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THE TRANSFER OF SODIUM IONS BETWEEN MAMMALIAN MUSCLE AND THE SURROUNDING MEDIUM

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Previous investigations[1,2] have dealt with the movement of potassium ions across the cell membrane separating the intracellular fluid of mammalian muscle from the extracellular space. These studies showed that the uptake of labelled K by muscle cells when the tissue is immersed in a saline medium containing a physiological concentration of K is a process strongly dependent on the supply of metabolic energy, and that any agency which interferes with the metabolism of the tissue inhibits the uptake of K. In contrast, the loss of K from the cells is by this criterion a passive process. It was further shown that when a muscle is incubated *in vitro* in a solution containing 5 mmoles/l of K, the exchange of tissue K proceeds until some 50% of the analytically determined K has turned over, after which no further exchange takes place. Increasing the concentration of K in the incubation medium brings about an increase in the extent of tissue K exchange, but does not affect the time constant governing the process. Complete exchange is obtained with an external K concentration of 20 mmoles/l.

These studies have now been extended to include the transfer of sodium ions in muscle. No evidence has been found for the presence of difficultly exchangeable Na in the muscle analogous to the inexchangeable K, such as has been reported by CONWAY AND CAREY[3] and by HARRIS AND STEINBACH[4] in frog muscle. The efflux of Na from the muscle and influx of K have been shown to be mutually dependent, inasmuch as changes in the external concentrations of either ionic species affect both fluxes similarly in most cases.

References p. 339.

METHODS

The tissue used in this work was the m. extensor digitorum longus of the rat. The animals were killed by decapitation, the muscles dissected out, and small cotton loops tied around the tendons at each end. They were then weighed on a small torsion balance, and immersed in a saline medium which contained a proportion of either the total Na or K ions labelled with a radioactive isotope. The method used to measure the influx of K has been fully described in an earlier paper[1]. The procedure for measurement of Na efflux is as follows. After a period of incubation in radioactive solution (not less than 4 h), the muscles were transferred to appropriate media containing no isotope. At intervals thereafter the muscles were removed from the incubation media, lightly dried with filter paper, and placed on flat plastic holders which had small pegs projecting, over which the loops at the ends of the muscles could be slipped. The holder was placed under an end-window Geiger counter, and the radioactivity of the sample determined for two one-minute periods. The muscle was then returned to the incubation medium. The plastic holders permitted reasonable reproducibility of position of the muscle under the counter. At the end of incubation the muscle was reweighed to determine the amount of swelling, the loops cut off and weighed separately, and the tissue dissolved in a few drops of silica-distilled nitric acid. The weight of the digest was made to 1 g, and the radioactivity of this solution measured. This was compared with the activity of a suitable dilution of the radioactive solution used in the initial part of the experiment. Since the total Na (or K) content of the radioactive saline was known, a relation could be established between counts/min in the muscle and its labelled ion content. The radioactive isotopes used were ^{22}Na and ^{42}K, which were obtained in the form of NaCl and K_2CO_3 respectively from Atomic Energy of Canada Limited, Ottawa, Ont.

The saline media used had the following composition: at 20° C HCO_3^- 43, Cl^- 110.5; for 0° C HCO_3^- 86, Cl^- 67.5; plus in both cases Na^+ 144, K^+ 5.5, Ca^{++} 2, Mg^{++} 1, SO_4^{--} 1, glucose 10 (all in mmoles/l). These solutions had a pH near 7.4 when equilibrated with 95 % O_2–5 % CO_2. For those experiments where the concentration of K in the medium was varied, the Na concentration was adjusted to maintain isotonicity. When the Na concentration was altered, the medium used contained no bicarbonate, and was bubbled with 100 % O_2; isotonicity was preserved by the use of choline chloride to make up the deficiency in NaCl. Total Na and K analyses of the muscle digests were performed with an EEL flame photometer.

RESULTS

When a rat muscle was incubated in a saline solution containing a proportion of ^{22}Na ions, the tissue Na undergoes exchange with the Na of the medium, until parallel estimations of the radioactivity and Na content of the muscle indicate that complete exchange has occurred. With the extensor digitorum longus this process requires about 4 h (a typical experiment gave values of 48% exchange in 1 h, 72% in 2 h, and 96% in 4 h).

