967; Howell & Jenden, 1967; Krolenko *et al.* 1967; Eisenberg & Gage,

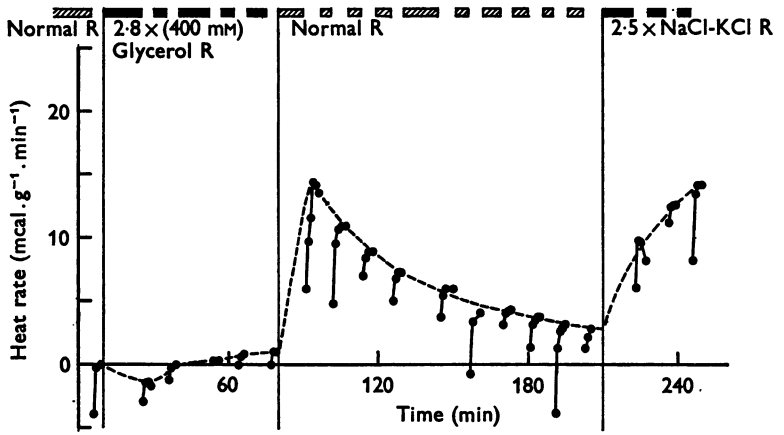

Fig. 3. The effect of 'glycerol' treatment. The muscle was immersed in Ringer solution with 400 mM glycerol (D) (osmolality 2·8 times normal) for 70 min with breaks for heat measurement as indicated along the top. This solution had almost no effect on the heat rate. On returning to normal Ringer the muscle showed an increased heat rate. After this had fallen almost to a normal value 2·5R NaCl–KCl (B) gave an increase of almost normal amount.

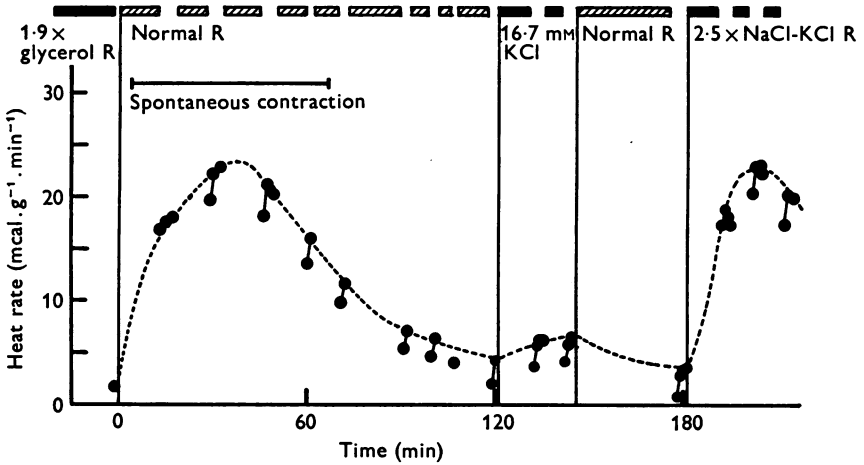

Fig. 4. The muscle was first exposed for 1 hr to Ringer with 200 mM glycerol (D). It was then returned to normal Ringer; the heat rate increased almost as much as in the hypertonic response, which is shown on the right. Weak twitches were observed after the return to Ringer from the glycerol solution. The addition of 16·7 mM-KCl to the Ringer (F) was almost without effect after 'glycerol treatment'.

1967; Eisenberg & Eisenberg, 1968*a*, *b*; Nakajima, Nakajima & Peachey, 1969; Howell, 1969).

The effect of procaine. As is shown in Table 1, 7 mM procaine HCl reduces the hypertonicity response substantially. Procaine is even more effective in suppressing the Solandt effect. Thus 7 mM procaine almost completely suppresses the effect of Ringer solution containing 20 mM-KCl. Novotný *et al.* (1962) reported that an even lower concentration of procaine (1 mM) was enough to suppress the extra oxygen consumption in high K solution.

