Selectivity of Sodium Channels in Denervated Tonic Muscle Fibre Membrane of the Frog

D. ZACHAROVÁ[1], J. ZACHAR[1], M. HENČEK[1] and G. A. NASLEDOV[2]

1 *Department of General Physiology, Centre of Physiological Sciences, Slovak Academy of Sciences, Vlárska 5, 833 06 Bratislava, Czechoslovakia*
2 *Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Thorez pr. 44, 194223 Leningrad, USSR*

Abstract. Ionic selectivity of sodium channels was examined under voltage clamp conditions in normal and denervated twitch fibres and denervated tonic fibres isolated from m. ileofibularis of the frog (*R. temporaria*). Membrane currents were recorded by means of the Hille-Campbell vaseline-gap voltage clamp method from muscle fibre segments exposed to a potassium-free artificial internal solution. Permeability ratio (P_S/P_{Na}) were determined from changes in the reversal potential after replacing all Na ions in the solution bathing the voltage clamped external membrane area with sodium substituting ions (S). The permeability sequence was: $Na^+ > Li^+ > NH_4^+ > K^+$. No inward currents were observed for Ca^{2+}. The permeability ratios were as follows. Denervated tonic fibres: 1:0.88:0.23:0.012; control twitch fibres: 1:0.94:0.22:0.076; denervated twitch fibres: 1:0.91:0.14:0.082. The permeability to Li^+ ions deviates from independence to a greater extent in tonic than in phasic fibres. Our results are consistent with the Hille model of sodium channel selectivity, and they support the hypothesis that sodium channels formed in denervated tonic muscle fibres of the frog are of the same genetic origin as Na channels expressed under physiological conditions.

Key words: Tonic (slow) muscle fibre — Phasic (twitch) muscle fibre — Denervation — Sodium channel selectivity — Voltage clamp — Hille's channel model

Introduction

The present paper examines the ionic selectivity of sodium channels formed in tonic muscle fibres of the frog after denervation (Miledi et al. 1971; Schmidt and Tong 1973; Nasledov and Thesleff 1974; Schalow and Schmidt 1975; Zachar et al. 1982). The main problem has been, whether the new sodium channels are similar to those present in phasic fibres of the same species. This study was prompted by the results of recent investigations (Zachar et al. 1982) on the kinetics of sodium channel conductances in tonic muscle fibre membranes after denervation; these

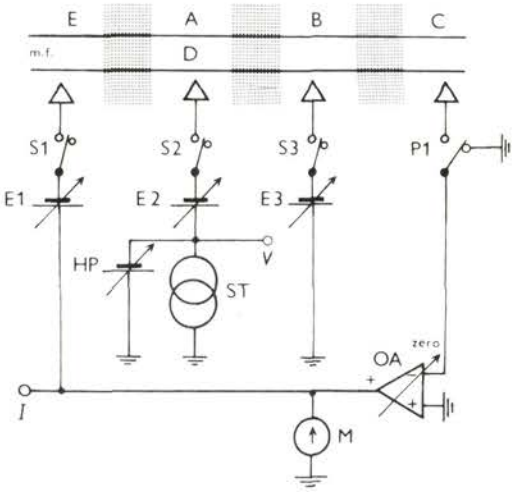

Fig. 1. Schematic representation of the muscle fibre segment (m. f.) in the experimental chamber, and of the voltage clamp circuit. The muscle fibre segment extends across three vaseline seals (stippling). A, B, C and E are solution-filled compartments in the chamber. Cut ends of the fibre lie in pools C and E, respectively. Point D is the fibre interior in the compartment A. OA — operational amplifier which keeps the D pool at virtual ground; M — offset indicator; P1 — switch in the input of the OA; S1, S2, S3 — switches for disconnection of electrodes; E1, E2, E3 — electronic voltage sources for the compensation of electrode potentials; ST — stimulator; HP-DC source of the holding potential; *V*, *I* — measurement of clamping voltage and membrane current, respectively.

results have brought evidence in support of the hypothesis that the new Na channels may represent the same molecular species as those expressed in phasic muscle fibres of the same animal species under physiological conditions.