If the muscle is then placed in a non-radioactive medium, the activity of the muscle declines as inactive Na replaces the labelled ions in the tissue. A semi-logarithmic plot of the tissue radioactivity remaining against time yields a curve of the type shown in Fig. 1. The initial non-linear portion represents diffusion of Na from the extracellular spaces—the movement of this fraction of the total muscle Na has been described elsewhere[5]. Extrapolation of the linear portion of the curve of Fig. 1 back to the ordinate gives a value of the muscle Na which is most probably associated with the cells themselves. The question whether this fraction of the Na is truly intracellular or is in part adsorbed to the cell surfaces will be further considered below. The slope of the line is a measure of the rate constant (k_2) governing the movement of this Na, which will hereinafter be referred to as Na_i. Mean values for Na_i and k_2 which have been found in the present work are, at 20° C, 13.8 μequiv./g and 1.59 h^{-1} respectively. Since it is the flux of an ion across a cell membrane which is of greatest interest, the Na flux in this case is $13.8 \times 1.59 = 21.9$ μequiv./g/h (11 determinations, S.D. ± 4.4). It was previously estimated that the volume/surface ratio for the

References p. 339.

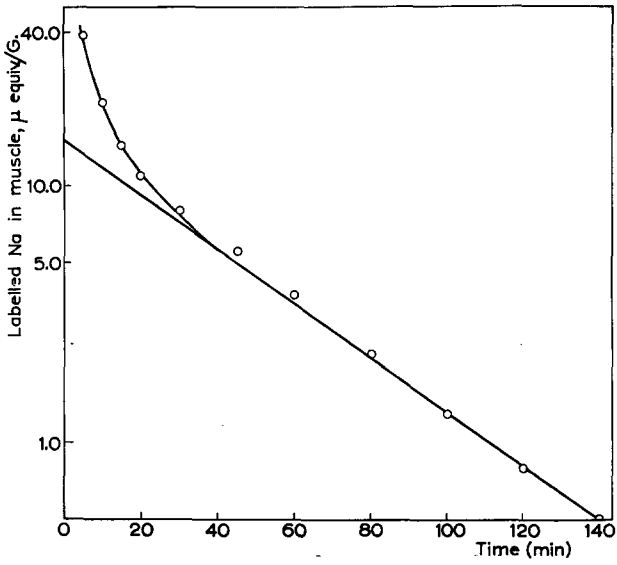

Fig. 1. The time course of loss of labelled Na from a muscle to saline solution at 20° C. The initial non-linear portion of the curve is made up by labelled Na in the extracellular fluid and in the water of swelling.

fibres of these muscles is 7.43×10^{-3} mm[1], so that the flux of Na per unit of area may be estimated as 4.5×10^{-8} μequiv./mm²/sec.—a value about twice that for K influx calculated previously[1]. It should be emphasized again that no distinction can be drawn from these results as to whether the above figure truly is a measure of exchange across a cell membrane or a desorption from the surface of the cell.

The extrusion of Na from the muscle has been found to be dependent on the K concentration in the bathing solution ($[K_e]$), and is also affected by the external Na concentration. Table I indicates that reduction of $[K_e]$ reduces the Na efflux, while increasing $[K_e]$ raises it. Reduction of the normally high $[Na_e]$ to 0 also brings

TABLE I

THE EFFECT OF CHANGES IN EXTERNAL K CONCENTRATION ON Na EFFLUX

$[K_e]$ mmoles/l	k_2 h^{-1}	$k_2 Na_i$ μequiv./mm²/sec × 10^8
0	1.50	4.25
5.5	1.62	4.60
11	1.77	5.05
22	2.72	7.75
0	1.54	5.45
5.5	1.82	6.30
11	2.22	7.15
22	3.00	9.40
0	1.00	3.70
5.5	1.46	4.15
11	1.60	9.30
22	1.94	8.55

TABLE II

THE EFFECT OF CHANGES IN EXTERNAL Na CONCENTRATION ON Na EFFLUX

$[Na_e]$ mmoles/l	k_2 h^{-1}	$k_2 Na_i$ μequiv./mm²/sec × 10^8
144	1.40	3.55
0	1.43	2.45
0	1.58	1.90
144	1.94	4.80
0	1.09	2.90
144	1.09	3.15
0	1.43	2.05

References p. 339.

about a drop in the Na efflux (Table II) although, as judged by analysis, the concentration of K in the tissue does not rise to compensate for the extrusion of Na which cannot be replaced from the solution. In this case therefore an anion must be excreted to accompany the Na.

Prior work showed that the extent of exchangeability of the total tissue K is dependent on $[K_e]$[1]. A possible explanation of the incomplete exchangeability in low K medium is provided by the "long pore" theory (see *e.g.*[6]), whereby if there is a slow net loss of intracellular K the flow of unlabelled intracellular ions down the pores might hamper the uptake of labelled K from the external medium. A higher $[K_e]$ provides both a larger number of labelled ions and as well tends to prevent a net loss of intracellular K. Earlier work showed that K influx at $[K_e]$ = 5 mmoles/l was $2.5 \cdot 10^{-8}$ μequiv./mm²/sec, and $3.9 \cdot 10^{-8}$ μequiv./mm²/sec at $[K_e]$ = 20 mmoles/l[1]. The present experiments have shown that the influx of K is affected also by changes in $[Na_e]$. Table III indicates that reduction of $[Na_e]$ to 0 results in a 50% diminution in K influx.