TABLE 1. The effect of procaine and ouabain on the Solandt effect and hypertonicity response, and the effect of previous depolarization with K_2SO_4 upon the hypertonicity response. Integrating thermopile. 17–19° C

Muscle	Solution	Maximum increase of heat rate* (mcal.g^{-1}.min^{-1})
1	Normal Ringer + 20 mM-KCl	26·4
	Normal Ringer + 20 mM-KCl + 1 mM procaine	19·4
	Normal Ringer + 20 mM-KCl + 3·5 mM procaine	11·7
	3R NaCl–KCl hypertonic solution	48·8
	3R NaCl–KCl + 3·5 mM procaine	26·6
2	Normal Ringer + 20 mM-KCl	29·0
	Normal Ringer + 20 mM-KCl + 7 mM procaine	1·3
	3R NaCl–KCl	59·2
	3R NaCl–KCl + 7 mM procaine	17·7
3	3R NaCl–KCl	49·4
	3R NaCl–KCl + 10^{-5} M ouabain	52·1
4	Normal Ringer + 19 mM-KCl	64·3
	Normal Ringer + 19 mM-KCl + 10^{-4} M ouabain	64·3
5	3R NaCl–KCl	46·0
	3R NaCl–KCl + 10^{-4} M ouabain	50·6
6	Normal Ringer + 15 g/100 ml. sucrose (3R sucrose)	60·2
	Isotonic K_2SO_4 + 15 g/100 ml. sucrose	53·0
7	Normal Ringer + 15 g/100 ml. sucrose	51·4
	Isotonic K_2SO_4 + 15 g/100 ml. sucrose	32·5

* The basal level of heat rate was about 2 mcal.g^{-1}.min^{-1} in the presence of oxygen.

The effect of procaine developed very rapidly as was noted by Novotný *et al.* (1962); for this reason the drug was applied to the muscle with the hypertonic solution.

The effect of cardiac glycoside (ouabain). The effect of ouabain was tested on both the hypertonicity response and the Solandt effect to see whether the ionic pump in the membrane is involved in these responses. As is shown in Table 1, 10^{-5} and 10^{-4} M ouabain has no effect on either type of response.

The hypertonicity response of a muscle depolarized in K_2SO_4. Since J. V. Howarth (unpublished results) has shown that the stretch response (Feng effect) remains normal in isotonic K_2SO_4, it is of interest to see whether the hypertonicity response is likewise still present in muscle depolarized by K_2SO_4. As is seen in Fig. 5, sucrose added to isotonic K_2SO_4 solution (E) still produced a substantial amount of increase in the heat rate. The

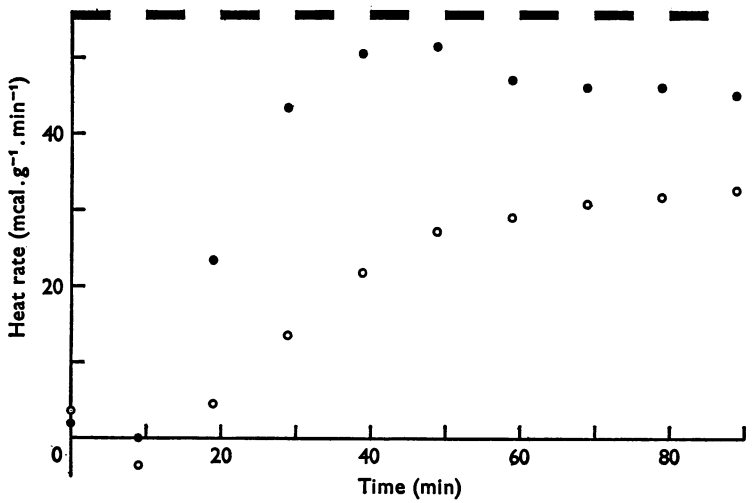

Fig. 5. Hypertonicity response produced by 3R sucrose (C) (●); and by isotonic K_2SO_4 (E) with 15 g sucrose/100 ml. (this has the same tonicity as 3R sucrose), (○) after the muscle had recovered from the brief contracture and associated temperature rise caused by depolarization. 19–20° C.

time course of this response is somewhat slower and the maximal value attained is also somewhat smaller than the normal hypertonicity response as seen in Fig. 5 and in Table 1.

The changes in membrane potential in hypertonic solutions. Changes in membrane potentials in sucrose (solution C) and NaCl–KCl (B) hypertonic solutions of various osmolality were recorded and are presented in Table 2. Depolarization of about 10 mV has been shown to occur in strong hypertonic solutions, as seen in the table; however in weak hypertonic solutions, hyperpolarization is more likely to be encountered. The smallest resting potential in a set of measurements in each hypertonic muscle is also shown; it never approaches the threshold of -65 mV required for the Solandt effect (Hill & Howarth, 1957).

