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ACTIVE TRANSPORT OF SODIUM AND POTASSIUM IN MAMMALIAN SKELETAL MUSCLE AND ITS MODIFICATION BY NERVE AND BY CHOLINERGIC AND ADRENERGIC AGENTS

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SUMMARY

1. Active transport of Na^+ and K^+ by Na-rich extensor digitorum and soleus muscles of rat was found to be increased considerably when muscles were innervated during enrichment with Na^+ in K-free modified Krebs solution containing 160 mM-Na at 2° C and recovery in a similar fluid with 10 mM-K and 137 mM-Na at 37° C, bubbled with oxygen.

2. Addition of acetylcholine (2·0 μg/ml.) to recovery fluid containing denervated extensors increased active transport, whereas addition of eserine (50 μg/ml.), decamethonium (0·1 μg/ml.) and to a lesser extent tubocurarine (0·26 μg/ml.) inhibited active transport. Blocking of nerve conduction in innervated extensor inhibited K^+ uptake more than Na^+ excretion.

3. The membrane potential of Na-rich extensor muscles measured soon after re-immersion in recovery fluid was higher in denervated than in innervated muscles. In the latter it was close to the K-equilibrium potential (E_K). It is suggested that denervation here makes the Na-pump electrogenic by decreasing K^+ uptake either by decreased permeability or by inactivating a K-pump. Evidence is presented that the latter is more likely.

4. Addition of isoprenaline to Na-rich soleus muscles in recovery fluid increased active transport and reduced the membrane potential measured soon after re-immersion in recovery fluid. The Na-pump still remained electrogenic in the presence of isoprenaline. It was suggested that isoprenaline might also stimulate the Na-pump, perhaps through activation of lactic dehydrogenase.

INTRODUCTION

The active transport of Na^+ and K^+ by Na-rich amphibian skeletal muscle has been extensively studied in the case of *Rana temporaria* (Carey, Conway & Kernan, 1959; Conway, Kernan & Zadunaisky, 1961; Cross, Keynes & Rybová, 1965; Dydynska & Harris, 1966) and of *Rana pipiens* (Mullins & Frumento, 1963; Mullins & Awad, 1965). The following work was undertaken to extend these investigations to mammalian tissue, where additional problems arise wth regard to its homeothermal nature and higher oxygen dependence (Creese, Scholes & Whalen, 1958). The methods of Na-enrichment already used for frog muscles (Desmedt, 1963), namely immersion of muscles overnight in cold K-free Ringer, proved unsuitable in the case of rat muscles, as they lost practically all their K^+ in exchange for Na^+ and failed to excrete Na^+ again even under the most favourable conditions. Enrichment with Na^+ was therefore confined to a 2 hr soaking period in cold, well-oxygenated Ringer solution. It will be seen that these muscles excreted Na^+ fairly well when re-immersed for 2 hr at 37° C in recovery fluid containing K^+, but uptake of K^+ was not impressive. In an attempt to overcome these difficulties a more complete preparation was employed in which companion leg muscles of the rat were left innervated and connected to the spinal cord during Na-enrichment and in some cases during Na^+ excretion. Better recovery was achieved with this preparation and this led us to investigate the nature of the influence of nerve on the process of active transport here.

There is already some evidence in the literature that nerve exerts an influence on the electrolyte content of leg muscle of rat. Freshly dissected muscles of the white fast-contracting type, such as extensor digitorum longus have been found to contain more K^+ and less Na^+ than the red slow-contracting type (Sréter & Woo, 1963). Also Drahota & Gutman (1963) have found that extensor digitorum muscles of rat contained more K^+ and glycogen than soleus muscles, but that they resembled soleus muscles in their content of these substances following long-term denervation. Cross-innervation experiments (Buller, Eccles & Eccles, 1960) involving the co-salled slow and fast muscles also indicate that the electrical and mechanical properties of muscles may be largely dependent on the properties of the nerve.

When we were satisfied that the nerve was having a positive action in promoting Na^+ excretion and K^+ uptake in our rat muscle preparations we examined the action of some cholinergic and adrenergic agents on the process. Finally, measurements of membrane potential and oxygen consumption were carried out during Na^+ excretion to elucidate the mode of action here of nerve and of isopropylnoradrenaline.

