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RESTING AND ACTION POTENTIALS IN RED AND WHITE MUSCLES OF THE RAT

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Classically, mammalian muscles have been differentiated into two types, red and white[12]. The red muscle, composed predominantly of small dark fibers, is capable of slow contracting activity. By contrast, the white muscle, consisting principally of pale fibers of a large diameter, can undergo strong rapid contraction[2]. It has also been known that, contrary to the difference between the slow tonic muscle fibers and the fast muscle fibers in the frog, the white and red muscles, e.g. the fast extensor digitorum longus muscle (EDL) and the slow soleus muscle (SOL) of the rat are both twitch muscles, i.e. they react with propagated action potentials to nerve stimulation[7].

It has recently been reported that red muscles have a significantly greater extracellular space than white muscles[9,18] and that muscles containing mostly white fibers have a lower intracellular Na concentration, [Na]i, and a higher K concentration, [K]i, than those of muscles containing red fibers[5,18]. DRAHOTA[5], who was the first to report differences in the ionic composition of red and white muscles, has suggested that various muscles can be subdivided into two grougs, based on their sodium and potassium concentration, [Na]i+[K]i, predominantly white muscles with a concentration of 174-180 mEq/l, and predominantly red muscles with a concentration of 154-156 mEq/l. SRÉTER and WOO[18], who confirmed the DRAHOTA's finding, have postulated that slow red fibers should have a slightly lower resting potential and a longer time course of the action potential than those of fast white fibers. However, no investigations on the difference in electrical properties between red and white muscles have so far been reported.

The present experiments were carried out to investigate the difference in the resting and action potentials between the red muscle and the white muscle. In addition, measurements of Na and K content in the two muscles were performed for the purpose of correlating the electrical properties of muscle fibers with the intracellular Na and K concentration.

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MATERIALS AND METHODS

Soleus (SOL) and extensor digitorum longus muscles (EDL) were obtained from rats of either Wistar-King or Sprague-Dawley strain, anesthetized with sodium amobarbital. The isolated muscle was stretched in a saline bath, the temperature of which was maintained at about 26°C throughout an experiment. Methods for measuring the resting and action potentials of rat muscle fibers were conventional[6]. The action potentials were obtained by penetration of a muscle fiber with two glass microelectrodes simultaneously, one of them being used for electrical stimulation and the other for recording changes in the membrane potential. The values of the resting potentials were measured by direct observation of the oscilloscope, and the action potentials by photo-recordings. Well trimming of the surface connective tissues of the rat muscle fibers, especially of the SOL fibers using a pair of scissors, was a necessary step before trials of microelectrode penetration, because the surface of the rat muscle did not permit an easy penetration of glass-microelectrode with 15–20MΩ in resistance without the trimming. The electrophysiological measurements were started usually 30 min after the excision of muscles and continued for the subsequent 1.5 hr.

The saline solution for soaking the muscles had the following composition (in mM): NaCl 114.0, KCl 5.0, $CaCl_2$ 2.5, $MaSO_4$ 1.2, $NaHCO_3$ 28.0, Na_2HPO_4 1.2, NaH_2PO_4 0.6 and glucose 11.0 with pH 7.4–7.8. During soaking of muscles, the gas-mixture of 95%O_2 and 5%CO_2 was bubbled as close to the surface of muscle fibers as possible, because in the mammalian skeletal muscle anoxia causes a rapid loss of K ions and a gain of Na[3].

After the measurements of the membrane potentials, the muscles were analysed for Na and K contents by means of a flame spectrophotometer (UNICAM SP 900), and the intracellular Na and K concentrations were calculated from their contents in muscles according to the conventional formula[4]. The extracellular space value used for the calculation was obtained from the empirical formula relating the space to the muscle weight[9] and the dry-to-wet-weight ratio was assumed to be constant, 0.223, for both kinds of muscles[9].