DISCUSSION

In the present study the rate of heat production of the resting frog sartorius was found to rise when the muscle was placed in a hypertonic solution. Three different types of solution have been used: A, in which the NaCl alone was increased; B, in which both NaCl and KCl were increased; and C, in which sucrose was added to normal Ringer. These would be

TABLE 2. Resting membrane potentials in normal Ringer and in hypertonic solutions

Muscle	Solutions	Number of fibres	Mean resting potential (mV)	± S.E. of mean	Lowest value in hypertonic solution (mV)
1	Normal Ringer	7	−89·7	0·9	
	3R sucrose, C	8	−82·0	1·3	−75·2
	Normal Ringer	5	−83·2	1·8	
2	Normal Ringer	6	−93·1	0·6	
	3R sucrose, C	9	−86·4	2·9	−70·8
	Normal Ringer	11	−87·0	0·8	
3	Normal Ringer	6	−90·5	0·9	
	3R sucrose, C	8	−79·8	2·2	−70·0
	Normal Ringer	9	−84·0	1·2	
4	Normal Ringer	6	−94·6	1·1	
	3R sucrose, C	8	−91·4	3·6	−76·7
5	Normal Ringer	7	−93·6	1·9	
	3R NaCl–KCl, B	9	−81·2	1·9	−75·2
6	Normal Ringer	7	−86·9	0·9	
	3R NaCl–KCl, B	6	−73·3	2·2	−65·6
	Normal Ringer	4	−74·4	0·9	
7	Normal Ringer	8	−93·1	1·1	
	2R sucrose, C	7	−103·9	0·9	−100·4
8	Normal Ringer	6	−91·5	1·1	
	2R sucrose, C	8	−97·6	1·3	−90·4
9	Normal Ringer	7	−90·7	2·5	
	2R sucrose, C	8	−87·9	2·1	−80·7

The solutions were applied in the order indicated.

expected to give rise to rather different Donnan equilibria (Boyle & Conway, 1941). K^+ and Cl^- would be expected to come out of the cell in increasing amount, in the order C > A > B the expected final state being that the membrane potential should be lowest with B; nevertheless, all three solutions had a similar effect on the heat production rate. This suggests that the hypertonic solutions exerted their effect on the resting

metabolism by their high osmolarity rather than by a change in ionic balance across the membrane.

The hypertonicity response is similar to the Solandt effect in several respects, and both of them differ from the Feng effect (Table 3). One puzzling feature is that the Feng effect is said not to depend on the presence of oxygen, so it is hard to imagine where the heat comes from under anaerobic conditions.

TABLE 3. Comparison between the hypertonicity response, the Solandt effect and the Feng effect

Stimulus	Hypertonicity of the medium	Depolarization of membrane [2 3]	Stretching[1 6]
Threshold	Less than 2 × normal osmolality	About −65 mV[2 3]	To 1·2 × normal length [6]
Maximal heat rate	30–50 mcal.g^{-1}.min^{-1}	40 mcal.g^{-1}.min^{-1}[3]	10–14 mcal g^{-1}.min^{-1}[6]
Effect of anaerobic condition	Reduced to 1/10	Reduced to 1/10[3]	Reduced to 1/2[1]
Effect of procaine (< 10 mM)	Substantially reduced	Suppressed completely [5]	Potentiated[6]
Effect of ouabain (10^{-4} M)	Not affected	Not affected	—
In K_2SO_4 (isotonic)	Remains substantial	—	Remains normal[4 6]

1. Feng, 1932; Euler, 1935.
2. Solandt, 1936.
3. Hill & Howarth, 1957.
4. Howarth, J. V., unpublished.
5. Novotný *et al.* 1962.
6. Clinch, 1968.

Changes in resting membrane potentials in hypertonic solutions

It is reasonably certain that the Solandt effect is produced by the depolarization of the surface membrane (cf. Hill & Howarth, 1957), and since the membrane is depolarized by hypertonic solutions (Ishiko & Sato, 1957), it was important to test whether or not the hypertonic solutions exert their effect on the resting metabolism simply by depolarizing the membrane. This possibility has been excluded for the following reasons:

1. The depolarization of about 10 mV does not nearly approach the amount required to 'trigger off' the Solandt effect (about 25 mV).

2. After the 'glycerol treatment' the muscle still responds to hypertonic solutions quite normally; however, solutions that merely depolarize the membrane are almost without effect.

3. A muscle completely depolarized with isotonic K_2SO_4 still gives a substantially normal hypertonic response.

Stimulation of oxidative phosphorylation

In view of its elimination by anoxia, the effect of hypertonic solutions must be produced via oxidative phosphorylation, the action taking place either *directly* on mitochondria or *indirectly* via some other process, for example a rise in the sarcoplasmic ADP level (Chance & Weber, 1963). This might in turn be produced by the increased activity of either membrane ATPase or actomyosin ATPase.