METHODS

Isolated companion extensor digitorum longus muscles of Wistar strain of rat were used in the first series of experiments. These were selected because they are small (about 100 mg) and therefore deeper fibres are less likely to suffer from anoxia in artificial oxygenated media. In later experiments the soleus muscle from the same animal (about 100 mg in weight) was also used.

The companion muscles were made Na-rich by immersion for 2 hr at about 2° C in aerated K-free modified Krebs solution (Table 1) containing 160 mM-Na. One muscle of each pair was then prepared for Na^+ and K^+ analysis while its companion was re-immersed for a further 2 hr at 37° C in oxygenated recovery fluid (Table 1) containing 10 mM-K and 137 mM-Na. These muscles were also prepared for analysis. In some experiments muscles were weighed immediately after removal from the animal and again before analysis to determine changes in hydration. They were also dried to constant weight to determine water content.

TABLE 1. Composition of modified Krebs solution used for the Na-enrichment and recovery of muscles

	K-free solution	Recovery fluids: Normal	Recovery fluids: Bicarbonate-free (for manometry)
	Concentrations (mM)		
Na^+	160	137	137
K^+	0	10	10
Cl^-	138·5	130·7	130·7
Ca^{2+}	2·5	2·5	2·5
Mg^{2+}	1·2	1·2	1·2
SO_4^{2-}	1·2	1·2	1·2
Bicarbonate	18·0	15·5	0
Gluconate	2·5	2·5	2·5
Glucose	30	56	56
Phosphate	1·2	1·2	9·7

Bubbled with 95 % oxygen and 5 % carbon dioxide

In later experiments companion extensor digitorum and soleus muscles were made Na-rich while still attached to the spinal cord through the peroneal and sciatic nerves. The rats were first killed by a blow to the head. The muscles and nerves were then dissected clear of the legs up to the point where the sciatic nerve enters the spinal cord. The hind legs were then removed from the animal leaving the muscles hanging by the nerves from the remainder of the animal (Kernan, 1965). The whole preparations was then enclosed in a Perspex moist chamber for the duration of the experiment, to ensure that the nerve did not become dry (Fig. 1). The muscles dipped into a Perspex bath (*B*, Fig. 1) containing the K-free or recovery fluids, but care was taken to ensure that as little of the nerve as possible was immersed. Using this arrangement a number of experiments were carried out with extensor and soleus muscles to determine the mean Na^+ and K^+ concentrations within the muscles after a 2 hr period of soaking in K-free solution (Table 1). Here both muscles were analysed for Na^+ and K^+ after immersion in this solution. In another series of experiments the effect of denervation on the recovery of Na-rich extensor and soleus companion muscles was examined. In this case Na-enrichment was carried out as above with both companion muscles in each case attached to the spinal cord. Then before re-immersion in recovery fluid containing 10 mM-K and 137 mM-Na, one companion muscle in each case was cut free of the nerve, while the other was left attached to the nerve during the 2 hr re-immersion at 37° C. The recovery fluid was bubbled with a gas mixture containing 95 % oxygen and 5 % carbon dioxide. Both

sets of muscles were then weighed and prepared for analysis. Following measurement of the distribution of muscle water between fibre and interstitial space by methods which will be mentioned later, the actual concentration of Na^+ and K^+ in the muscle fibres was determined and from a comparison with concentrations in Na-rich muscles the quantities of these ions transported by innervated and denervated muscles were compared.

In some experiments both extensor and soleus musles were attached to the same nerve during Na-enrichment and recovery.

