MOLECULAR PHARMACOLOGY, 14, 1204-1211

Denervation of Cat Fast- and Slow-Skeletal Muscles: Effect on Ouabain Binding

VIRENDRA K. SHARMA[1] AND SHAILESH P. BANERJEE

Departments of Pharmacology and Toxicology, University of Rochester School of Medicine and Dentistry, Rochester, New York, 14642

(Received January 19, 1978)
(Accepted June 19, 1978)

SUMMARY

SHARMA, V. K. & BANERJEE, S. P. (1978) Denervation of cat fast- and slow-skeletal muscles: Effect on ouabain binding, *Mol. Pharmacol.*, **14**, 1204-1211.

The specific binding of [^{3}H]ouabain in microsomal suspensions obtained from slow-contracting soleus and fast-contracting tibialis anterior is approximately 200% and 10%, respectively, of that seen in the microsomes of the mixed type gastrocnemius muscle. Chemical sympathectomy decreased specific binding of [^{3}H]ouabain by 90% in the soleus and 33% in the tibialis anterior muscle microsomes. Scatchard analysis revealed that the fall in specific binding of [^{3}H]ouabain in muscles obtained from sympathectomized cats was due to a marked decrease in the density of ouabain binding sites. The equilibrium dissociation constants decreased in all three types of muscles after administration of 6-hydroxydopamine. Ablation of the motor nerve, which involves surgical sympathectomy, increased specific binding of [^{3}H]ouabain by 20 to 40% in soleus and gastrocnemius muscle microsomal preparations. Kinetic analysis by reciprocal plots suggested that the enhancement of ouabain binding after denervation is due to changes in the total number of binding sites, and not the affinity constant. Since ouabain selectively binds to (Na^+ + K^+)-ATPase, these observations provide support for the hypothesis that the motor nerve may be involved in the regulation of the number of (Na^+ + K^+)-ATPase molecules in skeletal muscles. An increase in ouabain binding and (Na^+ + K^+)-ATPase activity following ablation of the motor nerve would suggest that the fall in resting membrane potential of denervated skeletal muscle may not be due to decrease in (Na^+ + K^+)-ATPase activity.

INTRODUCTION

Excitable cells, whether muscle or nerve, undergo dramatic changes in response to denervation (1, 2). Perhaps the most striking of these changes in mammalian skeletal muscle are a supersensitivity to acetylcholine (3), a decrease of end-plate cholinesterase activity (4) and development of spontaneous fibrillation (5, 6). An examination of events underlying the initiation of spontaneous action potentials or fibrillation in fibers of previously denervated rat diaphragm, maintained in organ culture for up to 10 days, revealed existence of two classes of spontaneously active fibers (7, 8). These are either rhythmically discharging fibers (9, 10) or fibers in which the action potential occurs at irregular intervals (7, 8). The discrete depolarizations found at sites of origin of action potentials in irregularly dis-

This study was supported in part by a United States Public Health Service Grant HL-18185 and by a grant-in-aid from the American Heart Association, with funds contributed in part by the New York Heart Association.

[1] Recipient of a fellowship from the Genesee Valley Heart Association.

0026-895X/78/0146-1204$02.00/0

charging fibers are of nearly constant amplitude outside of a refractory period, are reversibly abolished by tetrodotoxin or by removal of most of the Na^+ from the bathing fluid, and are insensitive to curare. These results have led to the suggestion that the spontaneous discrete depolarization that gives rise to fibrillation potentials in denervated muscle results from regenerative Na^+ conductance increases within the transverse tubular system of the muscle fibers (8).

Recently, Smith and Thesleff (11) examined the mechanism underlying the spontaneous discrete depolarizations that trigger action potential in chronically denervated skeletal muscle. The spontaneous activity may be enhanced by lowering the external Ca^{++} concentrations or by the addition of catecholamines. Ouabain or K^+-free solutions or tetrodotoxin block spontaneous activity. These results led Smith and Thesleff (11) to suggest that spontaneous action potentials in denervated muscle result from regenerative Na^+ conductance increases within the transverse tubular system of the muscle fibers, and catecholamines and ouabain could affect this activity either directly, through an action on membrane excitability, or indirectly via the $(Na^+ + K^+)$-ATPase.