RESULTS

Resting potentials in SOL and EDL muscle fibers. To obtain the resting potentials of SOLs and EDLs as exactly as possible, impalements with a recording electrode were performed on nearly all the distinguishable fibers in a muscle from one extreme side to the other in order, and then similar trials were made after turning the muscle over. Impalements were carried out only on the surface fibers, though there is a possibility that the relatively low and markedly variable potentials might be recorded from the surface fibers because of damage during trimming of the connective tissue[1]. FIG. 1 shows the frequency distribution of the resting potentials of 401 fibers from 8 EDLs and of 744 fibers from 12 SOLs. Both muscles do not show a normal distribution but a skewed distribution towards high values, raising the possibility that almost all tha values below −65 mV in EDLs and −50 mV in SOLs represent a different, and perhaps injured, population of fibers[14]. EDLs contain a distinct group of fibers showing the resting potential near to the mode value,

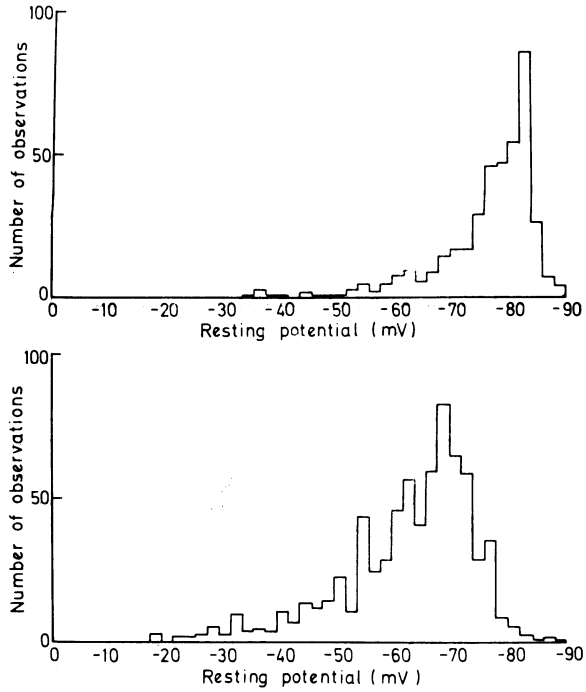

FIG. 1. The frequency distribution of the resting potential of 401 fibers in the EDL muscle (top figure) and of 744 fibers in the SOL muscle (bottom figure).

while the resting potentials of SOLs are more scattered than those of EDLs. The mode value of the frequency distribution in EDLs were −79 mV, while that for SOLs −68 mV. The resting potential magnitude obtained is about the same as that demonstrated by ZIERLER[14] on the rat EDL muscle, −74 mV on the average.

Action potentials in EDL and SOL muscle fibers. FIG. 2 shows typical action potentials of the EDL and SOL obtained by stimulation of the muscle fiber membrane with an intracellular electrode, the resting potentials of which were nearly at the mode value. It can be seen that the action potential amplitude and the max. rates of rise and fall are greater in the EDL than those in the SOL. However, configurations of the action potentials of fibers in both muscles differed greatly from each other because there was a great variability in the resting potential among fibers in each type of muscles, as shown in FIG. 1. In FIG. 3 the action potential amplitude was plotted against the resting potential magnitude for both types of muscles. Fibers of both kinds of muscles having a resting potential of less than about 55 mV did not elicit the action potential, as seen in this figure. The amplitude of the action po-

tential in EDLs ranged from 53 mV to 106 mV, while that in SOLs from 41 mV to 99 mV (FIG. 3), thus confirming that the former is, on the average, greater than the latter, although there is an overlap in the action potential amplitude between EDLs and SOLs. The mean values of the action potential magnitude