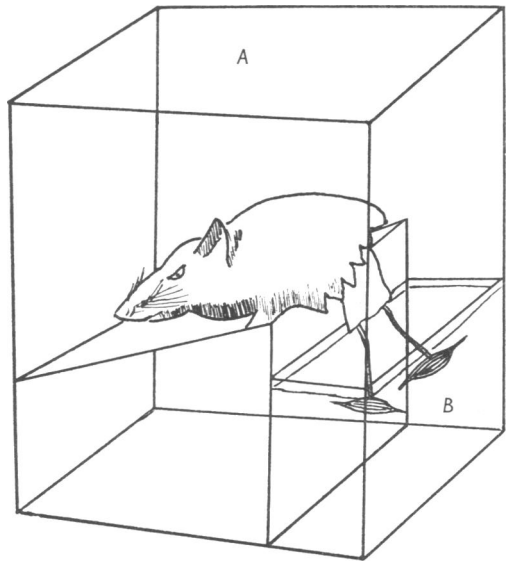

Fig. 1. Moist chamber (*A*) used for holding rat during Na-enrichment and recovery of nerve-muscle preparation. Solutions placed in chamber *B*.

Chemical methods. Before analysis, muscles were blotted on moist filter paper to remove excess moisture, weighed and digested in about 1 ml. of pure boiling concentrated nitric acid. When oxidation was complete the acid was evaporated and the residue dissolved in 10–15 ml. of de-ionized water, depending on the weight of the muscle. The concentrations of Na^+ and K^+ in this solution usually lay within the range of 0·2–0·8 m-equiv/l. This was then analysed for Na^+ and K^+ concentrations by means of the Beckmann flame photometer. In some cases muscles were dried to constant weight in an oven at 110° C to determine water content. The extracellular space in the muscles was measured by an inulin dilution technique (Dee & Kernan, 1963). When this technique was applied to innervated muscles special care had to be taken to ensure that inulin did not diffuse into the nerve, which of course was kept clear of the soaking fluid. A piece of nerve trunk adjacent to the muscle was analysed for inulin to ensure that none was lost by diffusion into the nerve.

Effect of nerve block by sucrose on active transport in extensor muscles. Companion extensor digitorum muscles were made Na-rich while attached to the C.N.S. Both were then re-immersed for 2 hr at 37° C in recovery fluid, but the nerve leading to one of the muscles was immersed for about 8 mm in isotonic sucrose solution contained in a small chamber. The object was to block electrical conduction through the nerve so as to see whether the nerve as a whole was involved in the effect on active transport in muscle. In another series of experiments some NaCl was added to the sucrose to give an isotonic solution containing 15 % of the normal plasma Na^+ concentration. Such a solution has been reported to block conduction

by γ and δ nerve fibres but not by α and β fibres (Nathan & Sears, 1960). All muscles were analysed for Na^+ and K^+ after the recovery period.

Effect of motor end-plate block on active transport of Na^+ by denervated muscles. Experiments were carried out to determine whether the higher concentration of Na^+ in denervated as compared with innervated muscles at the end of the recovery period might be due to increased influx of Na^+ in denervated muscles under the influence of excited motor end-plates. Here companion extensor digitorum and soleus muscles were made Na-rich while attached to the C.N.S. and were then cut free of the nerve and re-immersed in recovery fluid as already described. One muscle in each case was used as a control, while, to the recovery fluid containing the companion muscles, either of the end-plate blocking agents, 0·26 μg/ml. of tubocurarine (Eccles, Katz & Kuffler, 1941) or 0·1 μg/ml. of decamethonium iodide (Zaimis, 1951) were added. All muscles were analysed for Na^+ and K^+.

Membrane potential measurements. The micro-electrode technique of Graham & Gerard (1946) was used. The potential difference between micro-electrode and bath electrode was fed into the oscilloscope through a differential cathode-follower employing two ME 1400 valves. The micro-capillary electrodes were filled with 3M-KCl and had an impedance of 7–10 MΩ corresponding to a tip diameter of about 0·5 μ. Electrodes with tip potentials large enough to lead to significant errors in membrane potential measurement (Adrian, 1956) were rejected. The membrane potentials of the Na-rich extensor digitorum and soleus muscles were measured 5–10 min after re-immersion in recovery fluid containing 10 mM-K and 137 mM-Na. In the case of the extensor, potentials were measured on both the innervated and denervated muscles. About twenty measurements were made on each muscle. The muscles were then analysed for Na^+ and K^+ and the K-equilibrium potential was calculated by means of the Nernst equation,

$$E_K = RT/F \ln [K]_i/[K]_o, \quad (1)$$

where R, F and T are the Gas constant, Faraday constant and absolute temperature respectively and $[K]_i$ and $[K]_o$ are the potassium concentrations in the muscle fibres and bath fluid.