The above hypothesis has been further strenghtened by the results presented in the present paper showing that the selectivity of new sodium channels in tonic fibres after denervation is nearly identical to that observed in phasic muscle membranes.

A short account on this work was presented at the common meeting of the Czechoslovak Physiological Society and the Association des Physiologistes (Zacharová et al. 1982).

Material and Methods

Experiments were performed on single tonic and phasic muscle fibres isolated from the tonic bundle of m. ileofibularis of the frog (*Rana temporaria*) 15 days following denervation. The denervation and dissection procedures as well as the vaseline gap voltage clamp were described in detail in our previous

paper (Zachar et al. 1982). Fig. 1 shows for convenience the main set up of the method. Our method is in principle similar to the potentiometric voltage clamp method of Hille and Campbell (1976). The muscle fibre segment is compartmentalized by means of vaseline seals into four pools (A—E). The fibre interior represents and additional pool (D). Pool A (150 μm in length) contains the testing membrane area under voltage clamp, and it is filled with normal Ringer solution, or with a Ringer solution with all the sodium ions substituted by a test cation. The other three pools (B, C, E) contain artificial „internal" solutions. The solutions are referred to as external solution // internal solution. The Ringer solution contained (in mmol/l): Na^+ 120; K^+ 2.5; Ca^{2+} 1.8; Cl^- 121; $TRIS^+$ 4; pH 7.1. The composition of the standard "internal" solution (solution D) was as follows (in mmol/l): Cs glutamate 108; $MgCl_2$ 6.5; $CaCl_2$ 0.069; EGTA 0.2; ATP 5; glucose 5; PIPES 20; TRIS to obtain a pH of 7.1—7.2. The external K-free solution (solution A) used as the reference solution, contained (in mmol/l): NaCl 110; $CaCl_2$ 2; CsCl 4; TRIS 4; pH 7.2.

The test solutions contained the same salts as the solution A except that all of the NaCl was replaced by an osmotically equivalent amount of the respective test salt. Following salts have been tested: LiCl, KCl, $CaCl_2$, NH_4Cl.

Examination of selectivity

The selectivity of sodium channels to a sodium substitute (S) was calculated from changes in reversal potential acording to the procedure introduced by Hille (1971, 1972). It follows from the Goldman—Hodgkin—Katz equation (Goldman 1943; Hodgkin and Katz 1949; Cole 1968; Hille 1971) that changes in reversal potential, $\Delta E = E_S - E_{Na}$, are given by

$$\Delta E = \frac{RT}{F} \ln \frac{P_S [S]}{P_{Na} [Na]},$$

where R, T, and F have their usual meanings; P_S/P_{Na} is the ratio of the channel permeabilities to the substitute (test) ions and Na ions, respectively. Square brackets refer to cation concentrations (strictly activities) in Na and S solutions, respectively. For computations, the same activity coefficients were used as those in Campbell (1976).

The method of Hille was chosen for our experiments from at least two different reasons. First, it does not assume an independence, and does not depend on the number of open channels, as contrasted with other methods (for a review, see Edwards 1982). Second, the method has recently been used for quantitative estimation of sodium channel selectivity in a number of excitable cells.

Reversal potentials were determined graphically from the current-voltage relations as illustrated in Fig. 2—5. Measurements in the test solution were bracketed by measurements in the control (Na-containing) saline. Potential values were corrected for the two known systemic errors, the junction potentials, and the "attenuation artifact" (Hille 1971; Campbell 1976). Experiments were performed at 16 °C.

Results

Monovalent metal cations

Lithium. New sodium channels which appear in tonic muscle fibres following denervation, were nearly as permeable to lithium ions as they are to sodium ions. Fig. 2 shows families of voltage clamp currents recorded from a tonic muscle fibre

