J. Physiol. (1971), **216**, *pp.* 171–179
Printed in Great Britain

EFFECTS OF NERVE CROSS-UNION ON RAT INTRACELLULAR POTASSIUM IN FAST-TWITCH AND SLOW-TWITCH RAT MUSCLES

By J. F. Y. HOH and B. SALAFSKY*

From the Department of Pharmacology, College of Medicine, University of Illinois at the Medical Center, Chicago, Illinois 60680, U.S.A.

(*Received 29 December 1970*)

SUMMARY

1. Electrolytes of normal, self-innervated and cross-innervated extensor digitorum longus and soleus muscles of rats have been determined.

2. $[K]_i$ was 173 m-equiv/l. for both normal and self-innervated extensor digitorum longus. In cross-innervated extensor digitorum longus it was reduced to 159 m-equiv/l.

3. For normal and self-innervated soleus, $[K]_i$ was 150 m-equiv/l. and 154 m-equiv/l. respectively. In cross-innervated soleus it was increased to 182 m-equiv/l.

4. The content and distribution of most other electrolytes of cross-innervated soleus, as well as its weight, were not significantly different from those of controls. On the other hand, cross-innervated extensor digitorum longus weighed about half as much as controls and contained markedly elevated Na^+, Cl^- and extrafibre water and reduced non-collagenous protein and intrafibre water.

5. It is concluded that $[K]_i$ of fast-twitch and slow-twitch muscle fibres are under neural regulation. Possible mechanisms for this regulation are discussed.

INTRODUCTION

Mammalian fast-twitch and slow-twitch skeletal muscle fibres differ in a number of physiological and biochemical properties. By nerve cross-union experiments, in which nerve fibres normally innervating fast-twitch muscle fibres are made to reinnervate slow-twitch muscle fibres and vice versa, an increasing number of these differences (e.g. speed of contraction, post-tetanic effects on the isometric twitch, enzyme profiles) have now

* Present address: Visiting Professor, University of Pennsylvania Team, Pahlavi University, Shiraz, Iran.

been shown to depend on neural regulation (for review, see Guth, 1968; Close & Hoh, 1969; Prewitt & Salafsky, 1970).

Drahota (1960) reported that the potassium content of the fast extensor digitorum longus (hereinafter called toe extensor) muscle of the rat is 16% higher than that of the slow soleus muscle. Neural regulation of this difference in muscle potassium content was suggested by the finding of Drahota & Gutmann (1963) that the potassium content of the soleus following peroneal–tibial nerve cross-anastomosis increased to match that of the toe extensor. However, they found no change in the content of potassium in the toe extensor following this surgical procedure and attributed this to an ability on the part of the toe extensor to resist changes in potassium content.

Hoh (1969) has shown that the toe extensor was selectively reinnervated by its own nerve in experiments in which nerves to the toe extensor and the soleus were given equal chances to reinnervate it. This finding provides a plausible alternative explanation for the failure of Drahota & Gutmann to find any change in the potassium content of their reinnervated toe extensor: fibres in this muscle were probably not reinnervated by nerve fibres to soleus, but by nerve fibres which normally supplied fast-twitch muscle fibres. The question still remains as to whether the nerve to soleus could change the potassium content of the toe extensor following cross-innervation. In this report, effects of nerve cross-union on intracellular electrolytes in rat toe extensor and soleus muscles were investigated with special care to avoid selective reinnervation of muscle fibres. It will be shown that nerves to both muscles are able to regulate intracellular potassium in muscle fibres they innervate. A preliminary report has appeared (Hoh & Salafsky, 1971).

METHODS

Experimental design. The experiments were performed on nineteen male Wistar rats of body weight 365–520 g, thirteen of which were operated on at 4–5 weeks of age. The same operations were done on both sides in each rat. In six animals, nerves to toe extensor and soleus respectively were cut and the distal stump of the nerve to toe extensor was united with fine nylon to the proximal stump of the nerve to soleus. The soleus muscle was excised. In order to avoid reinnervation of toe extensor by fibres from the peroneal nerve, this nerve was tied, transected, reflected proximally and embedded in the biceps femoris muscle, to which it was secured by nylon suture. Stimulation of the appropriate nerves at the time of final experiment verified that this procedure achieved the desired exclusive functional reinnervation of toe extensor by nerve to soleus. These muscles are referred to as cross-innervated toe extensors.