In the case of soleus muscles, these were made Na-rich while attached to the C.N.S. but were denervated before immersion in recovery fluid. The mean membrane potential was measured as already described but in this case 0·05 μg/ml. isopropyl noradrenaline (Green & Kepchar, 1959) was added to the recovery fluid containing one set of muscles while their companion muscles used as controls were immersed in normal recovery fluid. Muscles were then analysed and E_K calculated.

Oxygen consumption measurements. The Warburg manometric technique was used, the bath temperature being set at 37° C. Calibration of the manometers was carried out by displacement of CO_2 from a standard bicarbonate solution by dilute HCl (Umbreit, Burris & Stauffer, 1957). In measuring the oxygen consumption of muscles, the centre chamber of the manometer flasks held filter paper soaked in KOH solution to absorb CO_2 liberated. The recovery fluid in which the muscles were immersed was therefore bicarbonate-free, this anion being replaced by phosphate (Table 1). Each manometer contained one muscle immersed in 4 ml. of fluid and the manometers and flasks were scrubbed with oxygen before the experiment. During equilibration of the manometers and flasks in the bath, the muscles were immersed in 2 ml. of K-free, high-Na fluid in the main chamber of the flasks, while the side arms of the flasks contained 2 ml. of a solution with 20 mM-K and low Na^+ concentration. When these solutions were mixed the 10 mM-K, 137 mM-Na phosphate recovery fluid (Table 1) was obtained. Where isopropyl noradrenaline was used this was added to the side arm. Oxygen consumption was measured in the case of Na-rich extensor and soleus muscle during active excretion of Na^+ and also on addition of isoprenaline to soleus muscles during active transport. Unfortunately the technique did not allow us to examine the effect of innervation on oxygen consumption during active transport. Some information was obtained from these

measurements concerning the maximum permissible thickness of muscle consistent with adequate oxygenation of tissue. Oxygen consumption was expressed as μl./mg dry weight/hr.

Effect of cholinergic and adrenergic agents on the active transport of ions by denervated muscles. The extensor and soleus muscles used in these experiments were made Na-rich while innervated but were denervated before re-immersion in recovery fluid. One muscle of each pair was used as a control while its companion was treated with isoprenaline (0·05 μg/ml.), adrenaline (0·01 μg/ml.) in the case of soleus muscles or with acetylcholine (2·0 μg/ml.) or eserine (50 μg/ml.) in the case of extensor muscles. The muscles were analysed for Na^+ and K^+ at the end of the recovery period and the amount of Na^+ and K^+ transported by control and treated muscles compared.

TABLE 2. The effect of denervation on the active transport of Na^+ and K^+ by Na-rich extensor digitorum muscles of rat

Electrolyte concentrations in muscles (m-equiv/l. fibre water ± S.E.)

	Na^+	K^+	Number of observations
Fresh muscle	14·0	165·0	10
Denervated muscles after 2 hr in K-free solution	71·5 ± 2·1	92·5 ± 2·0	8
Na-rich companions after 2 hr in recovery fluid	50·8 ± 3·4	97·5 ± 2·5	8
Net change	(−20·7)	(+5·0)	
Muscles made Na-rich while connected to C.N.S.	68·0 ± 2·2	103·0 ± 2·2	10
Na-rich muscles denervated before re-immersion in recovery fluid	45·1 ± 1·6	123·5 ± 3·0	10
Net change	(−22·9)	(+20·5)	
Companion Na-rich muscles connected to C.N.S. during recovery	29·4 ± 1·3	140·3 ± 2·5	10
Net change	(−38·5)	(+37·3)	