Direct stimulation of mitochondria. In the present study the effect of hypertonic solution on the resting metabolic rate has been found to be considerably reduced by procaine. It is unlikely that this is due to a direct action on the mitochondria, because procaine is known not to prevent the increase in respiration of a muscle caused by dinitrophenol (Novotný *et al.* 1962), which acts by an uncoupling of phosphorylation (Ronzoni & Ehrenfest, 1936). On the other hand, procaine abolishes or reduces the contractile response both to caffeine (in frog skeletal muscle, Schüller, 1925; Hardt & Fleckenstein, 1949; Feinstein, 1963; Caputo, 1966; Lüttgau & Oetliker, 1968; and in rat skeletal muscle, Gutmann & Sandow, 1965), and to long depolarizing pulses (in voltage-clamped snake twitch muscle fibres, Heistracher & Hunt, 1969). These facts suggest that hypertonic solutions operate on the part of the mechanism that is involved in the activation of contraction.

Indirect stimulation of mitochondria. 1. Membrane ATPase activity. Keynes (1954) found the rate of exchange of ^{24}Na in a frog's sartorius to be increased by raising the K^+ concentration outside from 2·5 to 10 mM. According to Keynes & Maisel (1954) the minimum power necessary for Na extrusion is about one tenth of the resting metabolic rate. Edwards & Harris (1957) also showed that Na exchange is increased by increasing the K^+ concentration outside from zero to 4 mM, and this flux is very sensitive to ouabain ($< 10^{-5}$ M). Harris (1954) also showed that stretch caused an increased rate of Na extrusion from the frog's sartorius. In view of these findings it is important to consider whether active extrusion of internal sodium might account for the increased metabolic rate observed. This point was tested by using ouabain and it was found that a concentration as high as 10^{-4} M did not suppress the hypertonicity response.

According to Skou (1964), the inhibitory effect of ouabain on the activity of the membrane [(Na^+–K^+)-activated] ATPase is dependent on the Na^+ and K^+ concentrations. E. J. Harris (personal communication) showed that the effect of ouabain on the sodium pump is readily suppressed in high K media. However, the suppression is of a competitive nature; for instance, the inhibition of Na extrusion by 10^{-6} M ouabain in the cat heart muscle is suppressed by 25 mM-K^+, whereas the effect of 10^{-5} M ouabain

is not (Page, Goerke & Strom, 1964). It is unlikely, therefore, that 10^{-4} M ouabain, which is ten times stronger than the concentration for complete suppression of the active Na extrusion in frog's sartorius (Edwards & Harris, 1957), failed to inhibit active transport in the present experiments. The persistence of the metabolic effect under these circumstances suggests that membrane ATPase is not the cause of it.

Indirect stimulation of mitochondria. 2. Stimulation of metabolic enzymes. It has already been seen that the hypertonic response is probably not due to an uncoupling of oxidative phosphorylation. On the other hand, one part of the metabolic chain that is influenced by Ca (and might therefore be affected by procaine) is phosphorylase *b* kinase (Green & Cori, 1943) which is known to be activated by even a low level of Ca. This in turn brings about an increased rate of glycogen break-down (Krebs, Graves & Fischer, 1959; Ozawa & Ebashi, 1967). It is possible, therefore, that hypertonic solutions act by raising the internal Ca^{2+} level and that this stimulates mitochondrial respiration indirectly via phosphorylase.

Indirect stimulation of mitochondria. 3. Actomyosin ATPase activated by an increase in sarcoplasmic Ca^{2+}. If the sarcoplasmic Ca^{2+} level is increased in hypertonic solutions, this would also give rise to actomyosin ATPase activity (Ebashi, 1961; Weber & Winicur, 1961), which in turn would stimulate mitochondrial respiration through an increased sarcoplasmic ADP level (cf. Chance & Weber, 1963).

To sum up, it seems probable that the hypertonic response is caused by an increase of intracellular Ca^{2+} which then stimulates oxidative phosphorylation through phosphorylase or actomyosin ATPase.

The site of action of hypertonic solutions. It has been shown that elements of the sarcoplasmic reticulum in frog's skeletal muscle are dilated in sucrose hypertonic solution (Huxley, Page & Wilkie, 1963; Freygang, Goldstein, Hellam & Peachey, 1964). More recently Birks & Davey (1969) using different fixation procedures from the previous authors have shown an even more marked swelling of the H-zone sacs and the longitudinal tubules of the sarcoplasmic reticulum. These striking deformations produced by hypertonicity might well trigger Ca release and the consequent increase in oxidative metabolism.

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