In four other animals cross-union of nerves to toe extensor and soleus were done without the special precautions. This procedure enabled the nerve to toe extensor to reinnervate soleus muscles exclusively (these muscles are referred to as cross-innervated solei), but the companion toe extensor was reinnervated by nerve fibres

from the peroneal nerve as well as by the nerve to soleus. These toe extensor muscles with mixed innervation were also studied, but data from these muscles were treated separately from those of cross-innervated toe extensor muscles with exclusive innervation from the nerve to soleus.

Self-union of nerves to toe extensor and soleus muscles respectively was performed on three rats. These muscles are referred to as self-innervated. Toe extensor and soleus muscles from six unoperated rats were also studied.

Experiments on operated rats were performed 106–144 days post-operatively. Rats were anaesthetized by intraperitoneal injection of sodium pentobarbitone (50 mg/kg body wt). The muscles were carefully excised, cleaned of visible fat and blotted free of visible blood, weighed and used for the measurement of total water, K^+, Na^+, and Cl^- contents (see below). A sample of serum was obtained from each rat for serum electrolyte analyses. The density and water content of the serum were measured by weighing a known volume of each serum from four different rats before and after drying in an oven. The mean value obtained for serum water content was used in calculating the extrafibre levels of Na^+, K^+, and Cl^- in each animal; the Donnan factors used for these electrolytes were those given by Manery (1954). Extrafibre water in muscles was calculated from Cl^- contents of muscle and serum by the method of Conway (1957). From these measurements intrafibre water, $[K]_i$, $[Na]_i$ and $[Cl]_i$ were calculated.

Chemical methods. Water, K^+, Na^+ and Cl^- were all determined from the same piece of muscle weighing up to about 100 mg by the method of Flear & Florence (1961). Muscles larger than 100 mg were bisected longitudinally and one portion used for electrolyte analyses; the remaining portion was used for the measurement of protein content. K^+ and Na^+ were measured by a Baird-Atomic flame photometer (Li^+ as internal standard) and Cl^- by the titrimetric method of Schales & Schales as described by Natelson (1961).

Initially normal muscle samples were dried and extracted with nitric acid in glass tubes instead of being dried in aluminium pans and extracted with acid in plastic tubes as recommended by Flear & Florence (1961). This gave very high and variable blank values for Na^+, presumably due to elution of Na^+ from glass or contamination of tubes by Na^+. The data for Na^+ from these muscles were rejected for this reason.

For the analysis of non-collagenous protein (Lilienthal, Zierler, Folk, Buka & Riley, 1950), muscles were cut into small pieces with scissors and extracted overnight with 5 ml. 0·05 N-NaOH. Portions from the same muscles (self-innervated muscles, cross-innervated solei) used for electrolyte measurements or whole muscles (normal muscles, cross-innervated toe extensors) were used. Aliquots of the extract were used for protein estimation by the method of Lowry, Rosenbrough, Farr & Randall (1951), bovine serum albumen (Pentex) being used as standard.

All electrolyte and protein measurements were done in duplicates.

The Student's t test (2-tail) for statistical significance was used, $P < 0{\cdot}05$ being taken as statistically significant.

RESULTS

Normal and self-innervated muscles

Table 1 shows that the composition and distribution of electrolytes (except non-collagenous protein) for self-innervated toe extensor and soleus muscles are very similar to those of corresponding normal muscles. The contents of K^+ for normal or self-innervated toe extensor and soleus muscles and those of Na^+ for self-innervated muscles are the same as those

TABLE 1. Properties of normal (N-EDL, N-SOL), self-innervated (S-EDL, S-SOL) and cross-innervated (X-EDL, X-SOL) extensor digitorum longus and soleus muscles of the rat. Values given are means ± S.E., the number of observations (n) being given below each value. Results of t tests between corresponding values for N-EDL and S-EDL, S-EDL and X-EDL, N-SOL and S-SOL, and S-SOL and X-SOL are indicated under the latter group in each pair, statistically significant values being in bold type

