Action Potentials in
Fast- and Slow-Twitch Mammalian Muscles
during Reinnervation and Development

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ABSTRACT   Action potentials (APs) were recorded from the extrajunctional membrane of surface fibers of the fast-twitch extensor digitorum longus (extensor) and the slow-twitch soleus muscles of adult rats. APs of the extensor muscle had a significantly faster rate of rise and fall, as well as a shorter duration, than those of the soleus. In addition, the overshoot of APs and the resting membrane potential was greater for the extensor. Whereas the soleus produced only one AP regardless of the stimulus duration, the number of extensor responses was directly proportional to the stimulus duration. This repetitive activity was greatly reduced by a concentration of tetrodotoxin (TTX) as low as 5×10^{-11} g/ml. Within 8 d after crush of the nerves to these two muscles, all differences in AP properties disappeared and both muscles became partially resistant to TTX. Reinnervation brought about a redifferentiation so that differences in AP were again significant at 22 d after nerve crush. However, the rate of rise of extensor APs did not attain normal values even as late as 60 d after nerve crush. APs were found to be the same for extensor and soleus muscles from 12-d-old rats. At 18 d after birth, rate of rise was equivalent to that of adult muscle for the soleus although 50–60 d were required before this parameter was fully mature for the extensor. Nevertheless, APs of the extensor and soleus were clearly differentiated within 25 d after birth. Differences in fast and slow muscle APs are discussed with regard to differences in ion gradients and sarcolemmal conductance.

INTRODUCTION

The extensor digitorum longus (extensor) and soleus muscles of the rat are classified as fast and slow twitch on the basis of differences in mechanical (Close, 1964), biochemical (Drahota, 1961; Kauffman and Albuquerque, 1970; Hoh and Salafsky, 1971; Mommaerts et al., 1977), morphological (Ellisman et al., 1976; McArdle and Sansone, 1977), and pharmacological (Gutmann and Sandow, 1965; Albuquerque and McIsaac, 1970; McArdle et al., 1977; D'Alonzo et al., 1979) properties. The observation that the rates of

rise and fall, as well as the amplitude, of the action potential (AP) and the resting membrane potential are greater for the extensor (Yonemura, 1967) is of particular interest to the present study. Yonemura (1967) and Akaike (1976, 1978) have demonstrated that these differences in membrane electrical properties depend upon a greater electrochemical gradient for Na^+ and K^+ across the membrane of fibers in the extensor muscle. Since denervated and neonatal muscles would not be expected to be differentiated with respect to membrane potentials (Redfern and Thesleff, 1971 a, b; Albuquerque et al., 1971; McArdle and Albuquerque, 1973; Vyskočil, 1974: Sellin and McArdle, 1977 a, b; McArdle and Sansone, 1977) a developmental study offers a means of further probing the basis for differences in AP. Thus, the primary objective of this study was to determine the time-course of differentiation of extensor and soleus APs during reinnervation and development. After having done this, we then attempted to correlate the differences with maturation of the concentration gradient for Na^+ across the sarcolemma of these muscles. To do this, we have utilized our electrophysiologic data to estimate the intracellular concentration of sodium.

METHODS

Preparation

All experiments were performed in vitro upon the fast-twitch extensor digitorum longus (extensor) and the slow-twitch soleus muscles of neonatal (10–60 d old) or adult (60–120 d old) rats of the Wistar strain. Studies of denervated and reinnervating muscles were restricted to adult rats. In these experiments rats were anesthetized with diethyl ether and the muscle nerves were crushed ~12 mm from their entry into the extensor and soleus, and the animals were allowed to recover. At various intervals after birth or nerve crush, the extensor and soleus muscles were excised under a continuous flow of oxygenated physiological solution.

Electrophysiological Measurements

The isolated muscles were stretched to about 5% beyond their resting length (Fatt and Katz, 1951) over a plano-convex lens in the center of a paraffin-lined Plexiglas plate. Stainless steel pins were inserted through the tendons to affix the muscles to the plate which was then inserted into an insulated chamber having a volume of 15 ml and superfused at a rate of 2–3 ml/min with an oxygenated (95% O_2 − 5% CO_2) solution containing (millimolar):NaCl, 135; KCl, 5; MgCl$_2$, 1; Na$_2$HPO$_4$, 1; NaHCO$_3$, 15; CaCl$_2$, 2; dextrose, 11. Unless otherwise stated all measurements were made at a temperature of 21–23°C.

Intracellular recordings were made from single fibers on the dorsal surface of the muscles with glass microelectrodes filled with 3 M KCl and having resistances of 5–15 MΩ and tip potentials of less than 3 mV (Adrian, 1956). Membrane potentials were amplified (W-P Instruments, Inc., New Haven, Conn., model M701), displayed on oscilloscopes, and photographed. The time constant of the entire recording circuit was ~ 40 μs. Jena (Jenoptic, Jena, East Germany) micromanipulators were used to insert the microelectrodes into transilluminated muscle cells visualized with a stereoscopic microscope.

To evaluate extrajunctional action potentials (APs), a recording electrode was inserted at least 1 mm from the endplate region. A stimulating electrode was then

inserted into the same fiber within 100 μm of the recording elecrode. All muscle fibers were hyperpolarized to −88 to −92 mV with anodal current delivered through the stimulating electrode 10–15 s before excitation with a depolarizing current pulse of 20 ms duration, unless noted otherwise. An on-line laboratory minicomputer (Hewlett-Packard Co., Palo Alto, Calif., model 21MX E-Series) digitized (50-μs sampling interval for 10 ms) the AP and listed the following parameters: (a) overshoot amplitude and duration at 0 mV membrane potential; (b) threshold that was the level of membrane potential at which the second derivative of membrane voltage change exceeded 0; (c) the maximum rate of rise (dV/dt) and fall ($-dV/dt$) of the AP as well as the membrane potential (V_{max}) at which dV/dt was maximal. Digitized images were differentiated and represented as phase plane trajectories (Jenerick, 1963).

In order to determine the sensitivity of APs to tetrodotoxin (TTX; Sankyo Co., Tokyo, Japan; mol wt, 319.3), muscles were exposed to varying concentrations of the toxin for 30 min before electrophysiological analysis. At least three animals (two extensor and two soleus muscles per animal) were studied at each concentration of TTX. APs were defined as those responses which crossed over the 0 level of membrane potential to become positive and then returned to some negative value. Thus, duration could be measured and local responses were excluded (Thesleff et al., 1974).

