MUSCLE RESPONSE TO LONG CHAIN QUATERNARY AMMONIUM IONS II

LADISLAV P. HINTERBUCHNER AND IRWIN B. WILSON

Department of Neurology, College of Physicians and Surgeons Columbia University, New York, N.Y. (U.S.A.)

(Received July 26th, 1958)

SUMMARY

1. Lipid-soluble quaternary amines produce an irreversible double twitch in frog sartorius and other muscles. The first twitch is relatively fast (30–40 sec) while the second is slow (peak at 2–3 min).

2. Neither curare nor atropine affects the response to lipid-soluble quaternary amines, indicating that the site of action is not the myoneural junction.

3. A muscle bathed in high potassium Ringer or eserine for a sufficiently long time will no longer give two twitches with NACh 12. Potassium abolishes the first twitch, while eserine gives a single twitch slower than the first and faster than the second twitch normally expected from a muscle with NACh 12.

4. Sodium-free Ringer has no effect on the first twitch, but the second contraction is larger and no relaxation takes place. Potassium-free Ringer abolishes the second twitch. Cold does not affect the double twitch beyond the expected prolongation.

5. This phenomenon is compared with the two-peak response obtained in denervated mammalian muscle and normal and denervated frog muscle by close intra-arterial injection of acetylcholine.

The differences are discussed.

INTRODUCTION

Recent studies with lipid-soluble quaternary amines such as β-acetoxyethyl dodecyl dimethyl ammonium ion (noracetylcholine 12, NACh 12), dodecyltrimethyl ammonium ion and N dodecyl pyridinium ion have shown that these compounds block conduction in lobster and crab nerve and depolarize the electroplax of the electric eel[1]. In the frog rectus abdominis muscle these compounds produce repeatable contractures[2,3]. Concentrations necessary to produce such effects were 10^{-3} to 10^{-4} M.

Since then it was found that much smaller concentrations of the lipid-soluble analogue of acetylcholine[4,5] as well as pyridine-2-aldoxime dodeciodide (a nerve gas antidote)[5] produced depolarization in the desheathed frog tibialis nerve. Physostigmine sulfate prevented depolarization by these compounds and largely reversed an already present depolarization[6].

This report is concerned with a description of the effect of the lipid-soluble analogue of acetylcholine and other long chain quaternary amine derivatives on frog striated muscle other than rectus abdominis.

References p. 380.

METHODS

Whole sartorius and biceps muscles as well as sartorius and semi-membranosus muscle strips were dissected and mounted in apparatus, where the tension which developed upon change of bathing fluid was measured by means of an electro-mechanical transducer (RCA 5734) and Varian pen recorder (G 10).

The Ringer solution contained Na^+ 116.55, K^+ 2.5, Ca^{++} 1.8, Cl^- 117.1, $H_2PO_4^-$ 0.45, HPO_4^- 2.55 mmoles/l.

Sodium-free Ringer solution contained K^+ 5.55, Ca^{++} 1.8, Cl^- 3.6, $H_2PO_4^-$ 0.45, HPO_4^- 2.55 and sucrose 232.55 mmoles/l. This solution did not produce any measurable change in the tension of the muscle despite the double content of potassium.

Potassium-free Ringer contained Na^+ 116.55, Ca^+ 1.8, Cl^- 114.6, $H_2PO_4^-$ 0.45, HPO_4^- 2.55 and sucrose 5.0 mmoles/l.

High potassium solutions were prepared by adding 20 or 40 mmoles/l of KCl to the Ringer or sodium-free Ringer solutions.

RESULTS

Noracetylcholine 12 in concentration of $2.3 \cdot 10^{-3}$ *M* repeatedly gave 2 twitches in the whole sartorius and biceps muscles as well as in strips of sartorius or semimembranosus muscles (Figs. 1–6). The first twitch usually reached its peak in 10–15 sec and abated within 40–50 sec from the onset. The second twitch was slower, reaching its peak in 2–3 min. The strength of both twitches varied from 1 to 3 g. To obtain control contractions we used the paired muscle from the opposite side of the same frog. But pairs differ considerably and therefore only large effects can be considered as significant. Identical response was obtained with N dodecyltrimethyl ammonium ion of similar concentration. Six and eight carbon chain derivatives of acetylcholine and tetramethyl ammonium in comparable concentrations produced no response in sartorius and biceps muscle, nor did they prevent the usual response to long chain derivatives on subsequent application.