TABLE III

THE EFFECT OF CHANGES IN EXTERNAL Na CONCENTRATION ON K INFLUX

$[Na_e]$ mmoles/l	k_2 h^{-1}	$k_2 K_i$ μequiv./mm²/sec × 10^8
144	0.29	1.70
72	0.38	1.55
36	0.38	1.55
0	0.52	0.85
144	0.34	2.90
14.5	0.63	2.15
0	0.53	1.80
0	0.59	1.70
144	0.54	4.55
0	0.78	2.10

The extrusion of Na ions from a muscle is against a gradient of concentration, and may be presumed therefore to be an energy-consuming process. Table IV shows that the rate constant governing efflux, and therefore the flux itself, is reduced at 0° C as compared with 20° C, this reduction being apparent whether the bathing solution was saline, K phosphate solution (154 mmoles/l with respect to K^+, pH 7.0), or 5% glucose. In the cases of the phosphate and glucose solutions the fluxes at 20° are also reduced compared with the flux in saline. This may, in the glucose solution at least, be in some way connected with the liberation of an anion from the tissue, since negative charges must be lost with the Na ions to maintain electrical neutrality.

DISCUSSION

The efflux of Na from rat muscle (diaphragm) has been studied by CREESE[7]. As in the present experiments, CREESE found that all of the tissue Na underwent exchange with the Na of the surrounding medium, and that there was no evidence for a dif-

References p. 339.

TABLE IV

EFFECT OF TEMPERATURE ON Na EFFLUX INTO VARIOUS SOLUTIONS

Bathing solution	*20° C*		*0° C*	
	k_2 h^{-1}	$k_2 Na_i$ μequiv./mm²/sec $\times 10^8$	k_2 h^{-1}	$k_2 Na_i$ μequiv./mm²/sec $\times 10^8$
Saline (5.5 mmoles/l K^+)	1.54	5.15	0.61	2.40
			0.59	1.75
	1.54	4.35	0.97	2.80
	1.94	4.80	1.22	3.10
	1.09	3.15	0.80	2.00
K phosphate	1.33	1.90	0.70	1.25
(154 mmoles/l K^+)	1.82	2.05	0.75	1.60
Glucose (0 mmoles/l K^+)	1.33	2.60	0.65	2.15

ficultly exchangeable Na fraction similar to that reported by CONWAY AND CAREY[3] and by HARRIS AND STEINBACH[4] in frog muscle. This difference between mammalian and amphibian muscle has no explanation at the moment. The efflux of Na from diaphragm calculated from CREESE's figures is $31 \cdot 10^{-8}$ μequiv./mm²/sec at 38° C, a value considerably higher than those reported here, even allowing for the difference in temperature of incubation.

The temperature coefficient for the movement of Na measured in the present series of experiments works out at a Q_{10} of 1.35 in saline solution. This is lower than that observed earlier for K influx ($Q_{10} = 2.4$[2]), and suggests that the rate-controlling step being measured here is not the transport of Na across a cell membrane by an active, *i.e.* energy-consuming, process, but rather a physical desorption of the ions from surfaces in the tissue, from which in turn exchange with the cellular contents take place.

HARRIS[8] has described this condition, where the rate constant governing desorption from a surface, designated "n", is slower than the rates of migration of the Na from the surface inwards (rate constant "n_1"), or from the interior outwards to the surface (rate constant "n_2"). If the labelled surface Na (Na_s^*) is a constant fraction "f" of the total labelled Na, extracellular Na being excluded; that is, if

$$Na_s^* = f Na_i^* = f(Na_s^* + Na_c^*) \qquad (1)$$

then the equations describing the time course of loss of Na* from the muscle to an inactive solution are:

$$\frac{dNa_c^*}{dt} = n_1 Na_s^* - n_2 Na_c^*$$

and

$$\frac{dNa_s^*}{dt} = n_2 Na_c^* - n Na_s^* + n_1 Na_s^*$$

Adding, one has

$$\frac{d(Na_s^* + Na_c^*)}{dt} = -n Na_s^*.$$

Substituting from (1) and integrating gives:

$$Na_s^* + Na_c^* = (Na_{s_0}^* + Na_{c_0}^*) \cdot e^{-fnt},$$

where the subscript $_0$ indicates the values at time 0.