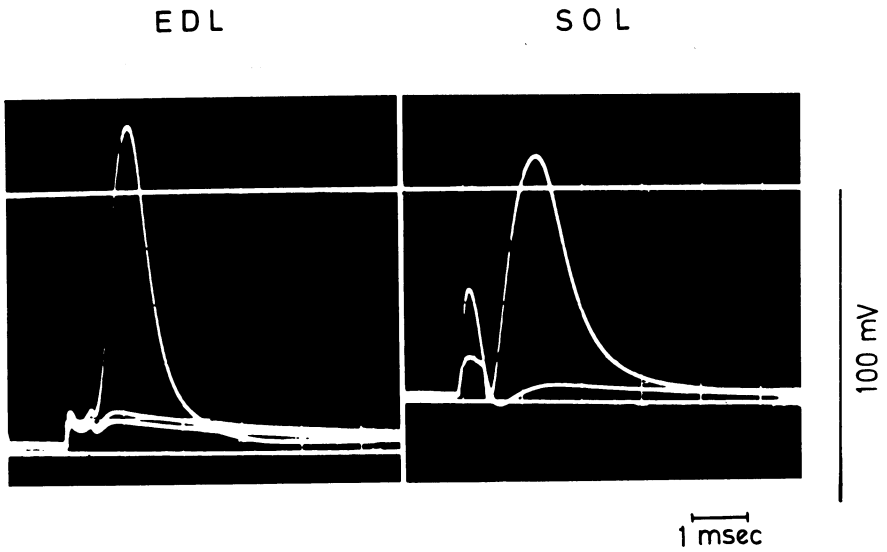

FIG. 2. The action potentials obtained by stimulation with an intracellular microelectrode of EDL (left) and SOL (right) muscle fibers, the resting potential of which was at nearly the mode value for each muscle (EDL −83 mV, and SOL −67 mV).

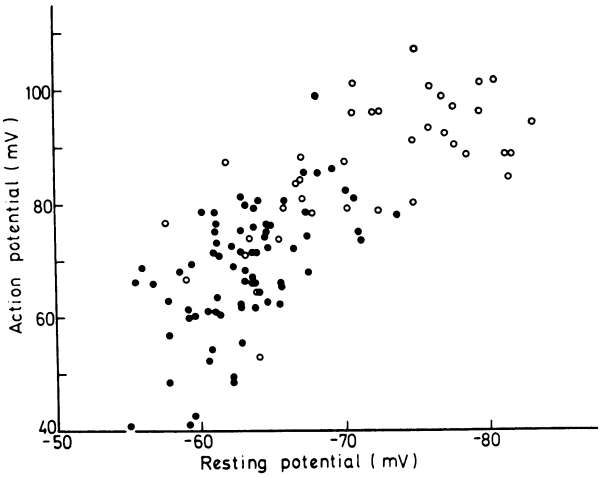

FIG. 3. The relationship between the action potential and the resting potential in EDL (○) and SOL (●) muscle fibers. Each symbol indicates the membrane potentials of each muscle fiber.

TABLE 1.
The average values of the resting and action potentials in EDL and SOL muscle fibers

Muscles		Weight (mg)	[K]i (mmole/kg f. w.)	[Na]i	Vr (mode) (mV)	Va(mV)	Vo(mV)	Immersion time
EDL	1	219.0	139.9	21.1	81			2°45′
"	2	154.9	147.3	24.7	79			2°16′
"	3	183.9	145.9	52.6	77			2°08′
"	4	199.8	129.4	27.2	77(13)	94.0(7)	22.2	2°28′
"	5	169.6	148.1	22.8	78(15)	79.2(9)	11.0	2°28′
"	6	117.5	149.7	16.6	81(13)	89.7(11)	11.8	2°00′
"	7	69.7	157.9	48.9	79(9)	88.1(15)	19.5	2°15′
"	8	95.4	154.0	21.9	74(16)	86.8(10)	18.3	2°19′
Mean		151.2	146.5	29.5	78.2	87.6	11.4	2°20′
S. D.			8.6	12.9	6.6	4.0		
SOL	1	100.1	117.4	32.8	73			2°45′
"	2	153.9	118.0	37.5	63			2°02′
"	3	176.4	116.3	35.3	58			2°51′
"	4	155.1	112.5	21.4	68			3°00′
"	5	122.3	111.8	53.1	68			2°34′
"	6	70.1	130.8	43.8	63(10)	52.0(4)	−6.4	2°52′
"	7	66.0	119.5	47.2	67(20)	70.8(15)	7.6	1°55′
"	8	69.0	123.6	40.0	68(26)	68.2(11)	4.1	2°35′
"	9	74.4	125.6	27.9	70(30)	67.0(16)	5.1	3°01′
"	10	135.8	117.7	31.3	67(18)	71.4(7)	6.8	2°02′
"	11	67.4	120.3	41.5	62(17)	55.8(5)	−7.0	1°45′
"	12	73.9	117.8	50.0	66(26)	72.9(16)	10.2	2°55′
Mean		105.4	119.3	38.5	66.0	65.4	2.9	
S. D.			4.9	8.9	3.7	8.0		

The average values (and ±S. D.) of [Na]i for 5 EDL muscles and 6 SOL muscles, on which the action potential was measured, are 27.5±11.2 and 40.2± 7.5 mmole/kg f. w., respectively. Figures in the parentheses indicate the number of fibers measured. Vo (the overshoot) is the mean value of $(Va-Vr)$ in individual fibers (Va; action potential amplitude, Vr; resting potential magnitude).

were 87.6 mV (5 muscles) for EDLs and 65.4 mV (7 muscles) for SOLs (TABLE 1). When all the measured values of the action potential amplitudes for both EDLs and SOLs are taken together, the action potential amplitude is approximately linearly related to the resting potential magnitude, as shown in FIG. 3.

