J. Membrane Biol. 4, 68–73 (1971)

Effect of Calcium Deprivation on Frog Skeletal Muscles at Different pH Values

JORGE ARGIBAY* and CARLO CAPUTO**

Instituto Venezolano de Investigaciones Cientificas,
Ap. 1827, Caracas, Venezuela

Received 16 July 1970

Summary. Whole sartorius muscles from the frog were exposed to calcium-free solutions at different pH values. The depolarizations caused by these solutions were measured 1 hr after the solution change. When the pH was 7.2, the mean depolarization was 8 mV. At pH values of 5.1 and 8.5, the mean depolarizations were 1 and 17 mV respectively. Similar experiments were carried out with solutions in which the main anion was sulfate instead of chloride. In these cases, the depolarization values caused by calcium deprivation at pH values of 7.2, 5.5, and 8.5 were 11, 4, and 20 mV, respectively.

Numerous authors have studied the effect of calcium on the properties of excitable cell membranes [2, 3, 7, 11, 18, 21]. It has been established that calcium deprivation of muscle fibers leads to progressive depolarization of the membrane accompanied by a fall of its electrical resistance [3, 7, 18, 21]. These effects are qualitatively similar to those that occur in nerve fiber membranes. To explain the effect of calcium on these membranes, it has been suggested that calcium ions are adsorbed on the external edge of the membranes [11]. There are several types of experimental evidence which appear to support this suggestion.

For example, it has been shown that isolated fragments of muscle fiber membranes can bind calcium ions and that the binding is pH dependent [5, 20]. This behavior is rather similar to that of phospholipid monolayers [23–25, 28]. Some years ago, Straub [27] reported that the depolarization of frog myelinated nerve fiber membrane, caused by calcium deprivation, was increased by alkaline and decreased by acid pH. Also it is

* Present address: National Institute of Medical Research, Mill Hill, London N.W. 7, England.

** Present address: Department of Physiology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14620.

known that after incubating whole muscles in a calcium-free EGTA medium, the membrane permeability to sodium ions is increased, that to potassium ions is decreased, and that to chloride ions remains practically unchanged [19]. The changes in the permeability to sodium and potassium ions could account for the depolarization of the membrane which occurs under these conditions. The present experiments were carried out to study the effect of pH on the depolarization of muscle fiber membranes caused by calcium deprivation. (A preliminary report of some of the results obtained has been presented at the XVIII Annual Convention of The Venezuelan Association for the Advancement of Science [1].)

Materials and Methods

Whole sartorius muscles dissected from *Rana pipiens* or *Leptodactylus insularis* were used. The muscles dissected from *Rana pipiens* were used only from October to April. The two sartorii of each animal were mounted in two separated lucite chambers. In most of the experiments, one muscle served as control of the other, and thus the two muscles of each animal were subjected to different experimental treatment. After dissection, the muscles were left in the experimental chambers immersed in normal Ringer's solution for 1 hr, after which the membrane potential of 15 to 20 fibers was measured. The effect of the different experimental solutions on the membrane potential of 15 to 20 fibers was tested after 1-hr incubation in the respective solution. The effectiveness of the solution change was assured by repeated changes of the solution at intervals of 10 min or less. After the measurements of the fiber membrane potentials in the experimental test solutions were made, the muscles were reimmersed in the normal Ringer's solution, and allowed to recover for 60 to 90 min. When the initial membrane potential values were not recovered after this period, the muscles were discarded. Normally in these cases, structural damage to the fibers was evident.

The normal Ringer's solution had the following composition in mM: KCl 2.5; NaCl 115; $CaCl_2$ 1.8; Na_2HPO_4 2.15; NaH_2PO_4 0.85. The sulfate Ringer's had the following composition in mM: K_2SO_4 1.25; Na_2SO_4 38.75; Na_2HPO_4 2.15; NaH_2PO_4 0.85; $CaSO_4$ 8.0; sucrose 113.

The calcium-free solutions were prepared simply by omitting the $CaCl_2$. The acid and alkaline solutions were prepared with the adequate phosphate buffer.

In some experiments, unbuffered alkaline solution was used and NaOH was used to increase the pH. In these cases, the solution was prepared immediately before the experiments, and was changed in the experimental chamber every 4 or 5 min.

Glass triple-distilled water was used. All the experiments were carried out between 20 and 23 °C.

Results

A preliminary set of experiments was carried out to find the time course of the depolarization caused by calcium lack.