In this investigation an attempt has been made to determine if the motor nerve is involved in the regulation of the number of $(Na^+ + K^+)$-ATPase molecules in fast-contracting and slow-contracting skeletal muscles. The method was measurement of the specific binding of [^{3}H]ouabain, a highly specific inhibitor of $(Na^+ + K^+)$-ATPase (12). The observations in this study were consistent with the idea that the motor nerve is involved in the regulation of $(Na^+ + K^+)$-ATPase present in the microsomal fraction of skeletal muscles. Preliminary reports of this work have already appeared (13, 14).

MATERIALS AND METHODS

Adult male cats, weighing 2.5 to 3.5 kg, were divided into two groups. In the first group, sympathetic nerve endings were effectively destroyed with intravenous injections of 6-hydroxydopamine hydrobromide, two doses of 20 mg/kg on the first day and two doses of 50 mg/kg one week later, as described by Thoenen and Tranzer (15). The second group, which served as the control, received equal volumes of normal saline instead of the drug. The animals were sacrificed two weeks after the first dose of 6-hydroxydopamine or normal saline. The effectiveness of the chemical sympathectomy was determined by measuring Na^+-dependent [^{3}H]norepinephrine uptake in hearts slices; it was inhibited by more than 85% in 6-hydroxydopamine-treated cats.

For the denervation of the three types of skeletal muscles (tibialis anterior, gastrocnemius and soleus), the cat was anesthetized with sodium pentobarbitone (30 mg/kg; Nembutal, Abbott), and under aseptic conditions, about 1 cm of the sciatic nerve from the thigh was removed. Denervation was carried out 14 days before sacrifice. The animals were killed by an overdose of sodium pentobarbitone. The tibialis anterior, gastrocnemius, and soleus were removed, and a microsomal preparation was made by the method of Schwartz et al. (16), as previously described (17, 18). Protein concentration was determined by the method of Lowry *et al.* (19). The assay for specific binding of [^{3}H]ouabain in microsomal fractions has been described (17, 18). Total binding was estimated in the presence of 4 mM Mg^{2+} and 1 mM inorganic phosphate in 0.05 M Tris HCl buffer. Corrections were made for nonspecific accumulation of radioactivity of [^{3}H]ouabain by assaying parallel incubations in which Mg^{2+} and inorganic phosphate had been replaced by 0.2 M Na^+. The concentrations of [^{3}H]-ouabain varied between 0.04 and 0.64 μM.

RESULTS

Two different groups of workers have reported that denervation of rabbit and rat skeletal muscle increases $(Na^+ + K^+)$-ATPase activity in the sarcolemmal membrane (20, 21). The molecular mechanism for the activation of $(Na^+ + K^+)$-ATPase following denervation of skeletal muscle is not known. Ouabain is a specific inhibitor of $(Na^+ + K^+)$-APase (12), and the specific binding of [^{3}H]ouabain has been used to estimate the number of $(Na^+ + K^+)$-ATP-

ase molecules (22-27). In order to obtain a better insight into the molecular mechanisms for the activation of skeletal muscle sarcolemmal membrane $(Na^+ + K^+)$-ATPase, specific binding of [^{3}H]ouabain in the microsomal fractions obtained from fast (pale) and slow (red) muscle fibers was measured (Table 1). Ouabain binding in the microsomal fraction from the soleus (slow-contracting) was 26 times that observed in the tibialis anterior (fast-contracting) muscle preparation. The specific binding of [^{3}H]ouabain in the microsomal fraction of the gastrocnemius (mixed type of muscle) was between the binding in the soleus and that in the tibialis anterior preparation. These results are consistent with the observation of Fesstoff and his associates (21), who reported that $(Na^+ + K^+)$-ATPase activity in the sarcolemmal membrane of red, slow muscle is considerably higher than that observed in pale, fast muscle. Two weeks after surgical ablation of sciatic nerve, ouabain binding increased by 20 to 40% in soleus and gastrocnemius muscles. The direction of these changes are in agreement with earlier observations of activation of $(Na^+ + K^+)$-ATPase activity following muscle denervation (20, 21). Quantitative differences are caused by the use of microsomal fractions instead of the sarcolemmal fractions employed by previous workers. Denervation of cat gastrocnemius muscle leads to 5 to 6-fold increase in ouabain binding in the sarcolemmal membrane preparation (manuscript in preparation).