Results were entered into a data-base management system which was programmed to sort and statistically analyze the data. Student's t test ($P < 0.01$) was used to evaluate the significance of differences.

RESULTS

Characteristics of Action Potentials from Innervated Extensor and Soleus Muscles from Adult Rats

A qualitative comparison (Fig. 1) of APs from fibers in an extensor and the ipsilateral soleus muscle reveals differences in time and voltage characteristics. Quantitative evaluation (Table I) shows that these differences are statistically significant ($P < 0.01$). For instance, the maximum rate of rise (dV/dt) was 459.4 ± 4.7 V/s (mean ± SEM) and 294.4 ± 3.5 V/s for fibers in the extensor and soleus muscles, rspectively. Fig. 2 shows that the distribution of dV/dt was skewed toward higher values for the extensor whereas that of the soleus was normal. It is interesting to note that at 12°C the differences in AP characteristics were not statistically significant (see Table I). Fibers in the extensor and soleus muscles also differed in their responses to stimuli of increasing duration (Fig. 3). For example, a stimulus of 50 ms duration produced an average of 3.5 ± 0.9 APs in fibers of the extensor and 1.0 ± 0.01 in fibers of the soleus (Fig. 4).

Single APs of these two muscles were equally sensitive to TTX. Approximately 1×10^{-9} g/ml (3 nM) of TTX caused a 50% reduction of dV/dt, and in the presence of $3–4 \times 10^{-8}$ g/ml, 50% of the fibers were unable to generate APs. However, at even lower concentrations of TTX the frequency response of the extensor muscle was reduced. For example, 87% of the fibers in the fast muscle produced only one AP during a 20 ms stimulus in the presence of 5×10^{-11} g/ml of TTX. At this concentration of TTX, all of the fibers examined produced APs although dV/dt was reduced to 391.6 V/s for the remaining extensor response and 268.4 V/s for that of the soleus.
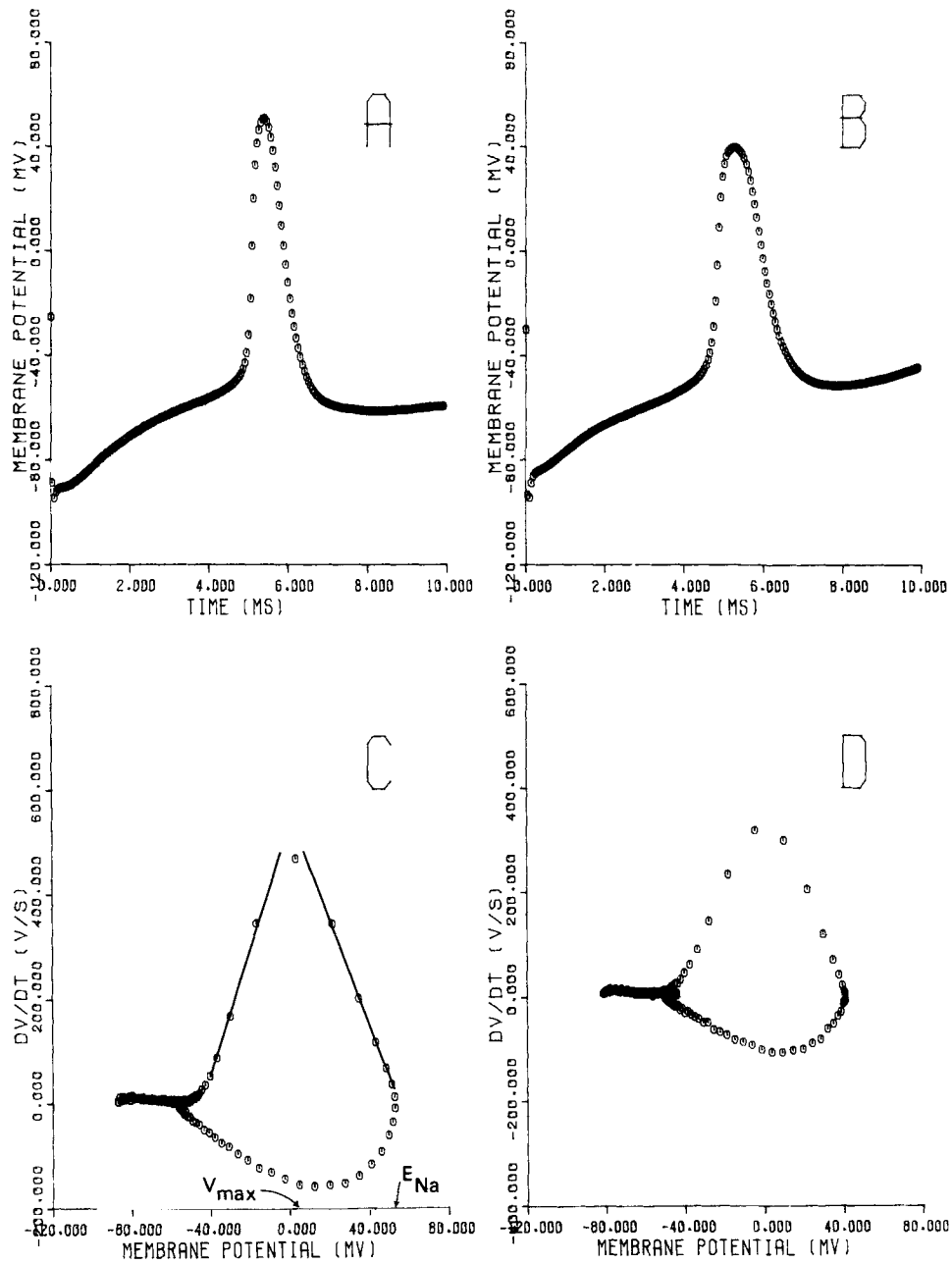
FIGURE 1. Representative action potentials (A and B) and phase plane trajectories (C and D) recorded from fibers in the extensor (A and C) and soleus (B and D) muscles of adult rats. C indicates how estimates of the reversal potential for Na^+ (E_{Na}), as well as the membrane potential at which dV/dt was maximum (V_{max}), were obtained.