RESULTS

Effect of denervation on active transport in Na-rich extensor digitorum and soleus muscles. Denervation before Na-enrichment did not alter significantly the uptake of Na in K-free solution, but it did increase significantly the loss of K^+ from the muscles (Table 2). It was also found that denervated muscles increased in weight by about 9·6% during Na-enrichment. The ratio of $3K^+$ lost from the muscles for $2Na^+$ taken up (Table 2) in the case of denervated extensors was similar to that found *in vivo* where rats were kept for several weeks on a K-deficient diet (Cooke, Segar, Creek, Coville & Darrow, 1952; Orloff, Kennedy & Berliner, 1953). Denervation carried out before or after Na-enrichment resulted in about the same degree of inhibition of active Na^+ transport when these denervated muscles were compared after recovery with muscles which had been innervated during the whole experiment, but in the case of K^+ the situation was quite different. Here K^+ uptake during recovery was 4 times greater if denervation was carried out after Na-enrichment. It is also evident that innervation

during the whole experiment greatly increased the amounts of Na^+ and K^+ actively transported by the muscles during recovery. It was impossible of course to weigh innervated muscles at the beginning of the experiment, so changes in weight of this preparation during Na-enrichment and recovery could not be determined. It was found however that muscles denervated before re-immersion were 8·3% heavier on the average than their innervated companions when both sets of muscles were weighed at the end of the experiment.

TABLE 3. Effect of denervation on the active transport of Na^+ and K^+ by Na-rich soleus muscles of rat

Mean electrolyte concentrations in muscles (m-equiv/l. fibre water ± S.E.)

	Na^+	K^+	Number of observations
Fresh muscle	22·6	153·8	*
Muscles innervated during Na-enrichment	76·0 ± 1·6	93·5 ± 2·0	11
Na-rich muscles denervated before re-immersion in recovery fluid	62·5 ± 1·8 (−13·5)	102·5 ± 2·2 (+9·0)	8
Na-rich companions connected to C.N.S. during recovery	40·0 ± 2·3 (−36·0)	117·0 ± 3·5 (+23·5)	8
Recovered soleus muscles where both extensor and soleus were connected to C.N.S. during re-immersion	26·8 ± 3·2 (−49·2)	123·0 ± 1·6 (+29·5)	6

* Taken from Sréter & Woo 1963.

In the case of soleus muscles (Table 3) it is evident that even with a preparation which had been connected to the C.N.S. during Na-enrichment and recovery, the uptake of K^+ during re-immersion in recovery fluid was a good deal less than the Na^+ excretion and the active transport of both ions fell considerably in the denervated preparation. There was an increase in active transport of Na^+ and K^+ when both extensor and soleus muscles were attached to the same nerve during the experiment.

Effect of nerve block by isotonic sucrose on active transport by extensor muscles. When the nerve leading from the spinal cord to the extensor muscles was bathed in isotonic sucrose solution, a procedure which would be expected to block electrical conduction through the nerve, a slight but highly significant inhibition of K^+ uptake occurred (Table 4) ($P < 0·01$), while inhibition of Na^+ excretion was fairly significant ($0·05 < P < 0·1$). The active transport of these ions was not appreciably altered when the isotonic sucrose solution bathing the nerve contained sufficient Na^+ to allow of conduction in the α and β nerve fibres. In this solution the

difference in Na^+ and K^+ concentrations between control and experimental muscles was only from 3 to 4 m-equiv/kg which was not significant ($0{\cdot}1 < P < 0{\cdot}2$ for Na^+; $0{\cdot}2 < P < 0{\cdot}3$ for K^+). It is therefore considered likely that the α and β fibres are involved in the promotion of ion transport observed in the innervated preparation.

TABLE 4. Effect of nerve block by isotonic sucrose solution on active transport by innervated Na-rich extensor muscles

Mean electrolyte concentration in muscles (m-equiv/l. fibre water ± S.E.)