The max. rates of rise and fall of the action potentials are plotted against the resting potential magnitude in FIGS 4 and 5, respectively, in which it is seen that EDLs show the greater max. rates of rise and fall than those in SOLs, though there is an overlap in these values between the two kinds of

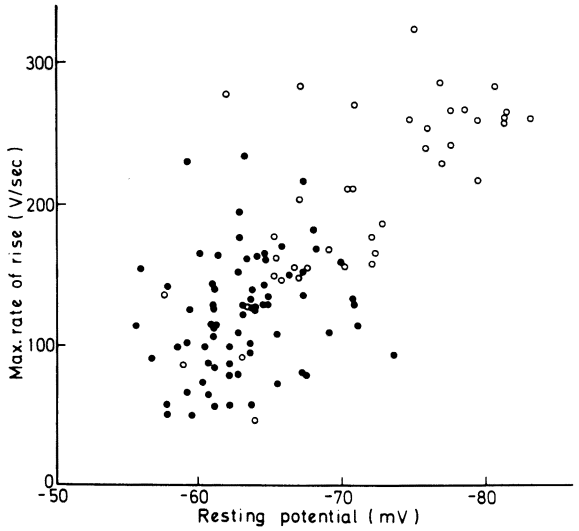

FIG. 4. The relationship between the max. rate of rise of the action potential and the resting potential in EDL (○) and SOL (●) muscle fibers. Each symbol represents an individual fiber.

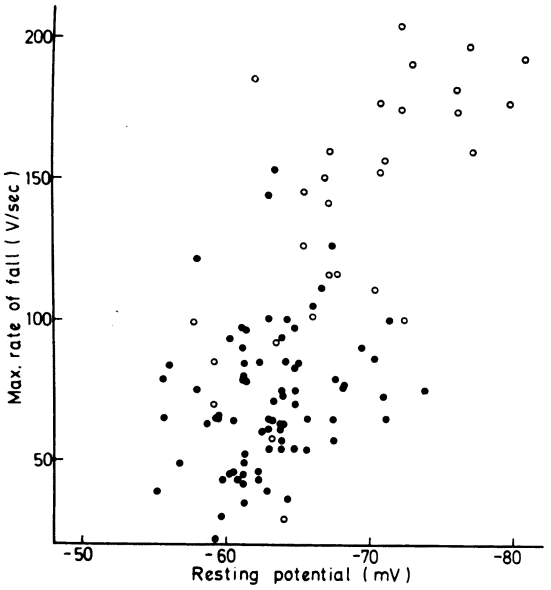

FIG. 5. The relationship between the max. rate of fall of the action potential and the resting potential in EDL (○) and SOL (●) muscle fibers. Each symbol represents an individual fiber.

muscles and that both parameters are linearly related to the resting potential magnitude when the max. rates of rise and fall for EDLs and SOLs are taken together, although there is a great variability in the values obtained.

Na and K contents in EDL and SOL muscles. In order to know the intracellular concentration of the freshly dissected muscles, the ionic concentration of the plasm is required. Therefore, the estimation of Na and K ion concentrations in the serum was carried out by means of flamephotometry and found to be 4.9 mmole/l serum for K ions and 143 mmole/l serum for Na (the average for three rats); these values are quite similar to K and Na concentrations of the saline solution. Then, using these values of the concentration of the plasm, the intracellular concentrations of Na and K ions in freshly

TABLE 2.
The average Na and K content in fresh EDL and SOL muscles

Muscles	Na	K	[Na]i	[K]i
EDL (10)	24.0±2.3	106.7±4.5	17.2±4.0	156.4± 7.8
SOL (19)	31.3±4.2	89.9±5.6	25.2±6.3	132.9±12.1

± refers to S.D.
Na and K refer to the ion content, expressed by mmole/kg w.w., while [Na]i and [K]i the ion concentration by mmole/kg f.w.