TABLE I

PROPERTIES OF RESTING (RMP) AND ACTION POTENTIALS RECORDED FROM SURFACE FIBERS OF THE EXTENSOR AND SOLEUS MUSCLES OF ADULT RATS AT 12, 22, AND 32°C

Temp	RMP	Overshoot	dV/dt	−dV/dt	Duration	Threshold	V_{max}	% Fibers with two or more AP*
°C	mV	mV	V/s	V/s	ms	mV	mV	
Extensor								
12	−82.8±0.6‡ (20)	67.6±1.8	145.2±5.7	57.1±2.0	3.51±0.12	−39.4±0.9	14.8±1.0	−
22	−83.5±0.2§ (864)	53.8±0.2§	459.4±4.7§	150.1±0.8§	0.99±0.06§	−52.4±0.2§	5.3±0.3§	91
32	−83.0±0.4§ (42)	47.3±0.8§	751.9±18.5§	265.6±3.0§	0.43±0.01§	−55.0±0.6§	28.0±2.3§	−
Soleus								
12	−80.8±1.0 (4)	55.5±8.8	115.0±15.4	42.0±7.2	3.70±0.24	−41.0±1.5	13.0±3.1	−
22	−79.2±0.2 (774)	41.8±0.4	294.4±3.5	103.0±0.9	1.25±0.01	−51.4±0.2	−1.6±0.3	6
32	−78.6±0.4 (40)	30.6±1.4	435.0±19.9	175.3±7.4	0.53±0.01	−30.6±1.4	7.0±1.9	−

* The duration of the stimulus pulse was 20 ms.
‡ The mean ± SEM. Numbers in parentheses are the number of fibers examined.
§ The differences between these values and those seen in the corresponding soleus muscle are significant (P < 0.01).
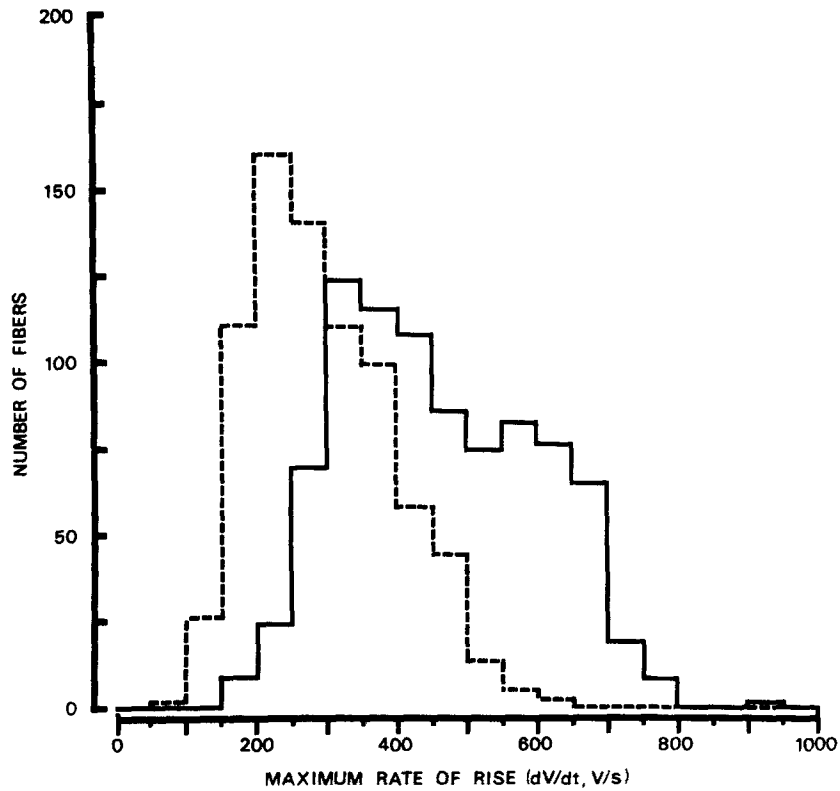

FIGURE 2. Distribution of dV/dt for the innervated extensor (solid lines) and soleus (dashed lines) muscles from the adult rats.

Assuming that the overshoot of the AP is a reasonable approximation of the reversal potential for Na^+ (E_{Na}; Hodgkin, 1951; Desmedt, 1953; Jenerick, 1963; Akaike, 1978), it is possible to estimate the intracellular concentration of Na^+ ($[Na]_i$) from the Nernst equation as expressed below:

$$E_{Na} = \frac{RT}{F} \ln \frac{[Na]_o}{[Na]_i}$$

where R, T, and F have their usual meaning. When T is 22°C this expression reduces to

$$[Na]_i = [Na]_o/(10)^{E_{Na}/58.4},$$

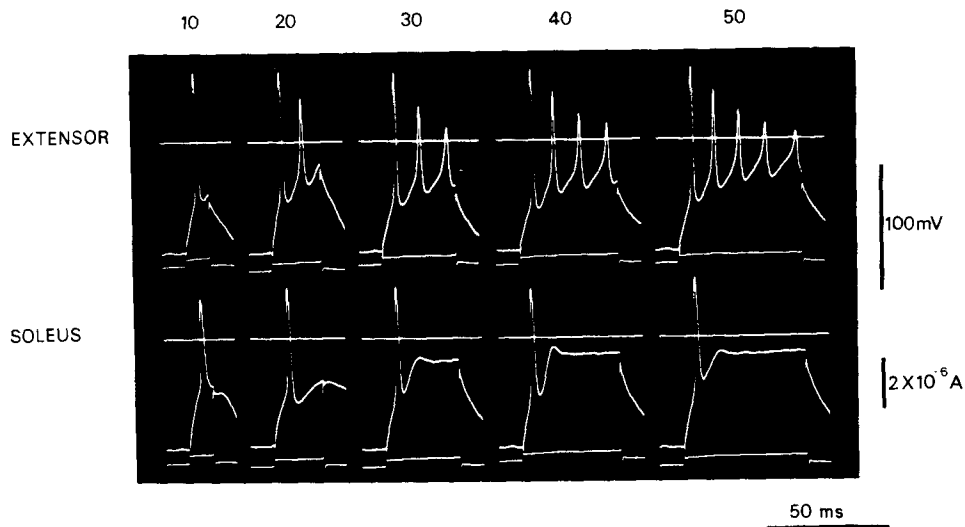where the extracellular concentration of Na^+ ($[Na]_o$) is 152 mM. Thus, $[Na]_i$

FIGURE 3. Typical action potentials from fibers in extensor and soleus muscles from adult rats at stimulus durations of 10, 20, 30, 40, and 50 ms. Lower trace is the current stimulus, the middle trace is membrane potential, and the upper trace is 0 mV membrane potential.

was 18.2 mM for the extensor and 29.2 mM for the soleus at 22°C. It must be emphasized that these and subsequent similar estimates of $[Na]_i$ are only aproximations, because the overshoot of the AP does not equal E_{Na} (see Discussion).