Increase in ouabain binding following denervation of skeletal muscle may be due to changes in apparent affinity of $(Na^+ + K^+)$-ATPase for ouabain or alterations in the maximal number of binding sites for ouabain. For example, thyroid hormones increase ouabain binding in cardiac and skeletal muscle membrane preparations by enhancing apparent affinity of $(Na^+ + K^+)$-ATPase toward ouabain (28). On the other hand, chronic alcoholism increases ouabain binding in cardiac microsomes by increasing the maximal number of binding sites for ouabain (29). The equilibrium dissociation constants and densities of ouabain binding sites in microsomal fractions derived from innervated and denervated cat soleus, gastrocnemius, and tibialis anterior were determined by measuring specific binding in the presence of five different concentrations of [^{3}H]ouabain and analyzing the data by reciprocal plots (Figs. 1-3). Kinetic constants obtained from Figures 1-3 are provided in Table 2. Although denervation of soleus or gastrocnemius or tibialis anterior does not alter apparent affinity of microsomal $(Na^+ + K^+)$-ATPase for ouabain, there is a 33 to 50% increase in the density of ouabain-binding sites. These observations suggest that enhancement of ouabain binding following ablation of motor nerve is due to increase in the number of such binding sites.

It has been reported that chemical sympathectomy by the administration of 6-hydroxydopamine leads to a marked reduction in specific binding of [^{3}H]ouabain in the microsomal fractions of various sym-

TABLE 1

[^{3}H]Ouabain binding in microsomal fractions obtained from innervated and denervated cat skeletal muscles

Values are means ± standard errors of 9 determinations obtained from 3 cats. The concentration of ouabain was 80 nM. Cat skeletal muscles were denervated by the ablation of sciatic nerve two weeks before the animals were sacrificed.

Muscle	Specific binding of [^{3}H]ouabain		D/I × 100
	Innervated (I)	Denervated (D)	
	pmoles/mg protein		%
Soleus	21.72 ± 1.22	29.54 ± 1.26 ($p < 0.05$)	136
Gastrocnemius	9.10 ± 0.48	12.72 ± 0.92 ($p < 0.05$)	140
Tibialis anterior	0.83 ± 0.29	0.99 ± 0.31 ($p > 0.05$)	119

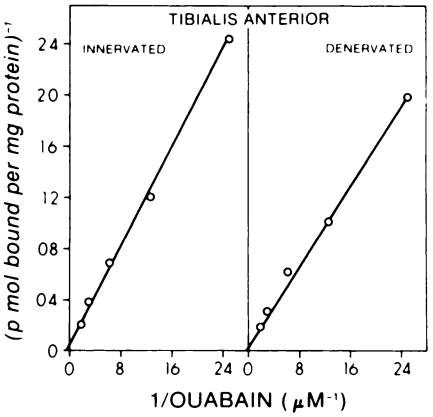

FIG. 1. *Double reciprocal plots of specific binding of [^{3}H]ouabain in microsomal fractions derived from innervated and denervated tibialis anterior muscle.*

Specific binding of [^{3}H]ouabain in microsomes obtained from innervated and denervated tibialis anterior muscles was measured as described in the text. Each experimental point is the average of 9 determinations of three separate measurements, which varied less than 10%.

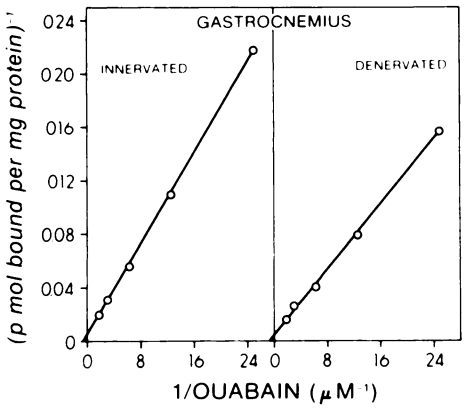

FIG. 2. *Double reciprocal plots of specific binding of [^{3}H]ouabain in microsomal fractions obtained from innervated and denervated gastrocnemius.*