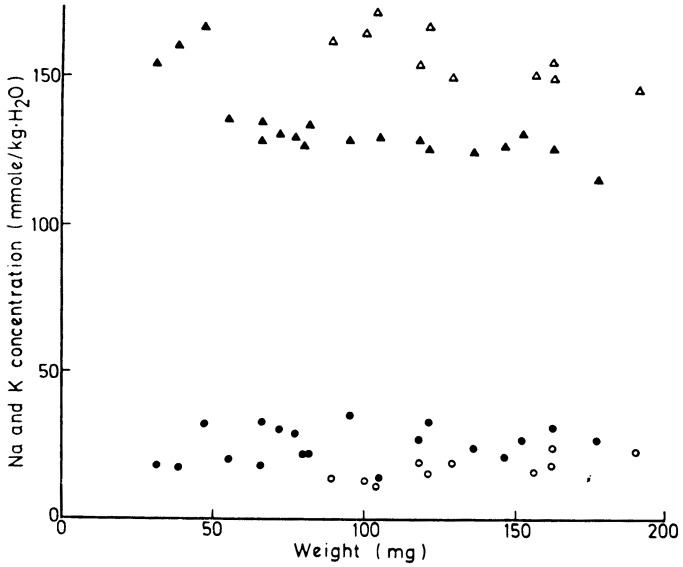

FIG. 6. Na and K concentrations in fresh EDL and SOL muscles, plotted against the muscle weight. ○; Na (EDL), ●; Na (SOL), △; K (EDL) and ▲; K (SOL)

dissected rat muscles were calculated from the muscle content measured. The results are shown in TABLE 2 and FIG. 6. EDL muscles show, on the average, a lower value for Na and a higher value for K ion concentration than SOL muscles. This is in good agreement with the results of the previous investigators[5,13]. The sum [Na]i+[K]i is significantly greater in EDLs than that in SOLs[5]. In FIG. 6 there is a trend that [K]i decreases with an increasing muscle weight in both EDL and SOL muscles; this agrees with similar finding by KUSUMOTO[10].

TABLE 3 shows the Na and K concentrations in both muscles after immersion in the saline solution for 2-3 hr. Compared with TABLE 2, TABLE 3 indicates a decrease in [K]i and an increase in [Na]i in both EDLs and SOLs during 2 hr's immersion. This implies that the intracellular Na and K concentrations are not maintained constant in the saline solution, but decreases with a rate constant of about $0.05\,hr^{-1}$.

TABLE 3.
The average Na and K concentration in EDL and SOL muscles immersed in the saline solution for about 2 hr.

Muscles	Average immersion time	[Na]i	[K]i
EDL (8)	1°55′	33.1±11.1	148.9±6.7
EDL (8)	2°31′	27.7±10.2	143.1±10.5
SOL (9)	2°07′	35.8±8.0	119.4±8.6
SOL (10)	2°50′	40.8±11.6	119.1±5.6

± refers to S.D.
All values were expressed by mmole/kg f.w.

Relationships between the ionic concentrations and the resting potential, overshoot and the max. rates of rise and fall. Magnitudes of the resting and action potentials, [K]i and [Na]i in EDL and SOL muscle fibers are shown in TABLE 1. The mode value of the resting potentials (Vr) of each EDL and SOL was plotted against [K]i of each muscle in the left of FIG. 7, in which it is seen that EDLs have a greater Vr and a greater [K]i than those of SOLs. When EDLs and SOLs are taken together, an approximately linear relationship is observed between the Vr and the logarithm of [K]i.

In the right of FIG. 7, the mean value of the overshoot obtained from a number of fibers in each muscle was plotted against [Na]i. In general EDLs show a greater overshoot than that of SOLs corresponding to a smaller value of [Na]i in the former. The overshoot of all the muscles including EDLs and SOLs show a trend to become small with increasing [Na]i, although the observed values are scattered very much.