Since the product of membrane capacitance (C_M; 3.6 $\mu F/cm^2$; McArdle and Albuquerque, 1973) and dV/dt approximates the maximal sodium current (I_{Na}; Akaike, 1978; Walton and Fozzard, 1979), maximal sodium conductance (g_{Na}) can be estimated from the following expression (Hodgkin et al., 1952).

$$I_{Na} = C_M \, dV/dt = g_{Na} \, (E - E_{Na})$$

where E is V_{max}. It is important to point out that to set E equal to the level of

membrane potential from which the AP is triggered (−90 mV in this study) leads to an underestimate of g_{Na}. The reason for this is that E is constantly changing during the time-course of the AP and, unlike in voltage-clamp experiments, the driving force for sodium current is not constant. Thus, one must know the true value of $E - E_{Na}$ in effect at the instant of maximum rate of voltage change in order to use the following expression to accurately estimate maximum sodium conductance,

$$g_{Na} = C_M \, dV/dt/(E - E_{Na}).$$

Therefore, our ability to measure V_{max} increases the accuracy of g_{Na} estimates and reveals one advantage of analyzing the phase plane trajectories of APs.

I_{Na} was 1.6 mA/cm^2 for the extensor and 1.1 mA/cm^2 for the soleus at 22°C. The corresponding values of g_{Na} were 34.1 mmho/cm^2 and 24.4 mmho/cm^2, respectively. Using a voltage-clamp technique, Adrian and Marshall
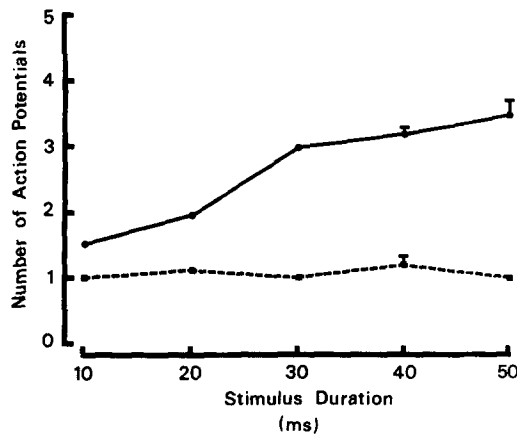

FIGURE 4. Number of action potentials in fibers of extensor (●) and soleus (■) muscles from adult rats at different stimulus durations. Each point represents the mean of at least 30 fibers from two or more muscles. Verticle bars indicate SEM greater than 0.1.

(1977) have estimated [Na]$_i$ to be 16 mM and I_{Na} to be 1.2 mA/cm^2 for the extensor.

Characteristics of Action Potentials from Denervated and Reinnervating Extensor and Soleus Muscles from Adult Rats

Denervation had the expected depressant effects upon muscle APs (Redfern and Thesleff, 1971 *a*). At 5 d after nerve crush, there were no qualitatively observable differences in the APs from extensor and soleus muscles (Fig. 5), and at 8 d any quantitative differences were not statistically significant (Tables II and III). This was associated with an estimated increase of [Na]$_i$ to 39.0 mM in the extensor and 39.5 mM in the soleus, as well as a decrease of g_{Na} to 18.9 mmho/cm^2 for both of these muscles. Also, TTX sensitivity was reduced in accord with the work of Redfern and Thesleff (1971 *b*). However,
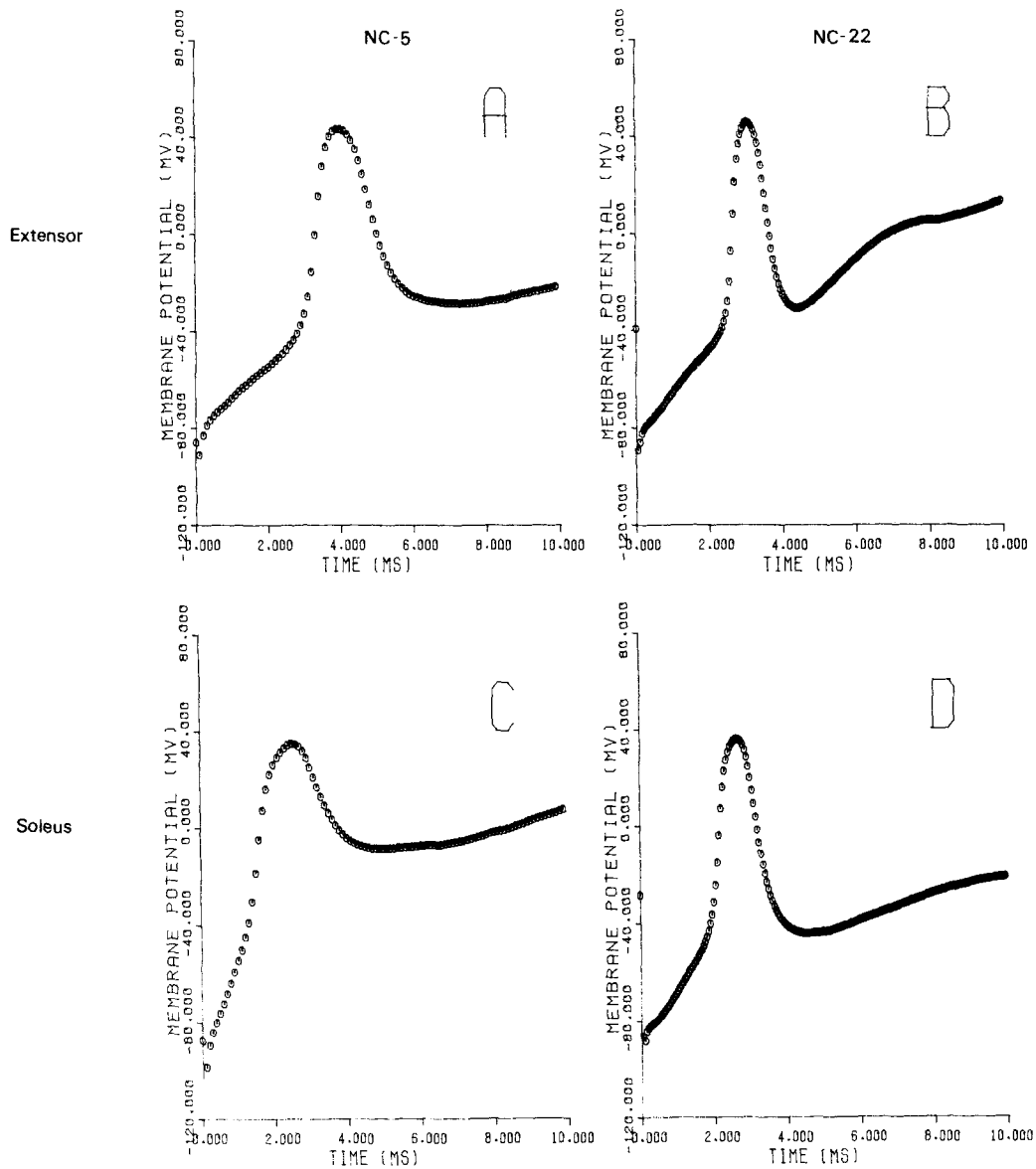
FIGURE 5.   Representative action potentials recorded from fibers in the extensor and soleus muscles at 5 and 22 d after nerve crush.