Specific binding of [^{3}H]ouabain in microsomes derived from innervated and denervated gastrocnemius muscles was measured as described in the text. Each experimental point is the average of 9 determinations of three separate measurements, which varied less than 10%.

pathetically innervated organs of cat (18). Since this decrease in binding of [^{3}H]ouabain has been suggested to be due to the localization of (Na^+ + K^+)-ATPase predominately in the noradrenergic nerve endings of some sympathetically innervated peripheral organs of cat (18), and skeletal muscle cell surface is not known to be directly innervated by catecholaminergic nerve terminals (30, 31), effect of chemical sympathectomy on specific binding of [^{3}H]ouabain in the microsomal fractions of cat soleus, gastrocnemius, and tibialis anterior muscles was determined (Table 3). Although there is a significant quantitative difference in the effect of chemical sympathectomy on ouabain binding to fast, pale and slow, red muscle fibers, qualitatively, administration of 6-hydroxydopamine markedly decreased ouabain binding in soleus, gastrocnemius and tibialis anterior muscle microsomes. While fast, pale muscle fiber (tibialis anterior) microsomes retained about two-thirds of specific binding of [^{3}H]ouabain following the administration of 6-hydroxydopamine, chemical sympathectomy decreased ouabain binding to less than 5% and 16% of control values in soleus and gastrocnemius muscle microsomal fractions, respectively. In order to evaluate the molecular mechanisms for the reduction of ouabain binding following chemical sympathectomy, the equilibrium dissociation constant and density of ouabain binding sites in different muscle microsomal frac-

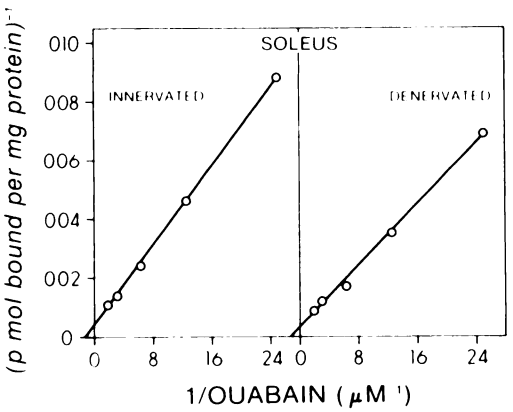

FIG. 3. *Double reciprocal plots of specific binding of [^{3}H]ouabain in microsomal fractions obtained from innervated and denervated soleus.*

Specific binding of [^{3}H]ouabain in microsomes obtained from innervated and denervated soleus muscles was measured as described in the text. Each experimental point is the average of 9 determinations of three separate measurements, which varied less than 10%.

TABLE 2

Apparent affinities and binding capacities of [^{3}H]ouabain in microsomal fractions of cat skeletal muscles

The specific binding of various concentrations of [^{3}H]ouabain in microsomal preparations from different skeletal muscles of the cat was assayed as described in MATERIALS AND METHODS. The equilibrium dissociation constant (K_d) and maximal number of binding sites (B_{max}) were estimated from reciprocal plots. Cat skeletal muscles were denervated by the ablation of sciatic nerve. Values are averages of three separate determinations which varied less than 10%.

Muscle	Innervated		Denervated	
	K_d	B_{max}	K_d	B_{max}
	μM	*pmoles/mg*	*μM*	*pmoles/mg*
Soleus	0.83	250	0.83	334
Gastrocnemius	1.47	187	1.47	250
Tibialis anterior	2.50	20	2.90	30

TABLE 3

Specific binding of [^{3}H]ouabain in microsomal fractions obtained from skeletal muscles of control and 6-hydroxydopamine-treated cats

Values are means ± standard errors of 9 determinations. The concentration of [^{3}H]ouabain was 80 nM. The procedures for the administration of 6-hydroxydopamine and the measurement of specific binding of [^{3}H]-ouabain are described in the text.