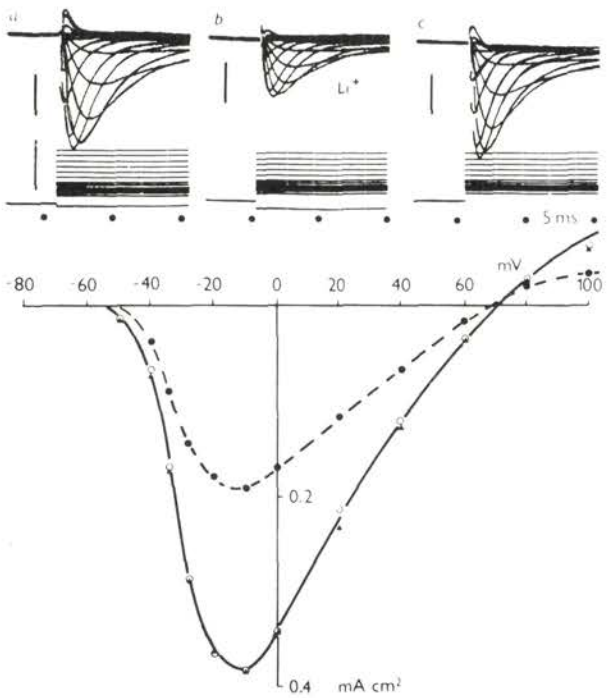

Fig. 2. Sodium and lithium currents in the sodium channel. Tonic muscle fibre of the frog 15 days after denervation. *Top*: superimposed voltage clamp recordings from a cut muscle fibre segment for voltage steps as recorded on the lower beam of the storage oscilloscope. *a*, *c* — solution A // solution D; *b* — after replacement of Na^+ ions with Li^+ ions in solution A. *Bottom*: corresponding peak current-voltage relations. ○, ▲ and full curve: sodium currents; ● and interrupted curve: lithium currents. The curves were drawn by hand. $t = 16$ °C. Fibre: 231281. Current scales: 75 nA. Voltage scale: 200 mV.

segment bathed in a control sodium saline (*a*, *c*), and in a lithium containing solution (*b*). The corresponding current—voltage relation showed almost no change in reversal potential with all the external sodium substituted by lithium. In four different fibres, the addition of the lithium solution resulted in a lowering of the reversal potential by 4.7 ± 0.8 (mean ± SEM); this corresponded to a permeability ratio P_{Li}/P_{Na} of 0.83. Although the permeabilities to Na and Li ions of new sodium channels in denervated tonic muscle fibres were not very much different, lithium currents were much lower (about 50 %) than the control sodium ones, as clearly evidenced by records and current-voltage relations illustrated in Fig. 2. In this case, the independence principle (Hodgkin and Huxley 1952) was evidently violated; lithium ions block sodium channels in addition to passing through them.

In order to estimate the effect of denervation per se on the selectivity of sodium channels for Na^+ and Li^+ ions, the P_{Li}/P_{Na} ratio was also determined in

Table 1. Reversal potential changes (ΔE_r in mV) and permeability ratios (P_S/P_{Na}) in sodium channels

	Denervated tonic fibres		Denervated twitch fibres		Normal twitch fibres	
	ΔE_r	P_S/P_{Na}	ΔE_r	P_S/P_{Na}	ΔE_r	P_S/P_{Na}
Lithium	4.7 ± 0.8 (4)	0.88	2.3 ± 0.9 (6)	0.91	1.6 ± 0.9 (3)	0.94
Ammonium	36 ± 6 (4)	0.23	48 ± 8 (5)	0.14	37 ± 8 (5)	0.22
Potassium	51 (2)	0.12	62 (2)	0.082	64 (1)	0.076
Calcium	>81 (2)	<0.038	— —	—	$>67 \pm 11$ (3)	<0.067

m. ileofibularis, *R. temporaria*,
measured at 16 °C, mean ± SEM,
numbers in brackets indicate number of fibres examined,
denervated fibres were examined 15 days after the denervation.

normal and denervated twich (phasic) muscle fibres isolated from the same muscle, and using the same experimental procedure as that used for denervated tonic muscle fibres. As illustrated in Table 1, the relative permeabilities of sodium channels to Na^+ and Li^+ ions in phasic muscle fibres of the frog remained in fact identical after denervation. In lithium solution, the reversal potential was by 1.6 ± 0.9 mV ($n = 3$) and by 2.3 ± 0.9 ($n = 6$) lower in normal and denervated (15 days) frog phasic muscle fibres, respectively. The observed changes in the reversal potential corrresponded to permeability ratio P_s/P_{Na} of 0.94 and 0.91, respectively. The new sodium channels in tonic fibres are thus nearly as permeable to Li ions as sodium channels in phasic fibres. The deviation from independence was, however, less pronounced in phasic than in tonic fibres. The lithium currents were by 0.76 % and 0.79 % lower in denervated and control twitch muscle fibres, respectively.