Fig. 1 shows a typical experiment in which a whole sartorius muscle was exposed to a calcium-free solution. The points represent the mean

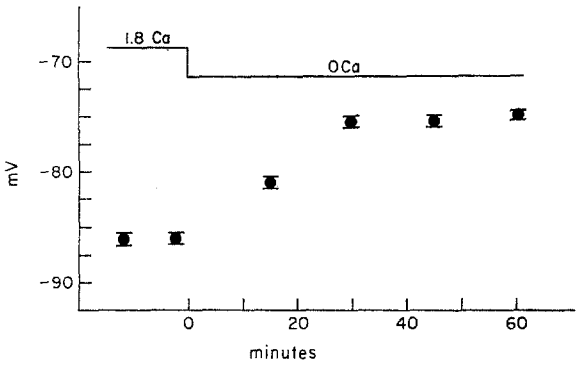

Fig. 1. Time course of fiber membrane depolarization in a whole sartorius muscle caused by calcium deprivation. Each point represent the mean ±SEM obtained by sampling 15 to 20 fibers

Table. *Results when membrane resting potential was measured 1 hr after calcium deprivation*

Principal anion in the medium	pH	Number of muscles	Initial resting potential (mV)	Membrane potential (mV) [a] calcium-free	Recovery
Chloride	7.2	14	−85±2	−77±2	−86±2
	5.1	7	−86±2	−85±2	−86±2
	8.5	7	−84±2	−67±2	−86±2
Sulfate	7.2	8	−90±2	−79±2	−92±2
	5.5	7	−91±2	−87±2	−92±3
	8.5	6	−87±2	−67±3	−90±4

[a] The membrane potential values listed here are the mean ±SD obtained by pooling the measurements from 15 to 20 fibers of each muscle.

values (± the standard error of the mean) obtained from the impalement of 10 fibers. Similar results were obtained with five other muscles. On the basis of these results, we decided to perform all measurements of the membrane potential 1 hr after the change to the test solutions. Next it was thought necessary to test the effect of acid (pH 5.1) and alkaline (pH 8.5) solutions on the membrane resting potential. In five muscles exposed for 1 hr in solutions at pH 5.1 or at 8.1, the membrane potential was not different within 1 or 2 mV from that measured initially.

The Table summarizes the results obtained when the membrane resting potential was measured 1 hr after calcium deprivation in solutions of pH 5.1, 7.2, and 8.5. The upper three rows show the results obtained when the solution contained chloride ions. The lower three rows show the results obtained when sulfate was substituted for chloride. In this case, the muscles

were immersed for 1 hr in a sulfate Ringer's with normal calcium-solution content and then exposed to the calcium-free medium. It appears that in both chloride and sulfate media acid pH reduces the depolarization caused by calcium deprivation; in contrast, alkaline pH increases it. It may be observed that in the sulfate solutions the depolarization values at acid, normal, and alkaline pH are somewhat greater than those obtained in the chloride media.

Discussion

In the range of values used in the present work, it was found that pH variations had no appreciable effect on the resting membrane potential of frog sartorius muscle fibers. These results are similar to those reported previously by other authors [9, 22]. Similarly, the depolarization of approximately 10 mV measured 1 hr after calcium deprivation at normal pH is consistent with previous experimental work. In accord with the concepts of the ionic theory, this depolarization could be explained in terms of an increase of the membrane conductance to sodium ions, to a decrease of the membrane conductance to potassium ions, or to a combination of both. It could also be explained assuming that the membrane conductances of all ions increase, with the sodium one increasing relatively more than the potassium one, so that the membrane selectivity is diminished. The first explanation would be in agreement with the work of Kimizuka and Koketsu [19], who found that calcium deprivation leads to an increase in sodium permeability and a decrease in potassium permeability, without affecting the chloride permeability. The second explanation would seem to agree with the results of Curtis [7] which show that the effective membrane resistance of muscle fibers bathed in sodium-free choline Ringer's decreases by about 10-fold after calcium deprivation. Knowledge of the effects of calcium deprivation on the permeability of muscle fiber membrane to choline would be especially useful for clarifying this point.

The principal finding of this work is the pH dependency of the effects caused by calcium deprivation. This phenomenon is qualitatively similar to that described by Straub [27] for the case of frog myelinated nerve fibers.

Recently, Hutter and Warner [15–17] have reported that the chloride conductance of frog muscle membranes is also dependent on the pH of the medium. It is important to note that the phenomenon reported here differs from that reported by Hutter and Warner. In fact, these authors showed that the pH dependency of the chloride conductance was not affected by changes in the external calcium concentration. Furthermore, the effect of

pH changes on the depolarization caused by calcium lack has been shown in the present work to occur independently of whether the main ion is chloride or sulfate.

The results reported here are in agreement with the findings of other authors who have studied the interaction of calcium ions, either with fragments of natural biological membranes [5, 20] or with artificial phospholipid membranes [23–25, 28]. In both cases, the interaction has been shown to be pH dependent. In the case of artificial phospholipid membranes the interaction has been ascribed specifically to the phosphate groups. Furthermore, the presence of negative fixed charges at the cell membranes has been well established [8, 10, 12].

The results obtained in the present work could be explained assuming that the membrane permeability to cations is dependent on the number of calcium ions bounded at the surface or, conversely, on the number of non-neutralized negative charges present on the membrane surface. This explanation appears to be consistent with the findings of Kimizuka and Koketsu [19] and with the hypothesis that calcium ions exert a stabilizing effect on excitable membranes [26]. It is interesting to notice that Chandler, Hodgkin and Meves [6] have shown that the presence of negative charges at the membrane boundary may affect the relationship existing between voltage and the mechanism which regulates the sodium permeability in the giant axon of the squid.