Muscle	Specific binding of [^{3}H]ouabain		T/C × 100
	Control (C)	6-hydroxydopamine treated (T)	
	pmoles/mg protein		%
Soleus	22.23 ± 1.28	0.91 ± 0.42	4.10
Gastrocnemius	9.36 ± 0.72	1.49 ± 0.87	15.90
Tibialis anterior	0.80 ± 0.34	0.54 ± 0.29	67.50

tions derived from control and 6-hydroxydopamine-treated cats were determined by measuring specific binding of different concentrations of [^{3}H]ouabain and analyzing the data by the method of Scatchard (Figs. 4-6). These results demonstrate that there is a single class of ouabain-binding sites in the microsomal fractions obtained from three skeletal muscle types. The equilibrium dissociation constants and densities of ouabain binding sites are provided in Table 4. Administration of 6-hydroxydopamine decreased the density of ouabain binding sites in the microsomal fraction of tibialis anterior by about 50% in contrast to more than 97% decrease in the maximal number of binding sites in the microsomes derived from soleus muscle. The effect of chemical sympathectomy on the density of ouabain binding sites in gastrocnemius muscle was in between those seen in soleus and tibialis anterior (Table 4). Administration of 6-hydroxydopamine either increased or decreased the dissociation constants of cat

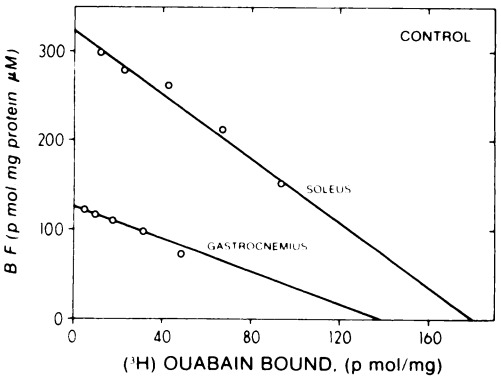

FIG. 4. *Scatchard plots of specific binding of [^{3}H]ouabain in microsomal fractions derived from soleus and gastrocnemius muscles of control cats.*

Specific binding of [^{3}H]ouabain was measured as described in the text. Each experimental point is the average of 9 determinations of three separate measurements, which varied less than 10%.

peripheral tissue microsomes toward ouabain (18). For example, the apparent affinity of (Na^+ + K^+)-ATPase derived from

cardiac and nictitating membranes toward ouabain increased following chemical sympathectomy, in contrast to a decrease seen with the microsomes obtained from salivary glands and vas deferens (18). The affinity constants of all three cat skeletal muscle microsomes for ouabain decreased following treatment with 6-hydroxydopamine, suggesting that the apparent affinity of $(Na^+ + K^+)$-ATPase located on skeletal muscle surface is greater than the apparent affinity of the transport enzyme system located in the sympathetic nerve endings for the cardiac glycoside (Table 4).

DISCUSSION

The tibialis anterior muscle consists mainly of white, or fast-contracting, fibers and the soleus, mainly of red, or slow-contracting fibers (32). The gastrocnemius muscle contains a rather even mixture of red and white fibers (32). The specific binding sites of ouabain appear to be located predominantly in the microsomal fractions of slow-contracting fibers (Tables 1 and 3). 6-Hydroxydopamine decreased the density of specific binding sites for ouabain by 50 to 97% in the microsomal fractions derived from these three types of skeletal muscle (Table 4). Since administration of 6-hydroxydopamine leads to chemical sympathectomy (15, 33), the decrease in the density of ouabain binding sites may be due either to degeneration of sympathetic nerve terminals innervating the blood vessels in the muscles or to toxic effects of the drug.

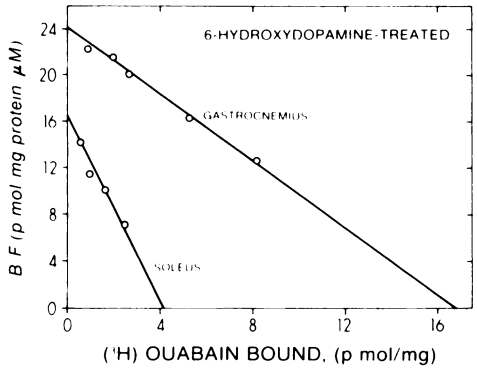

FIG. 5. *Scatchard plots of specific binding of [³H]ouabain in microsomal fractions derived from soleus and gastrocnemius muscles of 6-hydroxydopamine treated cats.*

Specific binding of [³H]ouabain was measured as described in the text. Each experimental point is the average of nine determinations of three separate measurements, which varied less than 10%.