References p. 339.

The total labelled Na thus falls exponentially with a time constant "fn", which is less than the true rate of desorption in the ratio $Na_s/(Na_s + Na_c)$. This treatment seems to satisfy the experimental results found in the present work, where the low Q_{10} value and single rate constant governing Na outflow alike indicate a physical desorption as the rate-controlling step.

Reduction of the external K concentration brings about a net loss of tissue K as determined by analysis. This K loss is made up by a gain of Na, and at the same time the turnover rate of the Na is lowered: this too has been reported to occur in frog muscle[8]. Raising $[K_e]$ in saline solution increases the rate of extrusion of Na (Table V); however in a solution of very high $[K_e]$ (K phosphate), the rate of desorption of Na is not increased, and the total flux is in fact approximately halved. Table V summarizes the values obtained in this and the preceding paper[1] for the fluxes of K and Na. It appears that, except in K phosphate solution, an increase in K flux is associated with an increased Na flux, and *vice versa*. The discrepant behaviour in K phosphate may possibly be explained as follows. If the effect of change in $[K_e]$ is not of the same magnitude for the various rate constants "n", "n_1" and "n_2", and in particular if "n_1" and "n_2" become greater than "n" in a solution with very high $[K_e]$, then the loss of Na* will be described by an equation involving two exponential terms with time constants "n_2" and "$n + n_1$"[8]. Of these, "$n + n_1$" will be greater than "fn" before measured. Since in the present series of experiments it has been the slow component only of the total Na* which has been followed, it may be that the flux described for K phosphate solution is in fact equal to n_2Na_c, instead of fNa_i $(= fn(Na_c + Na_s))$ as measured in saline solution. If this is so, the inferences seem to be (a) that "n_2" is little affected by $[K_e]$, with which the lack of effect of $[K_e]$ on the rate constant governing K influx should be compared[1]; and (b) that Na_c is of the order of $1/2Na_i$. HARRIS[8] has also estimated that in frog muscle "f" may be approximately 0.5.

TABLE V

THE INTERDEPENDENCE OF Na AND K FLUXES

Results in this table are from preceding tables, an earlier paper[1], and scattered individual experiments.
Mean values for the fluxes are presented; the numbers in parentheses are the numbers of determinations averaged.

Bathing solution	$[Na_e]$ *mmoles/l*	$[K_e]$ *mmoles/l*	*Influx* k_2K_i *μequiv./mm²/sec* $\times 10^8$	*Efflux* k_2Na_i *μequiv./mm²/sec* $\times 10^8$
Saline	144	5.5	2.7 (8)	4.5 (11)
K-free saline	144	0	—	4.5 (3)*
High K saline	127	22	3.9 (3)	8.6 (3)
K phosphate	0	154	3.5 (5)	1.6 (3)
Glucose + KCl	0	22	—	3.3 (1)
Choline "saline"	0	5.5	1.2 (4)	2.3 (4)
Glucose	0	0	—	2.8 (3)

* Although average values for the Na efflux in this solution are the same as in complete saline, Table I indicates that in each individual experiment reduction of $[K_e]$ to 0 reduces the Na efflux.

The parallelism observed between Na and K movements suggests that there exists a linkage between the two mechanisms. Specifically it would appear that

References p. 339.

two sodium ions are extruded for every potassium ion taken up. STEINBACH[9] has shown interdependence between Na and K movements in frog muscle; and HODGKIN AND KEYNES[10] that there exists a 1:1 connection between active K uptake and Na extrusion in squid nerve fibres.

It might be expected that the ratio Na efflux/K influx should be 1:1 in these muscles in the interests of electrical neutrality, and not 2:1 as has been found. However, the accuracy of measurement is probably insufficient to be certain of the ratio, although it is possible that even in saline solution some extruded Na ions may be accompanied by anions. This must certainly occur when tissue Na is lost to glucose solution, where there are no ions available for uptake; and that the coupling between Na extrusion and K uptake is not a rigid one is further indicated by the fact that Na exchange can occur in K-free saline media.

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SUMMARY

The kinetics of loss of Na ions from mammalian muscle have been described. Following the rapid movement of the extracellular Na, the remainder is extruded with a uniform time constant of 1.59 h^{-1} at 20° C. Determination of the temperature coefficient for the process suggests that the rate-limiting step is a physical desorption of the ions.

Na efflux is affected by the concentrations of both Na and K in the bathing solution, and K uptake by the muscles is conversely dependent on the presence of Na. The results suggest that the two processes are linked together in such a way that two Na ions are extruded for every K ion taken up.

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