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SODIUM INFLUX INTO DENERVATED RAT DIAPHRAGM MUSCLE FIBRE

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SUMMARY

The influence of denervation on the sodium influx into the rat diaphragm muscle fibre was studied. By analysing the wash-out curve of the ^{24}Na from the muscle strip preparation, both the amount of intracellular ^{24}Na obtained after a definite period of soaking in a radioactive medium, and the extracellular space of the muscle were determined. The sodium influx values calculated from these data for the normal and the denervated rat hemidiaphragm were found to be 62.8 ± 11.8 and 36.3 ± 5.8 ($\pm$ S.E.) pmoles$\cdot$cm$^{-2}\cdot$sec^{-1}, respectively. It seems that the decreased ^{24}Na influx after denervation is due also to a decrease in the sodium permeability of the muscle fibre membrane.

INTRODUCTION

After denervation of the skeletal muscle fibre some morphological as well as functional changes have been observed, *e.g.*, atrophy, which in the rat diaphragm is preceded by hypertrophy[1], and a spread of the chemical-sensitive area from the end plate region over the entire muscle fibre membrane[2]. It was also shown that after denervation the membrane potential of the rat diaphragm decreased[3,4]. This finding could not be interpreted as being due to the changes in the intracellular ion concentrations since these were found to be only slightly reduced[5]. The decrease in the membrane potential may be due to a reduction of the potassium permeability of the fibre membrane in the denervated muscle[6].

The aim of the present experiments was to see whether sodium permeability is also affected by denervation. To this end the influx of labelled sodium into the muscle fibre of the normal and denervated rat diaphragm was measured.

METHODS

Either the left or the right hemidiaphragm of an albino rat (100–150 g) was denervated by resection of the phrenic nerve 9–11 days before the experiment. The phrenic nerves were resected and the diaphragm strips prepared as already described[7]. Only one strip (about 30 mg) was dissected from each hemidiaphragm, mounted on a perspex frame and incubated in 5 ml of a solution containing ^{24}Na (10–20 mC/ml).

After 20 min the strip was blotted and transferred into 4 ml of an incubating solution devoid of radioactive sodium. The solution was changed at fixed intervals (Fig. 1) and the radioactivity of the washing solution determined. Krebs bicarbonate buffer[8] (pH 7.4), with 200 mg/100 ml of glucose was used throughout the experiment. Both incubation and washing were done at room temperature.

At the end of the experiment the strip was blotted, the rib and the central tendon were removed, and the muscle was weighed. Subsequently the muscle was ashed, the ash dissolved, and its radioactivity measured. Finally, the sodium concentration in the ash solution was determined by means of a flame photometer.

The radioactivity of ^{24}Na was measured by means of a scintillation counter (Ekco N-664 A) with a well-type sodium iodide crystal and automatic scaler (Ekco N-530 F). The results were corrected for the background and radioactive decay. ^{24}Na was obtained from the Nuclear Institute 'Jožef Stefan', Ljubljana.

The ^{24}Na wash-out curve plotted on a semilogarithmic scale was obtained by using the data of the radioactivity washed out during the experiment and the data of the muscle radioactivity at the end of the experiment. The curve was analyzed and the intracellular as well as the extracellular fraction of radioactive sodium at the beginning of the washing procedure was estimated.

The sodium influx into the diaphragm muscle fibre was calculated using the equation derived by KLAUS, LÜLLMANN AND MUSCHOLL[6] from the basis equation by KEYNES AND LEWIS[9].

The extracellular space of the muscle was calculated from the data on the radiosodium efflux from the muscle strip. In control experiments the inulin space was determined under the same conditions as referred to above. The inulin, again, was determined by the method of ROE, EPSTEIN AND GOLDSTEIN[10].

The sodium influx into the denervated hemidiaphragm was compared with that into the normal hemidiaphragm of the same animal and a statistical method for paired experiments applied[11].

RESULTS AND DISCUSSION

The determination of the sodium fluxes in the muscle is technically difficult because the sodium amount in the extracellular space represents the bulk of the total sodium amount in the muscle. In our experiments the intracellular radioactive sodium obtained after 20 min of incubation represented only 5 to 10 % of the total radioactive sodium in the muscle preparation. The intracellular radioactivity could be calculated as the difference between the total muscle strip radioactivity and the extracellular radioactivity estimated from the muscle extracellular space and the specific radioactivity of the incubating medium. This value, however, would be rather inaccurate since it would be obtained as a difference between two large quantities. Therefore, in our experiments the intracellular ^{24}Na was determined by analysing the wash-out curve.

The curve of the ^{24}Na efflux from the rat diaphragm strip displayed a rather constant shape (Fig. 1). By analyzing the curve, 3 fractions of washed-out ^{24}Na were determined. The fraction, with a time constant of about 0.5 min, was assumed to have been washed from the surface of the preparation. A similar fraction was found by MULLINS AND FRUMENTO[12] in experiments with the frog sartorius muscle.