Potassium. As evidenced by two successful experiments, new sodium channels in tonic muscle fibres were permeable to potassium ions to an extent similar to that observed in normal and denervated phasic (twitch) muscle fibres (Table 1). Although CsCl (4 mmol/l) was present in solution A, potassium selectivity in Na channels was difficult to determine. In agreement with the experience of other authors (Campbell 1976), muscle fibre segments deteriorated rather quickly in high-potassium saline. Trials where the effect of the potassium solution was reversible, were only taken into consideration; the reversibility was evidenced by the current-voltage relation in Na^+ solution (filled circles in Fig. 3). The reversal potential measured in potassium solution was by 51 mV lower as compared to Na^+

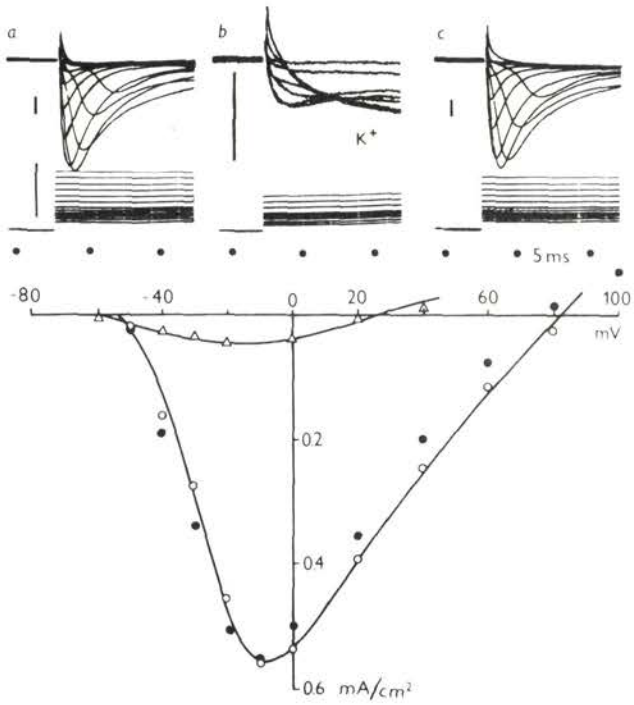

Fig. 3. Potassium currents in the sodium channel. Tonic muscle fibre of the frog 15 days after denervation. *Top*: superimposed voltage clamp recordings. *a*, *c* — solution A // solution D; *b* — Na^+ ions in solution A replaced with K^+ (K_2SO_4) ions. Note the change in gain when recording the potassium currents. *Bottom*: Corresponding peak current-voltage relation. Circles: control recordings before (○) and after (●) application of the test solution. Triangles (△): peak K^+ currents. The curves were drawn by hand. $t = 16$ °C. Fibre: 301281. Current scales: 75 nA. Voltage scale: 200 mV.

solution, corresponding to a permeability ratio P_K/P_{Na} of 0.12. The corresponding values for denervated and normal twitch fibres were 0.082 and 0.076, respectively.

Organic cations

Ammonium. NH_4 is known to permeate sodium channels in many excitable cells to a moderate extent (Hille 1971; Campbell 1976; Pappone 1980; Edwards 1982). In view of the fact that the ammonium effect is reversible, relative permeability of this organic cation was compared in denervated tonic, denervated phasic and normal phasic muscle fibres under identical experimental arrangement as described above. Results of these experiments are summarized in Table 1. Fig. 4 illustrates a typical experiment with the ammonium cation in frog tonic muscle fibres 15 days after denervation. Ammonium solution reduced resersal potential by 36 ± 6 mV,