resistance to TTX was never complete (Table IV). For instance, at 8 d after nerve crush only 43% of the extensor fibers produced APs in the presence of 1×10^{-6} g/ml of TTX and in these fibers dV/dt was reduced to 45% of the value seen prior to exposure to the toxin.

An earlier study (McArdle and Albuquerque, 1973) has indicated that at least 74% of the extensor and soleus fibers are reinnervated within 21 d after

TABLE II

PROPERTIES OF RESTING AND ACTION POTENTIALS RECORDED FROM SURFACE FIBERS OF THE REINNERVATING EXTENSOR MUSCLE

Days after nerve crush	RMP	Overshoot	dV/dt	$-dV/dt$	Duration	Threshold	V_{max}	% Fibers with two or more AP
d	mV	mV	V/s	V/s	ms	mV	mV	
5	−61.7±0.4* (134)	35.3±0.8	182.3±5.2*	56.2±1.4	1.77±0.03	−52.8±0.4	−2.5±0.6*	2
8	−60.7±0.5 (63)	34.5±1.2	194.7±9.1	61.6±2.7	1.69±0.05	−47.5±1.0	−2.6±0.8	0
10	−61.9±0.6* (66)	37.9±1.4*	179.7±7.8*	53.1±2.4	2.16±0.08	−52.2±0.9	−1.0±0.9*	0
13	−63.8±0.6* (47)	38.4±1.3	219.5±9.1	71.1±2.7	1.49±0.05	−46.3±0.7	−4.1±1.2	9
14	−64.5±0.7* (65)	35.9±1.0	271.7±10.2	80.0±4.0*	1.40±0.05*	−48.7±0.9	0.0±1.0	32
18	−70.6±0.7 (58)	43.9±1.1	267.6±10.0*	111.0±3.6*	1.24±0.05	−47.6±0.8	0.6±1.0	55
22	−79.3±0.5* (39)	43.3±0.9	333.2±11.0*	144.1±2.7*	0.99±0.01*	−50.8±0.6	−1.7±1.1	87
30	−81.0±0.3* (93)	46.6±0.8*	350.6±8.4*	140.1±1.8*	1.09±0.02*	−50.4±0.4*	5.2±0.9	67
60	−81.9±0.7 (40)	45.4±1.0	411.6±15.5*	149.4±2.8*	0.92±0.02*	−49.0±1.0*	2.2±1.1	79

* The differences between the values and those seen in the corresponding soleus muscle are significant ($P < 0.01$).

TABLE III

PROPERTIES OF RESTING AND ACTION POTENTIALS RECORDED FROM SURFACE FIBERS OF THE REINNERVATING SOLEUS MUSCLE

Days after nerve crush	RMP	Overshoot	dV/dt	$-dV/dt$	Duration	Threshold	V_{max}	% Fibers with two or more AP
d	mV	mV	V/s	V/s	ms	mV	mV	
5	−62.8±0.4 (93)	32.7±1.2	147.3±5.4	57.1±2.3	1.80±0.05	−50.4±0.9	−5.4±0.7	0
8	−61.7±0.7 (58)	34.2±1.5	179.5±7.3	63.8±2.7	1.58±0.07	−48.2±0.9	−4.7±1.1	0
10	−64.1±0.5 (86)	31.7±1.0	142.5±7.8	46.4±1.9	1.95±0.05	−50.0±0.8	−4.0±0.7	0
13	−68.6±0.6 (43)	39.1±1.5	205.6±7.6	73.0±3.0	1.38±0.04	−46.3±0.8	−6.3±1.0	10
14	−69.7±0.7 (62)	39.5±1.4	269.8±12.3	98.9±4.0	1.17±0.03	−46.0±1.0	0.9±1.0	20
18	−69.6±0.7 (57)	41.4±1.5	195.0±7.5	85.6±2.7	1.41±0.05	−45.7±1.0	−0.3±0.9	5
22	−75.2±0.8 (44)	42.2±1.6	254.9±17.0	95.4±3.9	1.28±0.03	−48.4±0.8	−2.4±0.9	0
30	−76.6±0.4 (78)	42.9±0.7	232.0±4.8	87.6±1.8	1.43±0.02	−47.5±0.4	3.1±0.7	2
60	−79.5±0.8 (33)	40.3±2.0	268.1±12.4	92.5±3.0	1.28±0.04	−44.9±1.0	2.0±1.4	3

nerve crush. Within a similar interval, reinnervation produces marked changes in the properties of the AP (see Tables II and III). TTX resistance is completely absent and the APs of extensor and soleus fibers are significantly different with respect to dV/dt at 22 d after nerve crush (see Fig. 5). Also, 87% of the extensor fibers generate two APs during a 20 ms stimulus. Estimated values of

$[Na]_i$ (29.9 mM) and g_{Na} (26.2 mmho/cm^2) are normal for the soleus. Likewise, g_{Na} (31.1 mmho/cm^2) is normal in 22-d extensor muscle although dV/dt did not achieve a control value even as late as 60 d after nerve crush (see Table II). This could be related to the persistance of an elevation of $[Na]_i$ (25.4 mM).