	Na^+	K^+	Number of observations
Control muscles connected to C.N.S. during recovery	28·4 ± 1·9 (−39·6)	138·0 ± 1·1 (+35·0)	10
Muscles as above but with nerve bathed in isotonic sucrose	35·0 ± 2·5 (−33·0)	126·0 ± 1·5 (+23·0)	10
Control as above	30·6 ± 1·7 (−37·4)	138·0 ± 2·8 (+35·0)	10
Muscles as in control but with nerve bathed in isotonic solution of sucrose and 15% of normal Na^+ concentration	33·8 ± 0·7 (−34·2)	134·0 ± 1·7 (+31·0)	10

Effect of motor end-plate block on the Na^+ concentration in denervated muscles following recovery. The action of tubocurarine in blocking neuromuscular transmission by combining with the receptor sites on the muscle membrane was made use of to determine whether the higher Na^+ concentration in denervated muscles compared with innervated ones was due to increased permeability of the muscle membrane to Na^+ brought on by increased excitability of the nerve and end-plate. The results in Table 5 suggest that if neuromuscular transmission has any effect it is to decrease rather than increase muscle Na^+ concentration after recovery. The action of the blocking agent decamethonium iodide is somewhat similar to that of curare but is complicated by its known depolarizing action on the muscle membrane. Its action on the extensor is much more pronounced than its action on soleus (Table 5). The over-all results suggest that acetylcholine action at the end-plate may facilitate active transport of Na^+.

Membrane potentials of Na-rich muscles during active transport of Na^+. In the case of the extensor digitorum muscles examined denervation resulted in a significant increase in the membrane potential measured about 10 min after re-immersion in the recovery fluid. The membrane potential of innervated muscles measured under these conditions was not significantly different from the E_K ($0{\cdot}5 < P < 0{\cdot}6$) but the potential of denervated muscles was much greater than E_K ($P < 0{\cdot}01$) (Table 6). It

seems likely then that the Na-pump is electrogenic in the latter preparation as was already found in frog sartorius muscle (Kernan, 1962*a*; Keynes & Rybová, 1963; Mullins & Noda, 1963).

TABLE 5. Effect of motor receptor blocking agents on the Na^+ concentration of Na-rich extensor and soleus muscles after 2 hr in recovery fluid

	Sodium concentrations (m-equiv/kg wet wt. of muscle ± S.E.)		
	Control	Tubocurarine (0·26 μg/ml.)	Number of observations
Denervated extensor muscles	49·9 ± 1·6	54·1 ± 2·4	8
Significance of difference	$0{\cdot}1 < P < 0{\cdot}2$		8
		Decamethonium (0·1 μg/ml.)	
Denervated extensor muscles	48·2 ± 2·2	66·9 ± 2·7	8
Denervated soleus muscles	51·8 ± 3·4	55·6 ± 2·8	8
Significance of difference	$0{\cdot}4 < P < 0{\cdot}5$		

TABLE 6. Membrane potentials of Na-rich muscles measured during active excretion of Na^+ into modified Krebs solution containing 10 mM-K and 137 mM-Na

	Measured potential E_m (mV)		Calculated E_K (mV)	Number of muscles used
	With nerve	Without nerve		
Extensor muscles	62·1 ± 0·57	68·0 ± 0·75	63·2 ± 1·75	12
Denervated soleus	Control	With isoprenaline (0·05 μg/ml.)		
	72·5 ± 0·8	66·9 ± 0·9	61·5 ± 2·0	12

In the case of soleus muscles, addition of the vasodilator substance isopropyl noradrenaline to the recovery fluid caused a fall of membrane potential. This substance was also found to stimulate Na^+ transport. In the presence of isoprenaline the potential still remained significantly greater than E_K so the Na-pump here was still electrogenic.

The effect of cholinergic and adrenergic agents on Na^+ transport. In the first part of Table 7 is shown the effect of addition of cholinergic agents to recovery fluid on the active excretion of Na^+ from Na-rich extensor muscles. The addition of acetylcholine decreased the amount of Na^+ remaining in the muscles at the end of recovery as compared with controls, the increase in active transport being statistically significant ($P < 0{\cdot}01$). Eserine on the other hand appeared to reduce Na^+ excretion significantly ($P < 0{\cdot}01$). Although not included in the Table the corresponding changes were seen in the potassium movements.

In the second part of Table 7 the effects of isoprenaline and adrenaline on Na^+ excretion from soleus muscles are shown. Here the stimulating effect of the former contrasts with the absence of stimulation in the

presence of adrenaline. The isoprenaline also increased oxygen consumption by about 27 % which is in keeping with its known influence on glycogenolysis in muscles with a predominance of red fibres.