FIG. 8 shows the relationship between the max. rate of rise of the action

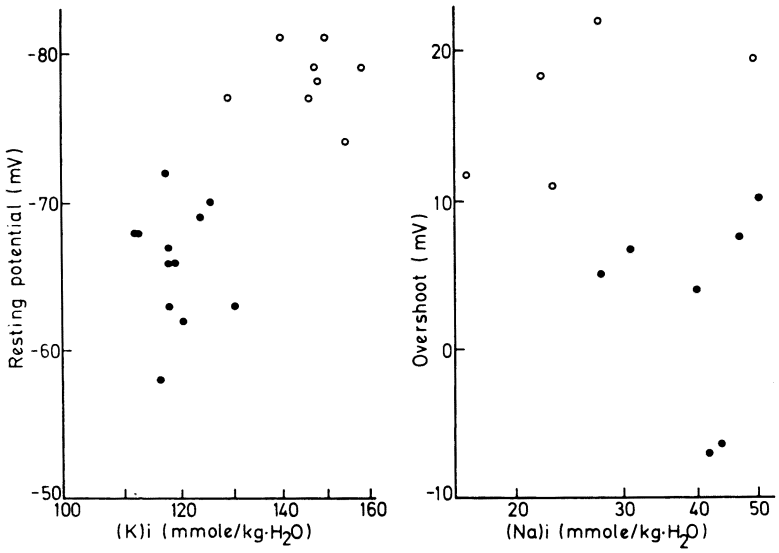

FIG. 7. Left: The relationship between the resting potential and [K]i in EDL (○) and SOL (●) muscles. Each symbol indicates the mode value of the resting potentials, measured on a number of fibers, and [K]i in a muscle. Right: The relationship between the overshoot of the action potential and [Na]i in EDL (○) and SOL (●) muscles. Each symbol indicates the mean value of the overshoots, measured on several fibers, and [Na]i in a muscle.

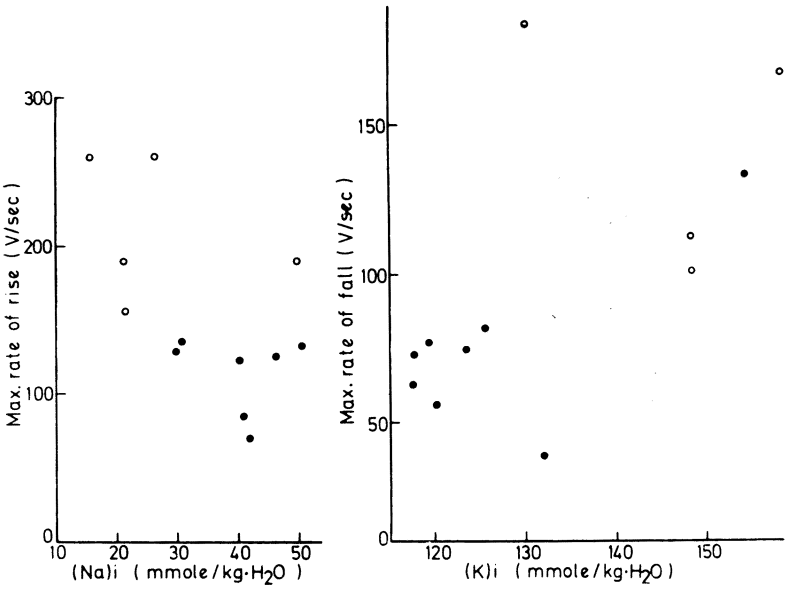

FIG. 8. Left: The relationship between the max. rate of rise of the acion potential and [Na]i in EDL (○) and SOL (●) muscles. Each symbol represents the mean value of the max. rate of rise of several fibers and [Na]i in a muscle. Right: The relationship between the max. rate of fall of the action potential and [K]i in EDL (○) and SOL (●) muscles. Each symbol represents the mean value of the max. rate of fall of several fibers and [K]i in a muscle.

potential and [Na]i (left figure) and that between the max. rate of fall and [K]i (right figure). The max. rate of rise decreases with increasing [Na]i and the max. rate of fall increases with increasing [K]i, when the values for EDLs and SOLs are taken together.