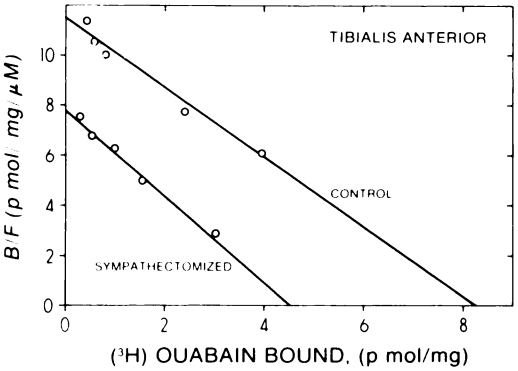

FIG. 6. *Scatchard plots of specific binding of [³H]ouabain in microsomal fractions obtained from tibialis anterior muscle of control and 6-hydroxydopamine treated cats.*

Specific binding of [³H]ouabain was measured as described in the text. Each experimental point is the average of 9 determinations of three separate measurements, which varied less than 10%.

TABLE 4

Equilibrium dissociation constants and maximal number of binding sites in microsomal fractions of skeletal muscles of control and 6-hydroxydopamine treated cats.

The specific binding of various concentrations of [³H]ouabain in microsomal preparations from different skeletal muscles of the cat was assayed as described in MATERIALS AND METHODS. The equilibrium dissociation constant (K_d) and maximal number of binding sites (B_{max}) were estimated by Scatchard analysis. Values are averages of three separate determinations which varied less than 10%.

Muscle	Control		6-hydroxydopamine treated	
	K_d	B_{max}	K_d	B_{max}
	μM	pmoles/mg	μM	pmoles/mg
Soleus	0.56	180	0.25	4.15
Gastrocnemius	1.10	137	0.69	16.80
Tibialis anterior	0.72	8.25	0.59	4.25

In an earlier report we showed that administration of 6-hydroxydopamine does not lead to decrease in ouabain binding to several organ membrane preparations such as highly innervated spleen and less highly innervated kidney (18). It is unlikely that toxic effects of 6-hydroxydopamine will be seen in some organs such as skeletal muscle and not in kidney or spleen. This conclusion is further substantiated by the fact that 6-hydroxydopamine leads to a decrease of about 33% in ouabain binding in microsomes from cat tibialis anterior, as compared with a decrease of over 95% in ouabain binding to membrane preparation from soleus (Table 3). This remarkable difference between these two skeletal muscle types is unlikely to be due to differential toxicity of 6-hydroxydopamine. Therefore, it may be concluded that the decrease in density of ouabain binding sites following chemical sympathectomy is due to destruction of $(Na^+ + K^+)$-ATPase transport enzyme system located in the adrenergic nerve terminals and not to the toxic effect of 6-hydroxydopamine.

There is considerable evidence that ablation of the motor nerve leads to a fall in resting membrane potential (34, 35). Since ouabain has been observed to reduce the resting membrane potential of normal muscle to the level of denervated muscle and to have little or no effect on the resting membrane potential of denervated muscles, it has been suggested that the nerve releases a trophic factor that regulates the activity of the electrogenic monovalent cation transport system intimately involved in the maintenance of a critical fraction of the membrane potential (34–38). Furthermore, the cause of decline in the resting membrane potential following denervation is believed to be related to a reduction in the active transport of Na^+ (36–38).

Ablation of the motor nerve increases binding of [^{3}H]ouabain in microsomes of fast-contracting muscle fibers (Table 1), and this increase appears to be due to changes in the density of ouabain binding sites (Figs. 1–3 and Table 2). Since ouabain binding is selectively restricted to $(Na^+ + K^+)$-ATPase (12) and measurement of ouabain binding is a useful method to determine the number of $(Na^+ + K^+)$-ATPase molecules (22–27), the present findings suggest that denervation of skeletal muscles leads to augmentation of the number of $(Na^+ + K^+)$-ATPase molecules. This is consistent with earlier reports on enhancement of $(Na^+ + K^+)$-ATPase activity of skeletal muscle sarcolemmal membrane following ablation of the motor nerve (20, 21). These observations provide further support for the hypothesis that the motor nerve releases a trophic factor that regulates the activity of $(Na^+ + K^+)$-ATPase. Nevertheless, it appears unlikely that the fall in the resting membrane potential following denervation is due to a reduction in the active monovalent cation transport.