TABLE 7. The effect of cholinergic and adrenergic agents on active excretion of Na^+ by Na-rich muscles denervated before re-immersion in recovery fluid containing 10 mM-K and 137 mM-Na

Na^+ concentrations in extensor muscles (m-equiv/kg wet wt. ± S.E.)

	Recovery		
Na-rich	Control	With acetylcholine (2·0 μg/ml.)	With eserine (50 μg/ml.)
59·1 ± 1·4 (20)	45·9 ± 1·1 (20)	38·8 ± 1·5 (10)	55·1 ± 2·5 (10)

Na^+ concentrations in soleus muscles (m-equiv/kg wet wt. ± S.E.)

	Recovery		
Na-rich	Control	With isoprenaline (0·05 μg/ml.)	With adrenaline (0·01 μg/ml.)
63·4 ± 1·6 (8)	47·0 ± 1·7 (9)	38·4 ± 1·3 (9)	46·1 ± 2·6 (12)
Oxygen consumption (μl./mg dry wt. hr)	1·56 (24)	1·98 (24)	

Number of observations given in brackets.

Creese *et al.* (1958) calculated that the maximum permissible thickness of diaphragm consistent with adequate oxygenation was 635 μ at 37° C where the preparation consumed oxygen at the rate of 7·07 μl./mg dry wt./hr in an atmosphere of 98 % oxygen. Assuming a similar Krogh diffusion constant applied in our case we calculated that the maximum permissible thickness for adequate oxygen of our soleus preparation was (0·635 × 7·07)/1·56 = 2·8 mm. Our preparation was never more than 2 mm in thickness, so we consider that even the deep fibres were adequately oxygenated. In the case of the extensor muscles, the rate of oxygen consumption during Na^+ excretion was only 0·8 μl./mg dry wt. hr which would correspond with a permissible thickness of 5·5 mm. This is also more than the average thickness of the muscles.

DISCUSSION

A comparison of the Na^+ excretion and K^+ uptake by innervated and denervated Na-rich muscles on re-immersion in recovery fluid (Tables 2 and 3), when considered in relation to the effects of tubocurarine, decamethonium and acetylcholine on active transport (Tables 5 and 7) would suggest that the nerve is having a synergic action on ion transport here.

The effect of nerve block by sucrose solution on active transport (Table 4) indicated that the net movement of K^+ was inhibited to a greater extent than was the Na^+ excretion. The effect of denervation of Na-rich extensor muscles on the membrane potential measured soon after re-immersion in recovery fluid was to raise it, thereby making the Na-pump electrogenic. In the innervated muscles on the other hand the membrane potential remained close to the K-equilibrium potential, E_K. In measurements of membrane potential (E_m) of isolated frog muscles during active excretion of Na^+ we have previously found that E_m was greater than E_K by about 11 mV (Kernan, 1962*a*). The potential difference E_m–E_K was due apparently to the activity of the Na-pump. Where the muscle fibres were permeable to K^+, it was expected to move passively into the fibres under the influence of this potential difference until at the end of recovery E_m was equal to E_K. In other words the Na-pump was doing the osmotic and electrical work for the net movement of both Na^+ and K^+. We have since confirmed (Kernan, 1966) that in frog also the mean membrane potential of Na-rich sartorius muscles is increased following denervation in recovery fluid.

The question then arises as to why the Na-pump in rat and frog muscles should become electrogenic following denervation, while at the same time less Na^+ and K^+ are transported. The most likely explanation perhaps is that K^+ uptake is inhibited in the denervated preparation either because of decreased permeability or because a K^+ pump is inactivated. Such a pump in innervated muscle might move K^+ into the fibres in step with Na^+ excretion making the process of active transport of these ions more or less electrically neutral. The potentials shown in Table 6 do not allow us to decide between these alternatives. If increased K^+ permeability reduces the potential at the beginning of recovery in the innervated muscle it reduces the total work which must be done by the Na-pump as expressed by the equation,