	N-EDL	S-EDL	X-EDL	N-SOL	S-SOL	X-SOL
Muscle weight (mg)	187·8 ± 10·8 n = 11	208·6 ± 6·8 n = 6	**100·1 ± 5·1** n = 12	130·1 ± 4·7 n = 12	120·2 ± 3·1 n = 6	135·7 ± 7·8 n = 7
Non-collagenous protein (g/kg wet wt.)	168·1 ± 6·2 n = 4	**146·3 ± 3·3** n = 6	**124·2 ± 3·3** n = 4	209·4 ± 4·2 n = 4	**157·6 ± 3·2** n = 6	150·3 ± 2·5 n = 6
K^+ (m-equiv/kg wet wt.)	104·7 ± 1·6 n = 7	101·1 ± 1·7 n = 6	**70·5 ± 2·1** n = 8	85·5 ± 2·3 n = 8	82·9 ± 0·9 n = 6	**97·2 ± 2·5** n = 7
Na^+ (m-equiv/kg wet. wt.)		25·1 ± 1·3 n = 6	**59·3 ± 4·6** n = 8		31·2 ± 1·4 n = 6	**40·5 ± 3·4** n = 7
Cl^- (m-equiv/kg wet wt.)	18·8 ± 1·3 n = 7	20·2 ± 1·0 n = 6	**36·7 ± 1·7** n = 8	24·0 ± 1·6 n = 8	26·9 ± 1·6 n = 6	30·4 ± 2·6 n = 7
Extrafibre water (ml./kg wet wt.)	147 ± 9 n = 7	158 ± 7 n = 6	**301 ± 13** n = 8	187 ± 12 n = 8	210 ± 12 n = 6	228 ± 20 n = 7
Intrafibre water (ml./kg wet wt.)	601 ± 11 n = 7	581 ± 9 n = 6	**435 ± 14** n = 8	567 ± 10 n = 8	**530 ± 13** n = 6	525 ± 16 n = 7
$[K]_i$ (m-equiv/l.)	173·1 ± 3·3 n = 7	172·8 ± 2·9 n = 6	**158·5 ± 1·8** n = 8	149·7 ± 2·4 n = 8	154·3 ± 2·9 n = 6	**182·4 ± 1·9** n = 7
$[Na]_i$ (m-equiv/l.)		3·9 ± 1·1 n = 6	**39·7 ± 9·7** n = 8		3·0 ± 1·1 n = 6	**15·6 ± 4·5** n = 7
$[Cl]_i$ (m-equiv/l.)	3·2 ± 0·3 n = 7	3·6 ± 0·2 n = 6	**8·8 ± 0·6** n = 8	4·4 ± 0·4 n = 8	5·2 ± 0·4 n = 6	6·0 ± 0·7 n = 7

obtained by Yonemura (1967) for normal toe extensor and soleus muscles respectively. However, values for extrafibre water obtained here by the method of Conway (1957) are substantially higher than the inulin spaces for toe extensor and soleus reported by Sréter & Woo (1963) and also by Kobayashi & Yonemura (1967). Nevertheless, in agreement with these authors, extrafibre water for normal soleus is significantly higher than that for normal toe extensor ($P < 0{\cdot}02$). Extrafibre water for self-innervated soleus is also significantly higher than that for self-innervated toe extensor ($P < 0{\cdot}005$).

The $[K]_i$ of normal and self-innervated toe extensors are significantly ($P < 0{\cdot}001$) higher than the corresponding values for soleus muscles (Table 1). These values are close to those reported by Sréter & Woo (1963) but appreciably higher than those obtained by Yonemura (1967; toe extensor = 156·4 m-equiv/l., soleus = 132·9 m-equiv/l.). Internal sodium concentrations were equally low in self-innervated toe extensor and soleus (Table 1). The higher values for $[Na]_i$ of toe extensor and soleus reported by Sréter & Woo and Yonemura are, at least in part, attributable to differences in the methods for estimating extrafibre water and hence the intrafibre water content.