TABLE IV
EFFECT OF TTX (1×10^{-6} g/ml) UPON THE ACTION
POTENTIALS OF DENERVATED AND REINNERVATING
EXTENSOR AND SOLEUS MUSCLES

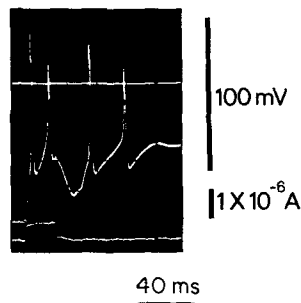| Days after nerve crush	% of fibers resistant to TTX	dV/dt as % of value prior to TTX
5	56	39
8	43	45
10	22	21
13	18	38
14	6	30
18	2	9
5	28	36
8	31	37
10	22	39
13	7	32
14	3	24
18	7	15

100 mV

1×10^{-6} A

40 ms

FIGURE 6.   Action potentials from an extensor fiber at 22 d after nerve crush. Note potentials occurring after the 20-ms stimulus has ended.

It is interesting to note that at 22 d after nerve crush 43% of the extensor fibers produced one or more APs after the 20-ms stimulus had ended (Fig. 6). Such after-activity was seen in only 6% of the control extensor fibers and even less frequently in the control and reinnervating soleus fibers.

Characteristics of Action Potentials from Extensor and Soleus Muscles from Neonatal Rats

In accord with earlier work (Harris and Marshall, 1973; Vyskocil, 1974), no TTX resistance was found during the time-course of the present neonatal

study. APs were not different for fibers in the extensor and soleus muscles from 12-d-old rats (Fig. 7). At this time, $[Na]_i$ was estimated to be 44.6 mM for the extensor and 54.5 mM for the soleus. Although dV/dt progressively increased for both muscles (Tables V and VI), the value of this parameter was significantly greater for the extensor as early as day 14. $[Na]_i$ had decreased to 32.8 mM in the extensor and 36.8 mM in the soleus at two weeks after birth.
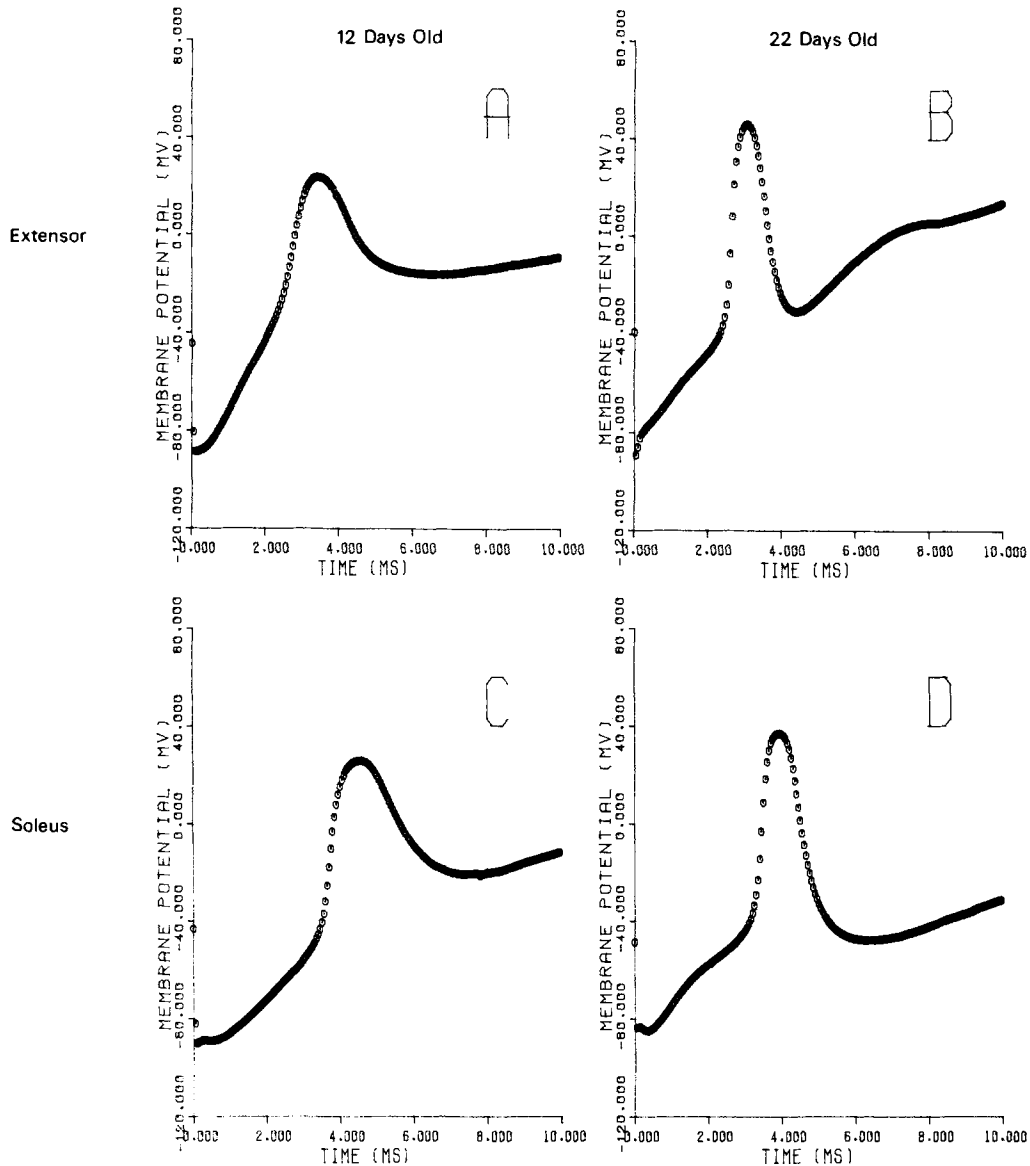

FIGURE 7. Representative actions potentials recorded from fibers in the extensor and soleus muscles at 12 and 22 d after birth.