$$dG/dn = RT \ln [Na]_o/[Na]_i + E_m F, \qquad (2)$$

where dG/dn is the free energy change per equivalent of Na^+ excreted, E_m the mean membrane potential of the muscle fibres (outside minus inside) measured in recovery fluid and $[Na]_i$ and $[Na]_o$ the sodium concentrations in muscle fibre water and bath fluid respectively. At the end of the recovery period $E_m = E_K$ and so equation (2) may be replaced by the following equation

$$dG/dn = RT \ln [Na]_o/[Na]_i + RT \ln [K]_i/[K]_o. \qquad (3)$$

When the final concentrations of Na^+ and K^+ in muscles after recovery (Table 2) were substituted in equation (3) it was found that the total energy required for active Na^+ excretion was 2·56 cal/m-equiv Na in the

innervated muscles compared with 2·22 cal/m-equiv Na in denervated muscles. This suggests that increased K^+ permeability alone does not account for the greater Na^+ excretion in the former.

Kirschner (1953) has reported that acetylcholine esterase inhibition by eserine decreased the active transport of Na^+ across frog skin. Our results on the stimulation of Na^+ transport by acetylcholine and its inhibition by eserine are also consistent with a role of acetylcholinesterase in active transport. In nerve it has been found that eserine antagonized the depolarizing action of acetylcholine at the nodes of Ranvier (Dettbarn, 1960) and in mammalian C fibres (Armett & Ritchie, 1960). Might not this be interpreted as inhibition by eserine of an electrogenic K-pump? If such a pump is to have functional significance it is under conditions where $E_m < E_K$. Cross *et al.* (1965) have reported active K^+ uptake in frog muscles with $E_m < E_K$, where muscles were made Na-rich in fluid containing little or no Ca^{2+}, and recovered in fluid containing Ca^{2+}. In low-Ca^{2+} medium the spontaneous release of acetylcholine at nerve endings is inhibited (Elmqvist & Feldman, 1965; Katz & Miledi, 1964). Perhaps the process may be augmented on return to recovery fluid containing Ca^{2+}, by the stimulation of a K-pump through increased release of acetylcholine.

An interesting property of acetylcholine which may be relevant to the present discussion is that it increases the amplitude of the positive after-potential in mammalian non-myelinated nerve fibres (Armett & Ritchie, 1960). The positive after-potential is believed to be due to the activity of an electrogenic Na-pump (Connelly, 1959; Greengard & Straub, 1962).

In the case of soleus muscles innervation also facilitated active transport of Na^+ and K^+. We tested the action of isoprenaline on Na^+ transport here because of muscle relaxing properties which in soleus are affected through the slow contracting red fibres (Bowman & Zaimis, 1958). Bülbring (1960) has suggested that the relaxing activity of adrenaline in smooth muscles might be due to stimulation of reactions supplying energy to an electrogenic Na-pump. In our case, however, having obtained stimulation of Na^+ transport in the presence of isoprenaline we found this to be associated with a decrease rather than an increase in membrane potential, at least when this was measured soon after re-immersion of the Na-rich denervated soleus muscles in recovery fluid. The Na-pump in the soleus muscles still remained electrogenic in spite of the fall of potential brought about by addition of isoprenaline. When Na^+ excretion is complete E_K is higher in the soleus muscles treated with isoprenaline than in the control muscles. If $E_m = E_K$ here then it follows that treatment of muscle with this adrenergic agent will in the long run increase the membrane potential as found by Bülbring in smooth muscle. The absence of an effect of adrenaline on active transport in our experiments may be due to its mixed constrictor

and dilator properties. It is more likely, however, that we did not select the optimum concentration of this drug because soleus has been shown to have a depressor response to adrenaline and isopropyl noradrenaline (Bowman & Zaimis, 1958).

Finally, it may be mentioned that isoprenaline causes release of lactic acid from tissues and is particularly potent in promoting muscle glycogenolysis. The potency of catecholamines in this respect is in the same order as their potency as vasodilators, muscle relaxants and cardiac stimulants. Lundholm (1956) and Allwood & Cobbold (1961) found that vasodilator response to adrenaline infusion was associated with release of lactic acid from the tissues. A connexion has already been found (Kernan, 1962*b*) between lactic dehydrogenase activity of muscles and active Na^+ excretion and between the latter and hyperpolarization of the muscle fibre membrane in frog.

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