More recently, Hille [13] using the frog myelinated nerve has shown that calcium ions selectively affect the sodium channels and pH changes preferentially affect the potassium channels. It remains to be proved if these effects on the properties of active membranes have any relevance for resting membranes as used in the present experiments. It is, however, satisfactory that a lowering of the pH compensates for the absence of calcium (*see* Hille [13]).

This phenomenon can be utilized to study the effect of calcium deprivation on the process of the excitation contraction coupling in frog muscle fiber, without the complications caused by depolarization of the fiber membranes [4].

References

1. Argibay, J., Caputo, C. 1968. Efecto de la falta de calcio y de cambios de pH sobre la membrana de fibras musculares. (Abstract). *Acta Cient. Venez.* **19**:8.
2. Brink, F. 1954. The role of calcium in neural processes. *Pharmacol. Rev.* **6**:243.
3. Bülbring, E., Holman, M., Lüllmann, H. 1956. Effects of calcium deficiency on the striated muscle of the frog. *J. Physiol.* **133**:101.
4. Caputo, C. 1968. The role of calcium in the processes of excitation and contraction in skeletal muscle. *J. Gen. Physiol.* **51**:180s.

5. Carvalho, A. P., Sanui, H., Pace, N. 1963. Calcium and magnesium binding properties of cell membrane materials. *J. Cell. Comp. Physiol.* **62**:311.
6. Chandler, W. K., Hodgkin, A. L., Meves, H. 1965. The effect of changing the internal solution on sodium inactivation and related phenomena in giant axons. *J. Physiol.* **180**:821.
7. Curtis, B. A. 1963. Some effects of Ca-free choline-Ringer solution on frog skeletal muscle. *J. Physiol.* **166**:75.
8. Davies, J. T., Haydón, D. A., Rideal, E. 1956. Surface behaviour of *Bacterium coli*. *Proc. Roy. Soc. (London)* **145**:375.
9. Del Castillo, J., Nelson, T. E., Sanchez, V. 1962. Mechanism of the increased acetylcholine sensitivity of skeletal muscle in low pH solutions. *J. Cell. Comp. Physiol.* **59**:35.
10. Elul, R. 1967. Fixed charge in the cell membrane. *J. Physiol.* **189**:351.
11. Frankenhaeuser, B., Hodgkin, A. L. 1957. The action of calcium on the electrical properties of squid axons. *J. Physiol.* **137**:217.
12. Haydón, D. A. 1961. The surface charge of cells and some other small particles as indicated by electrophoresis. 1) The zeta potential-surface charge relationship. *Biochim. Biophys. Acta* **50**:450.
13. Hille, B. 1968. Charges and potentials on the nerve surface. Divalent ions and pH. *J. Gen. Physiol.* **51**:221.
14. Hodgkin, A. L., Katz, B. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol.* **108**:37.
15. Hutter, O. F., Warner, A. E. 1967*a*. The pH sensitivity of the chloride conductance of frog skeletal muscle. *J. Physiol.* **189**:403.
16. — — 1967*b*. The effect of pH on the Cl efflux from frog skeletal muscle. *J. Physiol.* **189**:427.
17. — — 1967*c*. Action of some foreign cations and anions on the chloride permeability of frog muscle. *J. Physiol.* **189**:445.
18. Ishiko, N., Sato, M. 1957. The effect of calcium ions on electrical properties of striated muscle fibres. *Jap. J. Physiol.* **7**:51.
19. Kimizuka, H., Koketsu, K. 1963. Changes in the membrane permeability of frog's sartorius muscle fibers in Ca-free EDTA solution. *J. Gen. Physiol.* **47**:379.
20. Koketsu, K., Kitamura, R., Tanaka, R. 1964. Binding of calcium ions to cell membrane isolated from bullfrog skeletal muscle. *Amer. J. Physiol.* **207**:509.
21. — Noda, K. 1962. Membrane responses of frog skeletal muscle fibers in calcium-free media. *J. Cell. Comp. Physiol.* **59**:323.
22. Ling, G., Gerard, R. W. 1949. The normal membrane potential of frog sartorius fibers. *J. Cell. Comp. Physiol.* **34**:383.
23. Ohki, S., Goldup, A. 1968. Influence of pH, sodium and calcium ions on the D.C. resistance of black egg lecithin-cholesterol fibres. *Nature* **217**:459.
24. Rojas, E., Tobias, J. M. 1965. Membrane model association of inorganic cations with phospholipid monolayers. *Biochim. Biophys. Acta* **94**:394.
25. Shah, D. O., Schulman, J. H. 1967. Interaction of calcium ions with lecithin and sphingomyelin monolayers. *Lipids* **2**:21.
26. Shanes, A. M. 1958. Electrochemical aspects of physiological action in excitable cells. *Pharmacol. Rev.* **10**:59.
27. Straub, R. 1956. Der Einfluß von Lokalanaesthetika auf ionenbedingte Ruhepotentialänderungen von markhaltigen Nervenfasern des Frosches. *Arch. Int. Pharmacodyn.* **107**:414.
28. Tobias, J. W., Agin, D. P., Pawlowsky, R. 1962. Phospholipid-cholesterol membrane model. Control of resistance by ions or current flow. *J. Gen. Physiol.* **45**:989.