The ^{24}Na fraction with a time constant of about 4 min was assumed to have been washed out from the extracellular space of the muscle. This assumption was confirmed by the experiments presented in Table I. Since the extracellular space occupied by sodium in the muscle is generally somewhat larger than that occupied by inulin, both values from Table I are in reasonable agreement. This is also in keeping with experiments on kidney cortex slices[13]. The values for the extracellular space obtained in our experiments were larger than those obtained in the whole rat dia-

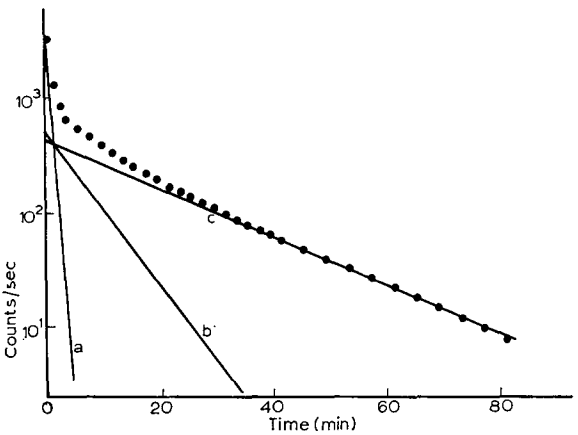

Fig. 1. The efflux of ^{24}Na from a rat diaphragm strip previously incubated for 20 min at room temperature in a ^{24}Na medium. The dots represent the values measured during the experiment. The solid lines show 3 fractions of ^{24}Na efflux with time constants of: 0.5 min (a), 4 min (b) and 14 min (c), respectively.

TABLE I

EXTRACELLULAR SPACE IN RAT DIAPHRAGM MUSCLE STRIPS OBTAINED BY TWO DIFFERENT METHODS

Method	*Number of experiments*	*µl/100 mg of wet weight* (± *S.E.*)
^{24}Na wash out	28	36.9 (± 2.25)
Inulin method	24	33.4 (± 2.45)

TABLE II

INFLUENCE OF DENERVATION ON SODIUM CONCENTRATION AND MOVEMENT ACROSS THE MEMBRANE OF RAT DIAPHRAGM MUSCLE FIBRE

The data represent mean values (± S.E.) for 14 paired experiments. ^{24}Na influx into the denervated fibre is significantly smaller ($P < 0.025$).

	Normal	*Denervated*
Rate constant for the ^{24}Na efflux (sec^{-1})	13.3 (± 0.5)	14.5 (± 0.4)
^{24}Na influx (pmole·cm^{-2}·sec^{-1})	62.8 (±11.8)	36.3 (± 5.8)
Intracellular Na concn. (mmole/l intracellular water)	46.4 (± 5.0)	45.5 (± 3.6)

phragm[5,14] or in the diaphragm strips[14]. This is probably due to the fact that some muscle fibres are damaged during strip dissection. Since in our experiments the strips were twice as small as those referred to above[14], the ratio between damaged and intact fibres was higher, and therefore the apparent extracellular space larger.

The third ^{24}Na-fraction with a time constant of about 14 min was assumed to be practically intracellular ^{24}Na. Using the data on this fraction, the sodium influx into the muscle fibres was calculated. The results are presented in Table II.

Our time constant of sodium efflux from the muscle fibre is close to 10.7 min, a value obtained by CREESE[14] with the same muscle. Since his experiments were done in a steady state, the influx should be approximately equal to the efflux, that is, about 28 pmoles cm^{-2} sec^{-1}. The influx obtained in our experiments with the normal diaphragm was approximately twice as large as the latter value.

Although the value of the sodium influx into the normal muscle fibre is only an approximate one, it still can be compared with that of the sodium influx obtained under identical experimental conditions on the denervated hemidiaphragm; the latter is significantly smaller than the former. The sodium influx is a passive process dependent mainly on the membrane permeability and the electrochemical potential across the muscle fibre membrane. It was shown that the intracellular sodium concentration remains unchanged after denervation[15,16]; in the rat diaphragm there was no change 9 days after denervation[5]. Also, at the end of our experiments the intracellular sodium concentration in the denervated muscle fibre was the same as in the normal muscle fibre (Table II). Since after denervation the membrane potential decreased about 10%, the electromotive force for the sodium influx must have diminished less than 10%. The fact that after denervation the ^{24}Na influx decreases by about one half, cannot be attributed solely to the decrease in the electromotive force, but probably also to changes in the membrane permeability.

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REFERENCES

1 O. M. SOLA AND A. W. MARTIN, *Am. J. Physiol.*, 172 (1953) 324.
2 A. G. GINETZINSKY AND N. M. SHAMARINA, *Adv. Mod. Biol.*, 15 (1942) 283.
3 E. MUSCHOLL, *Arch. Ges. Physiol.*, 264 (1957) 467.
4 H. LÜLLMANN AND W. PRACHT, *Experientia*, 13 (1957) 288.
5 H. LÜLLMANN, *Arch. Ges. Physiol.*, 267 (1958) 188.
6 W. KLAUS, H. LÜLLMANN AND E. MUSCHOLL, *Arch. Ges. Physiol.*, 271 (1960) 761.
7 Š. ADAMIČ, *Biochim. Biophys. Acta*, 102 (1965) 442.
8 H. A. KREBS AND K. HENSELEIT, *Z. Physiol. Chem.*, 210 (1932) 33.
9 R. D. KEYNES AND P. R. LEWIS, *J. Physiol. London*, 113 (1951) 73.
10 J. H. ROE, J. H. EPSTEIN AND N. P. GOLDSTEIN, *J. Biol. Chem.*, 178 (1949) 839.
11 A. GOLDSTEIN, *Biostatistics. An Introductory Text*, McMillan, New York, 1964.
12 L. J. MULLINS AND A. S. FRUMENTO, *J. Gen. Physiol.*, 46 (1963) 629.
13 A. KLEINZELLER AND A. KNOTKOVÁ, *Biochim. Biophys. Acta*, 126 (1966) 604.
14 R. CREESE, *Proc. Roy. Soc., London, Ser. B*, 142 (1954) 497.
15 L. EICHELBERGER, W. H. AKESON AND M. ROMA, *Am. J. Physiol.*, 185 (1956) 287.
16 Z. DRAHOTA, *Physiol. Bohemoslov.*, 9 (1960) 240.