TABLE V

PROPERTIES OF RESTING (RMP) AND ACTION POTENTIALS RECORDED FROM SURFACE FIBERS OF THE EXTENSOR MUSCLE OF NEONATAL RATS

Days after birth	RMP	Overshoot	dV/dt	−dV/dt	Duration	Threshold	V_{max}	% Fibers with two or more AP
d	mV	mV	V/s	V/s	ms	mV	mV	
10	−62.0±4.0 (3)	17.3±2.7	79.3±4.3	30.0±0.6	1.59±0.16	−38.0±1.7	−11.3±2.2	0
12	−72.1±1.3* (11)	31.1±4.1	164.9±15.6	70.4±29.5	1.25±0.05	−44.6±1.6	−10.4±1.8	18
14	−69.3±0.5* (97)	38.9±1.3*	199.3±5.7*	92.1±3.0*	1.27±0.03*	−44.1±0.05	−3.1±0.9	50
18	−73.9±0.3 (74)	47.2±1.0	274.6±7.7*	118.8±2.3*	1.12±0.02*	−46.1±0.6	2.7±0.8*	89
22	−76.0±0.4 (106)	49.5±0.8	322.9±10.0*	127.9±2.9*	1.22±0.09*	−49.4±0.5	0.6±0.8*	100
25	−78.2±0.2* (118)	48.2±0.8*	376.0±11.5*	134.4±2.2*	0.98±0.01*	−49.8±0.4	−2.0±1.0	96
30	−79.0±0.3* (109)	48.8±0.6*	386.4±8.6*	143.3±1.7*	0.94±0.01*	−48.9±0.4	2.9±0.7*	88
43	−79.8±0.2* (104)	50.3±0.5*	367.9±6.4*	146.2±1.6*	0.96±0.01*	−49.4±0.3*	4.9±0.5*	83
50	−78.5±0.2* (117)	48.5±0.6*	340.5±8.2*	137.7±1.6*	0.99±0.01*	−47.1±0.3*	3.1±0.7*	96
60	−81.9±0.3* (49)	53.3±0.9*	561.8±15.7*	168.9±3.2*	0.91±0.02*	−50.9±0.6	8.6±1.5*	100

* The differences between these values and those seen in the corresponding soleus muscle are significant ($P < 0.01$).

TABLE VI

PROPERTIES OF RESTING (RMP) AND ACTION POTENTIALS RECORDED FROM SURFACE FIBERS OF THE SOLEUS MUSCLE OF NEONATAL RATS

Days after birth	RMP	Overshoot	dV/dt	−dV/dt	Duration	Threshold	V_{max}	% Fibers with two or more AP
d	mV	mV	V/s	V/s	ms	mV	mV	
12	−64.1±1.8 (7)	26.0±5.6	117.3±17.3	46.6±9.8	1.50±0.15	−40.6±4.7	−10.7±1.9	0
14	−66.1±0.7 (72)	36.0±1.8	155.3±6.7	67.1±3.8	1.56±0.06	−43.9±0.7	−5.6±1.0	7
18	−72.8±0.4 (87)	44.9±1.1	235.0±9.4	100.3±3.9	1.38±0.04	−48.0±0.6	−0.8±0.6	38
22	−74.8±0.4 (89)	47.3±1.3	236.7±7.8	105.9±3.6	1.34±0.03	−50.1±0.7	−2.8±0.9	31
25	−75.6±0.3 (77)	41.4±1.3	280.2±11.0	114.7±3.9	1.12±0.02	−50.8±0.5	−4.4±0.9	17
30	−75.4±0.2 (106)	40.5±0.8	256.0±7.6	120.1±2.7	1.06±0.01	−47.4±0.5	−1.7±0.5	8
43	−75.4±0.3 (72)	37.0±1.2	222.1±7.2	92.0±3.2	1.26±0.02	−46.9±0.6	−2.2±0.6	7
50	−75.6±0.2 (77)	40.2±1.0	242.1±7.7	104.3±3.4	1.21±0.02	−46.4±0.5	−1.1±0.6	13
60	−76.3±0.5 (41)	40.6±1.3	313.6±13.6	110.0±4.1	1.16±0.03	−49.1±0.8	0.7±1.0	9

The increase of dV/dt for the soleus reached a plateau about 18 d after birth when [Na]$_i$ had attained a value (25.9 mM) equivalent to that of adult muscle. In contrast, dV/dt continued to increase for the extensor muscle until adult values were seen 50–60 days after birth. Between 18 and 60 d, [Na]$_i$ was

reduced from 23.6 to 18.6 mM for the fast muscle. It should be stressed that the APs of these two muscles were significantly differentiated with respect to all parameters within 25 d after birth.

Quite interestingly, an abnormally large percentage of soleus fibers produced two or more action potentials during a 20 ms stimulus between 18 and 25 d after birth.

DISCUSSION

The present data substantiate earlier findings that the APs of fibers on the surface of the extensor and soleus muscle are different. This region of these muscles is known to consist primarily of fast and slow fiber types, respectively, as defined by morphologic (McArdle and Sansone, 1977), physiologic (McArdle and Albuquerque, 1973), and pharmacologic (Albuquerque and McIsaac, 1970; McArdle et al., 1977) criteria.

Though the technique employed in this study is not sensitive enough to uncover the true cause of the differences, it did enable us to reveal the time-course of AP differentiation during reinnervation and development. Thus, some possible factors for the dissimilarities in extensor and soleus APs can be excluded. For instance, Ellisman et al. (1976) reported that the particular structure of the extensor sarcolemma differs from that of the soleus, and this might be taken as a morphologic correlate to the differences in APs. The results of our denervation studies make this unlikely since the "square arrays" of particles characteristic of the extensor do not change at a time after nerve section (8 d; Ellisman and Rash, 1977) when the APs are the same for the extensor and soleus.