Cross-innervated toe extensor muscles

The mean values of all properties listed in Table 1 for cross-innervated toe extensor differ significantly from those of self-innervated control. The mean weight of cross-innervated toe extensor is approximately half of that for self-innervated control, this difference being highly significant ($P < 0{\cdot}001$). Extrafibre water and the principally extrafibre Na^+ and Cl^- are markedly increased in cross-innervated toe extensor ($P < 0{\cdot}001$) at the expense of intrafibre water ($P < 0{\cdot}001$), non-collagenous protein ($P < 0{\cdot}005$) and K^+ content ($P < 0{\cdot}001$). $[K]_i$ is reduced compared with that of self-innervated toe extensor but not significantly different from $[K]_i$ of self-innervated soleus ($P < 0{\cdot}3$) which was reinnervated by the same nerve as cross-innervated toe extensor. $[Na]_i$ and $[Cl]_i$ are both increased relative to levels in self-innervated toe extensor ($P < 0{\cdot}01$ and $P < 0{\cdot}001$ respectively).

Toe extensor muscles which had been cross-innervated with the nerve to soleus without special precautions to avoid reinnervation by fibres from the peroneal nerve (i.e. companions to cross-innervated solei) were also studied. These muscles twitched when either the nerve to soleus or the peroneal nerve was cut, showing functional reinnervation by nerve fibres from both sources. Mean values ± S.E. ($n = 7$) were: muscle weight = 125·4 ± 17·6 mg, non-collagenous protein = 136·0 ± 2·5 g/kg wet wt., K^+ = 92·1 ± 2·3 m-equiv/kg wet wt., Cl^- = 36·9 ± 2·0 m-equiv/kg wet wt., Na^+ = 44·8 ± 2·5 m-equiv/kg wet wt. and extrafibre water = 271 ± 14 ml./kg. wet wt. Consistent with the mixed innervation of these muscles, nearly all these values lie

between those for self-innervated and cross-innervated toe extensors; those for K^+ and non-collagenous protein are significantly different from corresponding values obtained for both these groups of muscles ($P < 0.02$).

Cross-innervated soleus muscles

There is a significant increase in K^+ content and $[K]_i$ of cross-innervated soleus over values for normal soleus ($P < 0.005$ and $P < 0.001$ respectively and self-innervated soleus ($P < 0.001$). Compared to self-innervated soleus, Na^+ content and $[Na]_i$ of cross-innervated soleus are also significantly increased ($P < 0.05$), but in contrast to cross-innervated toe extensor, no significant changes occurred in muscle weight, non-collagenous protein and Cl^- contents, extrafibre water, intrafibre water and $[Cl]_i$.

DISCUSSION

The experiments reported above clearly show that after proper cross-union, both the nerve to toe extensor and the nerve to soleus significantly change the K^+ content and $[K]_i$ of muscles they innervate.

Yonemura (1967) and Federov (1969) have found that the mean resting membrane potential of fast-twitch muscle fibres of rats is higher than that for slow-twitch fibres. This difference in resting membrane potential correlates well with the difference in $[K]_i$ in accordance with ionic theory. The K-equilibrium potentials calculated by means of the Nernst equation using the mean values for $[K]_i$ given in Table 1 and the mean $[K]_0$ of 5·9 m-equiv/l. extrafibre water are -87.4 mV for normal toe extensor and -83.6 mV for normal soleus at 37° C. These values are in very good agreement with the mean resting membrane potentials recorded *in situ* by Federov (1969) from rat plantaris (-85 ± 0.4 mV) and soleus (-79 ± 0.44 mV) muscles.

The changes in $[K]_i$ observed in cross-innervated muscles would be expected to be accompanied by a fall in the resting membrane potential of fast-twitch fibres and a rise in that of slow-twitch fibres. Differences in the characteristics of action potentials between fast-twitch and slow-twitch muscle fibres (Yonemura, 1967; Federov, 1969) would also be expected to be altered following nerve cross-union, since they depend, in part, on $[K]_i$ and $[Na]_i$.