The resting membrane potential of electrogenic membranes is a function of a sustained electrochemical gradient for certain ions, a resting conductance for K^+ ions (gK), and a leakage current (39). There is evidence that following denervation, the K^+ permeability of the muscle membrane is decreased (40, 41); this decrease in permeability may contribute to the fall in membrane potential by altering the ratio between Na^+ and K^+ permeabilities of the resting membrane. The observation that tetrodotoxin raises the resting membrane potential of denervated muscle supports this possibility (42). Thus, mechanisms other than inhibition of active Na^+ transport may be involved in the fall of resting membrane potential following ablation of the motor nerve.

In general, the sympathetic nerve fibers to skeletal muscle pass from the main sciatic trunks to the blood vessels, around which they form plexuses (43, 44). Therefore, surgical sectioning of the sciatic nerve would lead to surgical sympathectomy. Thus, it appears surprising that chemical sympathectomy would decrease [^{3}H]ouabain binding and that ablation of the motor nerve coupled with surgical sympathectomy would increase the number of $(Na^+ + K^+)$-ATPase molecules on the skeletal muscle surface. Since ablation of the motor nerve increases the density of [^{3}H]ouabain binding sites on the muscle cell surface, and sympathectomy leads to destruction of ouabain binding sites at the nerve endings, sectioning of the sciatic nerve will give an algebraic sum of these two opposite effects.

This provides an explanation for the several-fold increase in $(Na^+ + K^+)$-ATPase activity and [^{3}H]ouabain binding in skeletal muscle sarcolemmal membrane following ablation of the motor nerve (20, 21, manuscript in preparation). Another interpretation for quantitative differences in ouabain binding and $(Na^+ + K^+)$-ATPase activity between microsomal and sarcolemmal fractions of denervated muscles relates to a shift in the distribution of binding sites between compartments rather than a change in the actual number of binding sites. This possibility appears unlikely because [^{3}H]ouabain binding in the whole homogenates of denervated soleus muscle was about 30% greater than the binding seen in the innervated muscle preparation (results not shown). This suggests that the quantitative differences in ouabain binding in microsomal and sarcolemmal fractions of innervated and denervated muscles is probably related to the inclusion of sympathetic nerve endings in the microsomal fractions of the skeletal muscle.