The permeability of the sarcolemma to K^+, Cl^-, and Na^+ would have a marked effect upon the voltage and time characteristics of the AP. However, a related study from our laboratory[1] suggests that steady state differences in K^+ and Cl^- permeability do not contribute to differences in extensor and soleus APs. On the other hand, our data and that of Akaike (1976, 1978) indicate that peak g_{Na} during the AP is greater for the extensor muscle. Though our technique may provide only a qualitative comparison of g_{Na}, the result does suggest differences in the nature and/or density of the Na conductance site for these two muscles. The similarity in the sensitivity of the extensor and soleus muscles to TTX indicates that this conductance site is the same. TTX-binding studies (Colquhoun et al., 1974) are needed to determine the density of Na channels along the sarcolemma of these muscles.[2] Also, comparative studies of the activation and inactivation of Na conductance as well as of anomalous and delayed rectification are required to further understand the difference in g_{Na}.

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[1] D'Alonzo, A. J., J. J. McArdle, and L. Michelson. Properties of fast-and slow-twitch muscles from rats with experimental myotonia. Manuscript submitted for publication.

[2] While this manuscript was in press, C. M. Hansen Bay and G. R. Strichartz (1980. Saxitoxin binding to sodium channels of rat skeletal muscles. *J. Physiol. [Lond.]*. **300**:89–103) presented data showing that the extensor muscle has a greater capacity to bind saxitoxin than the soleus.

It is possible that the ionic conductance mechanisms are identical for the extensor and soleus muscles. However, the underlying molecular structures may be inserted into membranes having different biophysical properties. This would be expected to induce differences in the kinetic parameters used to study such mechanisms. For instance, Gage and his colleagues (Gage et al., 1974, 1975; Gage and Hamill, 1975) have shown that alteration of the fluidity of the membrane in which the acetylcholine receptor is located changes the kinetics of the receptor activated ionic channel. Quite interestingly, preliminary voltage-clamp studies from our laboratory (D'Alonzo et al., 1980) indicate that the acetylcholine-activated endplate channel closes more rapidly for the extensor than for the soleus muscle.

Hodgkin and Nakajima (1972) showed that the conduction velocity of the sarcolemma varies inversely with the capacitance. Though low frequency capacitance is equivalent for the extensor and soleus (McArdle and Albuquerque, 1973), the high frequency capacitance of these muscles must be determined. This will allow evaluation of the role of the T tubule in producing the differences of APs.

The capacitative influence upon the AP raises another limitation of the technique employed. Valdiosera et al., (1974) have shown that sarcomere length can influence membrane cable properties. Thus, variations in the stretch of isolated muscle preparations would cause variations in membrane capacitance and propagation of APs.

The greater concentration gradient for Na^+ across the sarcolemma of the extensor muscle (Table VII) undoubtedly contributes to the differences of extensor and soleus APs. For example, the driving force for Na^+ is greater across the extensor sarcolemma and this would cause dV/dt to be faster (Hodgkin et al., 1952). In addition, the overshoot of the extensor AP will approach a more positive E_{Na} (see Table VII; Hodgkin, 1951; Desmedt, 1953). Since nerve section decreases the Na^+ gradient (Robbins, 1977), it is reasonable to hypothesize that some of the effects of denervation upon the AP are secondary to this change. The observation that activation of an electrogenic pump in the membrane of denervated muscles partially restores $[Na]_i$ and dV/dt to normal[3] supports this hypothesis. A corollary would be that the recovery of the AP during reinnervation is in part due to the reestablishment of the Na^+ gradient. This hypothesis may also be extended to the effects of cross-reinnervation. Hoh and Salafsky (1971) demonstrated that implantation of the nerve of a fast or slow muscle into a denervated muscle of the opposite type caused the electrolyte content of the reinnervated muscle to approach that of the donor muscle. Such a cross-reinnervation procedure is also known to convert the mechanical properties of the muscle (Close, 1969). It is conceivable that the AP (and its frequency response [see Fig. 4; Duval and Leoty, 1978]) of the cross-reinnervated muscle are first converted via a neural influence upon ion gradients and other excitability properties of the sarco-

[3] McArdle, J. J., and A. J. D'Alonzo. Effects of terbutaline, a B_2-adrenergic agonist, upon the membrane potentials of innervated and denervated fast- and slow-twitch muscles. *Exp. Neurol.* In press.

lemma so that the responsiveness of the muscle is matched with that of the innervation (Eccles et al., 1958; Ridge, 1967; Huizar et al., 1975). In contrast to this neural hypothesis, Westgaard (1975) and Lømo and Westgaard (1975) postulated that muscle activity alone is necessary for the maintenance of membrane electrical properties. However, their observation can also be interpreted to support the neural influence upon muscle since in the normal situation it is the nerve which determines the pattern of muscular activity.

We must emphasize that the estimates of $[Na]_i$ presented in this study depend upon the degree to which the overshoot of the AP approaches E_{Na}. Since various other events occurring during the AP may regulate the overshoot, such physiological estimates must be regarded with caution even though they agree reasonably well with chemically determined values.

TABLE VII

COMPARISON OF CHEMICALLY DETERMINED VALUES FOR THE INTRACELLULAR CONCENTRATION ([ ]$_i$) OF Na$^+$ OF THE NORMAL EXTENSOR AND SOLEUS MUSCLES WITH THE ESTIMATES DERIVED FROM THE PRESENT ACTION POTENTIAL DATA

$[Na^+]_i$	E_{Na}*	$[Na^+]_i$	E_{Na}*	Reference
mM	mV	mM	mV	
Extensor		Soleus		
9 .9	67.5	13.0	60.1	Drahota, 1961
14.0	—	22.6	—	Streter and Woo, 1963
17.2	53.8	25.2	44.1	Yonemura, 1967
16.4	53.9	24.2	44.1	Akaike. 1976, 1978
23.0	—	—	—	Kernan and MacDermott. 1976
16.0	—	—	—	Adrian and Marshall, 1977
18.2‡	53.8	29.2	41.8	Present study§
9.6‡	67.6	15.6	55.5	Present study‖

* Estimated from Nernst equation for those studies where extracellular concentration of Na$^+$ is given. $T = 22°$C.
‡ Assuming the overshoot value as an estimate of E_{Na}.
§ 22° C.
‖ 12° C.

In conclusion, differences in the concentration gradient for Na$^+$ and g_{Na} contribute to the dissimilarities of APs of the fast-twitch extensor and the slow-twitch soleus muscles of the rat. However, vigorous analysis of sarcolemmal properties is likely to reveal other more subtle factors.

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