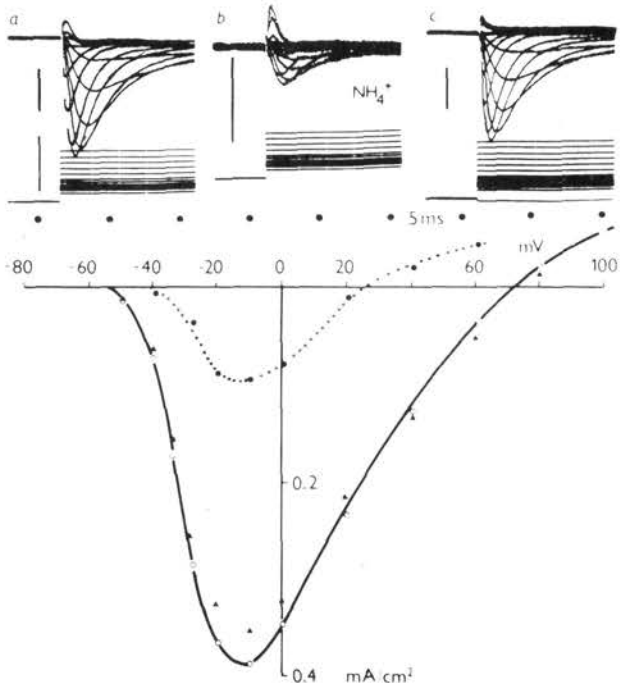

Fig. 4. Ammonium currents in the muscle sodium channel. Tonic muscle fibre of the frog 15 days after denervation. *Top*: superimposed voltage clamp recordings. *a*, *c* — solution A // solution D; *b* — Na^+ ions in solution A were replaced with ammonium (NH_4^+) ions. Note the change in gain in *b* in comparison with *a* and *c*. *Bottom*: corresponding peak current-voltage relations. ○, ▲ and full curve: sodium currents; ● and dotted curve: peak ammonium currents. The curves were drawn by hand. $t = 16$ °C. Fibre: 231281. Current scales: 75 nA. Voltage scale: 200 mV.

and gave a permeability ratio of 0.23. Peak currents recorded in ammonium solution satisfactorily fit the predictions of the independence principle ($I_{NH_4}/I_{Na} = = 0.17$). The relative permeability of new sodium channels for NH_4 ions was in fact the same as observed in normal phasic fibres ($P_{NH_4}/P_{Na} = 0.22$; Table 1); however, as compared to the denervated phasic fibres, the former value was higher ($P_{NH_4}/P_{Na} = 0.14$). On the other hand, the latter value is close to that observed in normal phasic muscle fibres of *R. pipiens* (Campbell 1976).

Divalent cations

Calcium. As shown in Fig. 5, no inward current could be recorded in Ca^{2+} solution. In addition, a threshold-raising effect of Ca^{2+} solutions could be observed (Frankenhaeuser and Hodgkin 1957). In the absence of inward current, the