Differences in $[K]_i$ presumably reflect differences in active transport and/or membrane permeability, which in turn are determined by differences in chemical composition of muscle membranes. There is evidence for a difference in the composition of the membrane between toe extensor and soleus muscle fibres: soleus muscle fibres are less sensitive to the destructive action of phospholipase C on muscle membrane than toe extensor muscle

fibres (Albuquerque & Thesleff, 1967). Furthermore, soleus muscle fibres are responsive to acetylcholine along their entire length, whereas responsiveness to acetylcholine of toe extensor muscle fibres is confined to the neuromuscular region (Miledi & Zelená, 1966). This difference in sensitivity to acetylcholine has been shown to reverse following nerve cross-union (Miledi, Stefani & Zelená, 1968).

It is suggested that nerves to fast-twitch and slow-twitch muscle fibres differ in their respective influences on muscle membrane permeability and/or active transport. Dockry, Kernan & Tangney (1966) have adduced evidence for a neural influence on the active transport of electrolytes in rat muscles. They found that innervated and Na^+-enriched toe extensor or soleus muscles extruded more Na^+ and took up more K^+ during recovery than acutely denervated companion muscles under the same experimental conditions. Their data also indicated that the passive permeability of K^+ into soleus was lower than that into toe extensor (for discussion, see Kernan, 1969). Kernan (1969) has shown that soleus accumulated caesium or rubidium ions to a greater extent than toe extensor of rats given drinking water containing these ions; he attributed this difference in distribution to the difference in passive permeability of these muscles to K^+.

In addition to the marked decrease in $[K]_i$ discussed above, cross-innervated toe extensor muscles failed to reach the size of control muscles as described by Close (1969). Furthermore, these muscles differed from controls by their increased extrafibre water, Na^+ and Cl^- content, decreased intrafibre water, non-collagenous protein and K^+ content. Similar reciprocal changes in Na^+ and K^+ contents have been reported for muscles from human beings with progressive muscular dystrophy (Horvath, Berg, Cummings & Shy, 1955); from mice with hereditary muscular dystrophy (Baker, Blahd & Hart, 1958; Young, Young & Edelman, 1959); from rabbits and calves with nutritional muscular dystrophy (Fenn & Goettsch, 1937; Blaxter & Wood, 1952); and from puppies with denervation atrophy (Eichelberger, Akeson & Roma, 1956). While these changes may in themselves be non-specific in nature, and may reflect merely a replacement of muscle fibres by connective tissue, their occurrence in cross-innervated toe extensor and not in cross-innervated soleus in an otherwise normal animal raises interesting questions as to the nature of the defect in the former muscle and its relationship to muscular dystrophy.

This work is supported by USPHS NINDS grant NB-05679. The authors thank Mrs Josephine Lai for technical assistance and Dr K. Unna and Dr E. Anderson for reading the manuscript.

REFERENCES

ALBUQUERQUE, E. X. & THESLEFF, S. (1967). Influence of phospholipase C on some electrical properties of the skeletal muscle membrane. *J. Physiol.* **190**, 123–137.

BAKER, N., BLAHD, W. H. & HART, P. (1958). Concentrations of K and Na in skeletal muscle of mice with a hereditary myopathy (dystrophia muscularis). *Am. J. Physiol.* **193**, 530–533.

BLAXTER, K. L. & WOOD, W. A. (1952). The nutrition of the young Ayrshire calf. 9. Composition of the tissues of normal and dystrophic calves. *Br. J. Nutr.* **6**, 144–163.

CLOSE, R. (1969). Dynamic properties of fast and slow skeletal muscles of the rat after nerve cross-union. *J. Physiol.* **204**, 331–346.

CLOSE, R. & HOH, J. F. Y. (1969). Post-tetanic potentiation of twitch contractions of cross-innervated rat fast and slow muscles. *Nature, Lond.* **221**, 179–181.

CONWAY, E. J. (1957). Nature and significance of concentration relations of potassium and sodium ions in skeletal muscle. *Physiol. Rev.* **37**, 84–132.