DISCUSSION

The present investigation has confirmed the finding by DRAHOTA[5] and SRÉTER and WOO[18] that muscles containing mostly white fibers have a lower [Na]i and a higher [K]i than those of muscles containing red fibers, and has further demonstrated that the EDL muscle shows a greater value in the resting potential magnitude, the action potential amplitude and the max. rates of rise and of fall than the SOL muscle. The greater resting potential magnitude and the greater max. rate of fall in the EDL has been correlated with the higher K concentration in this muscle, and a greater magnitude of the action potential and the greater value of the max. rate of rise in the EDL has been related to the lower Na concentration in the muscle. Further, when the relationships between these electrical parameters and the intracellular Na and K concentrations were examined for all the muscle fibers, the relationships were found to accord with those predicted by the ionic theory[8]. Such facts indicate that the mechanisms generating the resting and action potentials in the two kinds of muscles are not qualitatively different from each other, and that the quantitative difference between the two muscles is a consequence of the difference in [K]i and [Na]i. In this respect the present study has confirmed the classical notion that the mammalian red and white muscles can react with an action potential to external stimuli and that the difference between the two is not the same as the difference between the slow and fast muscle fibers in the frog.

According to the ionic theory[8], the resting potential (Vr) is expressed,

$$Vr = \frac{RT}{F} \ln \frac{b[\mathrm{Na}]o + [\mathrm{K}]o}{b[\mathrm{Na}]i + [\mathrm{K}]i},$$

if distribution of Cl ions and equal activity coefficients for Na and K ions inside and outside the cell are assumed. The permeability ratio b (P_{Na}/P_K) can be calculated for the EDL and SOL, by employing the average values obtained in the experiments: For the EDL [Na]i=27.5 mmole/kg f. w., [K]i =146.5 mmole/kg f. w. and $Vr=-78.2$ mV, and for the SOL [Na]i=39.7 mmole/kg f. w., [K]i=120.8 mmole/kg f. w. and $Vr=-66.4$ mV. The calculation yielded a value of 0.015 for b in the EDL and 0.030 for the SOL, indicating that the relative permeability of Na ions is greater in the SOL than in the EDL. However, the values of the resting potential, [K]i and [Na]i, quoted above, were derived from the muscles, which had been immersed in the saline solu-

tion at 26°C for 2hr. Therefore, the resting potential of the *in vivo* muscles should be about −83 mV for the EDL and −73 mV for the SOL, if the [Na]i and [K]i values in the fresh muscles are introduced into the above equation and correction for the difference in temperature is made. Similarly the P_{Na}/P_K ratios during the activity are 2.24 for the EDL and 1.28 for the SOL, which yielded values of 14 mV and 4 mV for the overshoot of the action potential in *in vivo* muscles, respectively. The resting potential of −78 mV in *in vitro* EDL muscles and 83 mV in *in vivo* EDL muscles is small compared with −100 mV obtained on the *in vivo* tibialis anterior muscles of mice by BENNETT, WARE, DUNN and MCINTYRE[1]. However they obtained this from the fibers deep inside the muscle, while from the surface fibers they got an average value of −83 mV, which is very close to the value found in the present experiments.

According to SRÉTER and WOO[18], 33.4% of the superficial bundle of the EDL are dark fibers while in the SOL dark fibers occupy 56.5% of the superficial bundle. This is consistent with the finding in the present experiments that the resting potential, action potential and the max. rates of rise and of fall in individual EDL and SOL muscle fibers overlap with each other, thus indicating no clear demarcation in the electrical properties between the EDL and SOL muscle fibers.

The distribution of the resting potentials in both EDLs and SOLs has been shown to be unimodal. This indicates further that the electrical properties in fibers of each type of muscles can neither be divided sharply into two distinct groups. NAGAKI[11] has recently measured the fiber diameter of EDL and SOL muscles and shown that the distribution of the fiber diameters is unimodal in both kinds of muscles or approximately a normal distribution, the mean value of the diameter being 35.2μ in the EDL and 30.3μ in the SOL. Therefore, there is little evidence that dark and pale fibers in the EDL and SOL muscle can be divided sharply regarding the fiber diameter and the resting and action potential magnitude.

SUMMARY

The resting and acting potentials of the extensor digitorum longus (EDL) and soleus (SOL) muscle fibers were measured with a glass-capillary microelectrode, EDL muscle fibers show, on the average, a greater resting potential magnitude and a larger action potential amplitude than those in SOL muscle fibers. The max. rates of rise and of fall is greater in the former than in the latter. The differences in the electrical properties between the EDL and SOL were found to be correlated with a greater [K]i and a smaller [Na]i in the former than in the latter.

Electrical parameters in individual muscle fibers showed an overlap between

the EDL and SOL, suggesting that no clear demarcation can be made between the red and white muscle fibers. It is concluded that the red and white muscles are not essentially different from each other and that the difference is only quantitative.

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