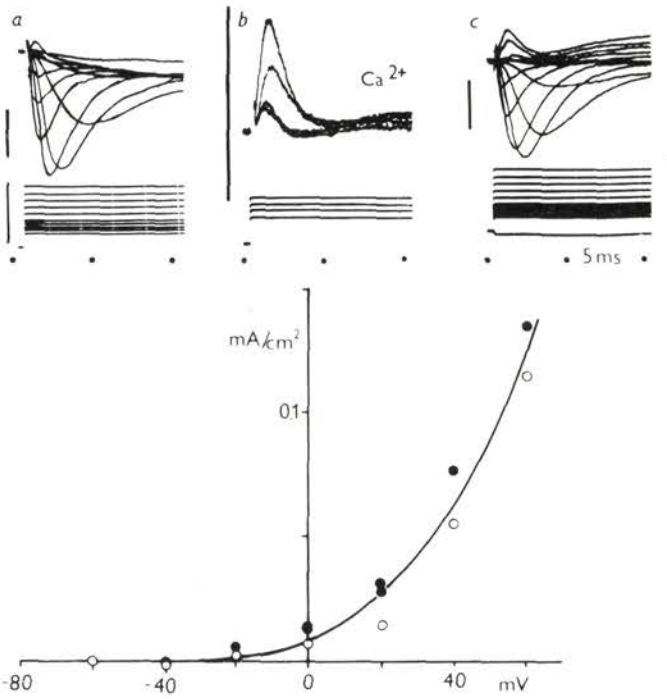

Fig. 5. Calcium currents in the sodium channel. Tonic muscle fibre of the frog 15 days after denervation. *Top*: superimposed voltage clamp recordings. *a*, *c* — solutions A // D; *b* — Na^+ ions in solution A were replaced with Ca^{2+} ions (89.5 mmol/l). Note the change in gain when recording in Ca^{2+} solution. *Bottom*: peak current-voltage relations of the outward calcium currents. Two muscle fibre segments (○, ●). The curve was drawn by hand. $t = 16$ °C. Fibre: 211081. Current scales: 75 nA. Voltage scale: 200 mV.

reversal potential cannot be determined. However, the lower limit in the reversal potential can be estimated from the current-voltage relation as the first potential to generate outward current. The apparent reversal potential estimated in this way was about 81 mV and 67 mV in denervated tonic and normal phasic muscle fibres, respectively, corresponding to P_{Ca}/P_{Na} less than 0.038 and 0.067, respectively (Table 1). These values are comparable with those (<0.093) reported for normal phasic muscle fibres of *Rana pipiens* (Campbell 1971).

Discussion

The main conclusion which could be drawn from experiments reported herein is that the ion selectivity sequence of new sodium channels formed after denervation in slow (tonic) muscle fibres of the frog, is the same as that found in sodium

Table 2. Sodium channel selectivity. Permeability ratios P_S/P_{Na}

S-ion	Frog Muscle				Rat	Node of
	Twitch	Twitch	Twitch	Tonic	Muscle	Ranvier
	(1)	(2)	(3)	(4)	(5)	(6)
Lithium	0.96	0.94	0.91	0.88	1.14	0.93
Hydroxylammonium	0.94	—	—	—	0.90	0.94
Hydrazinium	0.31	—	—	—	0.30	0.59
Ammonium	0.11	0.22	0.14	0.23	0.15	0.16
Tetraethylammonium	0.008	—	—	—	0.012	0.008
Potassium	0.048	0.076	0.082	0.12	0.045	0.086
Calcium	—	0.067	—	0.038	—	—

(1) Campbell (1976); *R. pipiens*
(2) this paper; *R. temporaria*
(3) this paper; *R. temporaria*; denervated
(4) this paper; *R. temporaria*; denervated
(5) Pappone (1980); *Rattus*
(6) Hille (1971, 1972); *R. pipiens*

channels in other cell membranes under comparable methodical conditions. Ions tested in the present work were chosen as representative cations from a larger list of inorganic and organic cations studied by Hille (1971, 1972) in myelinated nerve. Cations tested in his experiments were chosen so as to reveal the geometry and the nature of chemical and electrical forces that characterize the selectivity mechanism (selectivity filter) of sodium channels in the node of Ranvier. Hille used changes in the reversal potential (zero current potential) to estimate selectivity; this method has also enabled determination of deviations from the independence principle, and consequently, of a model that can discriminate between the energy barriers represented by the selectivity filter, and the binding sites in the channel (Hille 1975). The same method of selectivity determination was used in the present study. In the absence of previous selectivity studies on sodium channels in tonic muscle fibres, this enables a direct comparison of Na channel selectivities with those in other sodium channel membranes. The comparison has been made easier by the fact that our results of Na channel selectivity measurements were obtained in both the normal and denervated phasic muscle fibres from the same animal species, from the same muscle type, and under identical methodical conditions.

The first systematic study of Na channel selectivity in phasic muscle fibres of the frog (*R. pipiens*) under comparable methodical conditions was performed by Campbell in 1976. The selectivity sequence as well as the permeability ratios listed in Table 2, column 1 were in fact identical to those reported for the nerve membrane of the same animal species (column 6), with the exception of hyd-