DOCKRY, M., KERNAN, R. P. & TANGNEY, A. (1966). Active transport of sodium and potassium in mammalian skeletal muscle and its modification by nerve and by cholinergic and adrenergic agents. *J. Physiol.* **186**, 187–200.

DRAHATA, Z. (1960). Changes in the potassium content of various muscles immediately after denervation. *Physiologia bohemoslov.* **6**, 1–4.

DRAHOTA, Z. & GUTMANN, E. (1963). Long-term regulatory influence of the nervous system on some metabolic differences in muscles of different function. In *Proceedings of Symposium on the Effects of Use and Disuse on Neuromuscular Functions*, pp. 143–148, ed. GUTMANN, E. & HNIK, P. Prague: Czechoslovak Academy of Sciences.

EICHELBERGER, L., AKESON, W. H. & ROMA, M. (1956). Effects of denervation on the histochemical characterizations of skeletal muscle during growth. *Am. J. Physiol.* **185**, 287–298.

FEDEROV, V. V. (1969). Pecularities of bioelectric activity of fibres from fast and slow muscles in rat. *Fiziol. Zh. SSSR* **55**, 588–596.

FENN, W. O. & GOETTSCH, M. (1937). Electrolytes in nutritional muscular dystrophy in rabbits. *J. biol. Chem.* **120**, 41–50.

FLEAR, C. T. G. & FLORENCE, I. (1961). A rapid and micro method for the analysis of skeletal muscle for water, sodium, potassium, chloride, and fat. *Clinica chim. Acta* **6**, 129–135.

GUTH, L. (1968). 'Trophic' influences of nerve on muscle. *Physiol. Rev.* **48**, 645–687.

HOH, J. F. Y. (1969). The problem of selectivity during reinnervation of fast and slow rat skeletal muscles. *Aust. J. exp. Biol. med. Sci.* **47**, 17.

HOH, J. F. Y. & SALAFSKY, B. (1971). Neural regulation of intracellular potassium in fast and slow twitch muscle. *J. gen. Physiol.* **57**, 256.

HORVATH, B., BERG, L., CUMMINGS, D. J. & SHY, G. M. (1955). Muscular dystrophy. Cation concentrations in residual muscle. *J. appl. Physiol.* **8**, 22–30.

KERNAN, R. P. (1969). Accumulation of caesium and rubidium *in vivo* by red and white muscles of the rat. *J. Physiol.* **204**, 195–205.

KOBAYASHI, N. & YONEMURA, K. (1967). The extracellular space in red and white muscles of the rat. *Jap. J. Physiol.* **17**, 698–707.

LILIENTHAL, J. L. JR., ZIERLER, K. L., FOLK, B. P., BUKA, R. & RILEY, M. J. (1950). A reference base and system for analysis of muscle constituents. *J. biol. Chem.* **182**, 501–508.

LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

MANERY, J. F. (1954). Water and electrolyte metabolism. *Physiol. Rev.* **34**, 334–417.

MILEDI, R., STEFANI, E. & ZELENÁ, J. (1968). Neural control of acetylcholine sensitivity in rat muscle fibres. *Nature, Lond.* **220**, 497–498.

MILEDI, R. & ZELENÁ, J. (1966). Sensitivity to acetylcholine in rat slow muscle. *Nature, Lond.* **210**, 855–856.

NATELSON, S. (1961). *Microtechniques of Clinical Chemistry*, 2nd edn., pp. 175–178. Springfield: Charles C. Thomas.

PREWITT, M. A. & SALAFSKY, B. (1970). Enzymic and histochemical changes in fast and slow muscles after cross-innervation. *Am. J. Physiol.* **218**, 69–74.

SRÉTER, F. A. & WOO, G. (1963). Cell water, sodium, and potassium in red and white mammalian muscles. *Am. J. Physiol.* **205**, 1290–1294.

YONEMURA, K. (1967). Resting and action potentials in red and white muscles of the rat. *Jap. J. Physiol.* **17**, 708–719.

YOUNG, H. L., YOUNG, W. & EDELMAN, I. S. (1959). Electrolyte and lipid composition of skeletal and cardiac muscle in mice with hereditary muscular dystrophy. *Am. J. Physiol.* **197**, 487–490.