REFERENCES

1. Thesleff, S. (1960) *Physiol. Rev.*, **40**, 734–752.
2. Guth, L. (1968) *Physiol. Rev.*, **48**, 645–687.
3. Axelsson, J. & Thesleff, S. (1959) *J. Physiol. (Lond.)*, **147**, 178–193.
4. Guth, L., Albers, R. W. & Brown, W. C. (1964) *Exp. Neurol.*, **10**, 236–250.
5. Tower, S. S., Howe, H. & Bodian, D. (1941) *J. Neurophysiol.*, **4**, 398–401.
6. Tower, S. S. (1939) *Physiol. Rev.*, **19**, 1–48.
7. Purves, D. & Sakmann, B. (1974) *J. Physiol. (Lond.)*, **237**, 157–182.
8. Purves, D. & Sakmann, B. (1974) *J. Physiol. (Lond.)*, **239**, 129–153.
9. Thesleff, S. (1963) in *The Effects of Use and Disuse on Neuromuscular Functions* (Gutmann, E. & Hnik, P., eds.), pp. 41–51, Elsevier, Holland.
10. Thesleff, S. & Ward, M. R. (1975) *J. Physiol. (Lond.)*, **244**, 313–323.
11. Smith, J. W. & Thesleff, S. (1976) *J. Physiol. (Lond.)*, **257**, 171–186.
12. Schwartz, A., Lindenmayer, G. E., & Allen, J. C. (1975) *Pharmacol. Rev.*, **27**, 3–134.
13. Banerjee, S. P., Sharma, V. K. & Dasgupta, S. R. (1976) *Soc. Neurosci.*, **2**, 409.
14. Sharma, V. K., Dasgupta, S. R. & Banerjee, S. P. (1977) *Eur. J. Pharmacol.*, **43**, 273–276.
15. Thoenen, H. & Tranzer, J. P. (1968) *Naunyn-Schmiedebergs Arch. Pharmacol. Exp. Pathol.*, **261**, 271–288.
16. Schwartz, A., Nagano, K., Nakao, M., Lindenmayer, G. E., Allen, J. C. & Matsui, H. (1971) in *Methods in Pharmacology*, Vol. 1 (Schwartz, A., ed.), pp. 361–388, Meredith, New York.
17. Sharma, V. K. & Banerjee, S. P. (1977) *Eur. J. Pharmacol.*, **41**, 417–429.
18. Sharma, V. K. & Banerjee, S. P. (1977) *Mol. Pharmacol.*, **13**, 796–804.
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265–275.
20. Severin, S. E., Boldyrev, A. A. & Tkachuk, V. K. (1974) *Comp. Gen. Pharmacol.*, **5**, 181–185.
21. Fesstoff, B. W., Oliver, K. L. & Reddy, N. B. (1977) *J. Memb. Biol.*, **32**, 345–360.
22. Hoffman, J. F. (1969) *J. Gen. Physiol.*, **54**, 343–353S.
23. Ellory, J. C. & Keynes, R. D. (1969) *Nature*, **221**, 776.
24. Baker, L. F. & Willis, J. S. (1969) *Biochim. Biophys. Acta*, **183**, 646–649.
25. Baker, P. F. & Willis, J. S. (1972) *J. Physiol. (Lond.)*, **224**, 441–462.
26. Boardman, L. J., Lamb, J. F. & McCall, D. (1972) *J. Physiol. (Lond.)*, **225**, 615–635.
27. Lamb, J. F. & McCall, D. (1971) *J. Physiol. (Lond.)*, **213**, 57p.
28. Sharma, V. K. & Banerjee, S. P. (1978) *Mol. Pharmacol.*, **14**, 122–129.
29. Banerjee, S. P. & Sharma, V. K. (1978) *Br. J. Pharmacol.*, **62**, 475–479.
30. Bowman, W. C. & Raper, C. (1967) *Ann. N. Y. Acad. Sci.*, **139**, 741–753.
31. Bowman, W. C. & Nott, M. W. (1969) *Pharmacol. Rev.*, **21**, 27–72.
32. Denny-Brown, D. E. (1929) *Proc. Roy. Soc. B.*, **104**, 371–411.
33. Thoenen, H. & Tranzer, J. P. (1973) *Annu. Rev. Pharmacol.*, **13**, 169–180.
34. Locke, S. & Solomon, H. C. (1967) *J. Exp. Zool.*, **166**, 377–386.
35. Albuquerque, E. X., Schuh, F. T. & Kauffman, F. C. (1971) *Pfluegers Arch.*, **328**, 36–50.
36. McArdle, J. J. & Albuquerque, E. X. (1975) *Exp. Neurol.*, **47**, 353–356.
37. Sellin, L. C. & McArdle, J. J. (1977) *Eur. J. Pharmacol.*, **41**, 337–340.
38. Sellin, L. C. & McArdle, J. J. (1977) *Exp. Neurol.*, **55**, 483–492.
39. Hodgkin, A. L. & Katz, B. (1949) *J. Physiol. (Lond.)*, **108**, 37–77.
40. Klaus, W., Lüllmann, H. & Muscholl, E. (1960) *Pfluegers Arch. Ges. Physiol.*, **271**, 761–775.
41. Kernan, R. P. & McCharty, I. (1972) *J. Physiol. (Lond.)*, **226**, 62.
42. Grampp, W., Harris, J. B. & Thesleff, S. (1972) *J. Physiol. (Lond.)*, **221**, 743–754.
43. Tower, S. S. (1931) *J. Comp. Neurol.*, **53**, 177–203.
44. Tiegs, D. W. (1953) *Physiol. Rev.*, **33**, 90–144.