razinium and potassium ions, that show higher permeabilities in the node of Ranvier (Hille 1971, 1972). Interspecies variations seem to be negligible, as evidenced by the data on Na channel selectivity (Pappone 1980) reported for mammalian muscle membranes (m. sternocleidomastoideus and m. extensor digitorum longus of the rat). The only slight difference concerns a higher permeability of Na channels in the rat muscle membrane for Li^+ as compared to Na^+ ions. Our measurements in phasic muscle fibres of the frog (*R. temporaria*) are in a good agreement with the previous experience, as evidenced by all the ionic probes for different permeability ratios: high (Li^+), moderate (NH_4^+), low (K^+), and negligible (Ca^{++}). Similar probes were used in experiments to determine selectivity of new sodium channels formed in tonic muscle fibres after denervation. The results obtained in this experimental model, can be said to be basically identical. Only the anaesthetic action of Li^+ ions on Na channels in tonic fibres seems to be more pronounced in comparison with phasic fibres. Edwards (1982) brings in his review several other examples in support to the conclusion that sodium channels selectivity data may be fitted by the assumption of a single channel. On the other hand, data on sodium channels formed in tonic muscle fibres after denervation support the hypothesis, which has been put forward as the outcome of kinetic analysis of sodium conductances of sodium channels in tonic muscle fibres (Zachar et al. 1982). According to this hypothesis, new sodium channels in frog tonic muscle fibres may be a result of the expression of sodium channel protein genes, induced by the denervation.

Acknowledgment. The authors wish to acknowledge the technical assistance of Mrs. G. Sedláriková and Mr. A. Melota throughout the experiments.

References

Campbell D. T. (1976): Ionic selectivity of the sodium channel of frog skeletal muscle. J. Gen. Physiol. **67**, 295—307

Cole K. S. (1968): Membranes, ions and impulses. University of California Press, Berkeley, California.

Edwards C. (1982): The selectivity of ion channels in nerve and muscle. Neuroscience **7**, 1335—1366

Frankenhaeuser B. and A. L. Hodgkin (1957): The action of calcium on the electrical properties of squid axons. J. Physiol. (London), **137**, 217—243

Goldman D. E. (1943): Potential, impedance, and rectification in membranes. J. Gen. Physiol. **27**, 37—60

Hille B. (1971): The permeability of the sodium channel to organic cations in myelinated nerve. J. Gen. Physiol. **58**, 599—619

Hille B. (1972): The permeability of the sodium channel to metal cations in myelinated nerve. J. Gen. Physiol. **59**, 637—658

Hille B. (1975): Ionic selectivity, saturation, and block in sodium channels. A four barrier model. J. Gen. Physiol. **66**, 535—560

Hille B., Campbell D. T. (1976): An improved vaseline gap voltage clamp for skeletal muscle fibers. J. Gen. Physiol. **67**, 265—293

Hodgkin A. L., Huxley A. F. (1952): Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo. J. Physiol. (London), **116**, 449—472

Hodgkin A. L., Katz B. (1949): The effect of sodium ions on the electrical activity of the giant axon of the squid. J. Physiol. (London), **108**, 37—77

Miledi R., Stefani E., Steinbach A. B. (1971): Induction of the action potential mechanism in slow muscle fibres of the frog. J. Physiol. (London), **217**, 737—754

Nasledov G. A., Thesleff S. (1974): Denervation changes in frog skeletal muscle. Acta Physiol. Scand. **90**, 370—380

Pappone P. A. (1980): Voltage-clamp experiments in normal and denervated mammalian skeletal muscle fibres. J. Physiol. (London), **306**, 377—410

Schalow G., Schmidt H. (1975): Effect of temperature and length of nerve stump on development of action potentials in denervated frog slow muscle fibres. Pflügers Arch. **335**, (Suppl.) R57

Schmidt H., Tong E. Y. (1973): Inhibition by actinomycin D of the denervation induced action potentials in frog slow muscle fibres. Proc. Roy. Soc. B (London), **184**, 91—95

Zachar J., Zacharová D., Henček M., Nasledov G. A., Hladký M. (1982): Voltage-clamp experiments in denervated frog tonic muscle fibres. Gen. Physiol. Biophys. **1**, 385—402

Zacharová D., Henček M., Nasledov G. A., Zachar J. (1982): Courants ionique et sélectivité des fibres lentes et rapides de la Grenouille. J. Physiol. (Paris) **78**, 52A-53A

Received July 26, 1982 / Accepted May 20, 1983