Neither twitch could be produced more than once in the same muscle. If the stimulating compound was washed out at the peak or at the end of the first twitch, a subsequent trial yielded only the second twitch. The response to direct electrical stimulation was preserved through the first and the second twitch, however, it declined at the time of the second relaxation and eventually disappeared. In one sartorius washed for 8 h following exposure to 2 μmoles/ml of noracetylcholine 12, the electrical response returned, however, no second response could be obtained with the stimulating compound. To possibly gain some insight into the nature of the double response, solution with changed ionic composition were applied in association with noracetylcholine 12. Curare and atropine were used to determine to what degree the endplate and the nerve remaining within the isolated muscle participate in the double response.

d-Tubocurarine in various concentrations (100, 200, 1000 μg/ml for 40–70 min) failed to influence in any definite way the double twitch (Fig. 2) despite the fact that 2 μg/ml of d-tubocurarine abolishes about 60% of the response to indirect stimulation in less than 15 min[7].

Atropine in concentration of $3 \cdot 10^{-4}$ blocks up to 0.33 μmoles/ml of acetylcholine

References p. 380.

chloride (60 μg/ml) in the rectus abdominis muscle. This block is completely reversible after 30–45 min of washing with Ringer solution. In sartorius muscle, however, no definite influence of 0.6 μmoles/ml of atropine on the double response was observed.

Sodium-free Ringer failed to influence the first response, however, the second twitch became larger and no relaxation took place after the second contraction unless the sodium-free Ringer was replace by normal Ringer. Then a rapid relaxation ensued (Fig. 3). Upon exposure of the muscle to the high potassium Ringer there appears a fast twitch followed by rapid relaxation to the original tension. If the muscle is

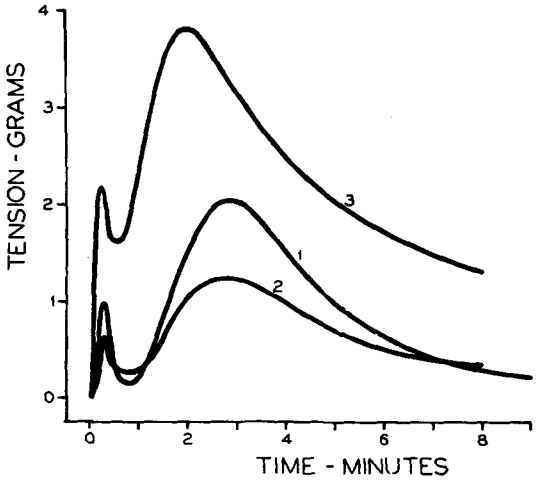

Fig. 1. Two-peaked response of sartorius muscle strips[1,2] and biceps muscle[3] to $2.3 \cdot 10^{-3}$ NACh 12.

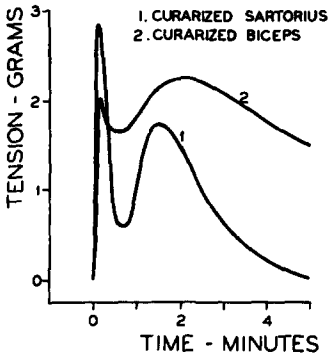

Fig. 2. Response of curarized muscles to $2.3 \cdot 10^{-3}$ NACh 12: (1) sartorius muscle curarized with 200 μg/ml of d-tubocurarine for 60 min and (2) biceps muscle curarized with 200 μg/ml of d-tubocurarine for 130 min prior to test with NACh 12.

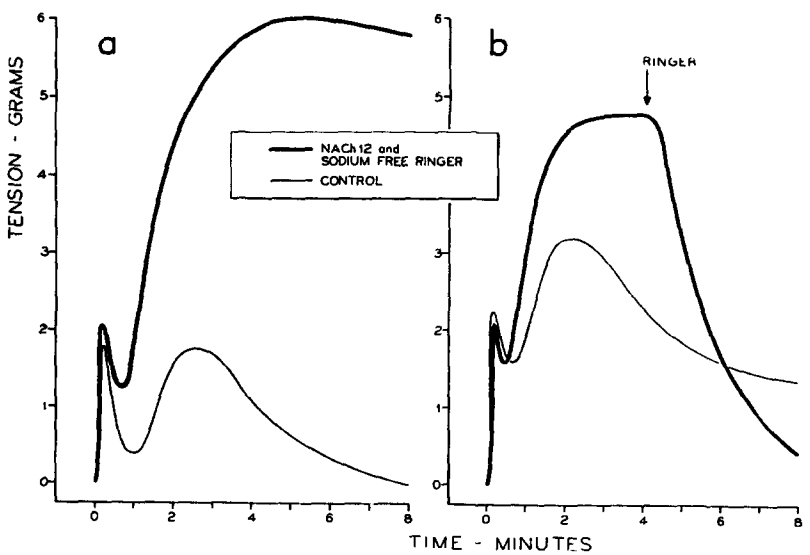

Fig. 3. Response to $2.3 \cdot 10^{-3}$ NACh 12 modified by prior bathing of the muscle in sodium-free Ringer solution for 10 min: (a) sartorius, (b) biceps. Arrow in *b* indicates replacement of the testing solution by normal Ringer. Control obtained with paired muscles from the same animal.

References p. 380.

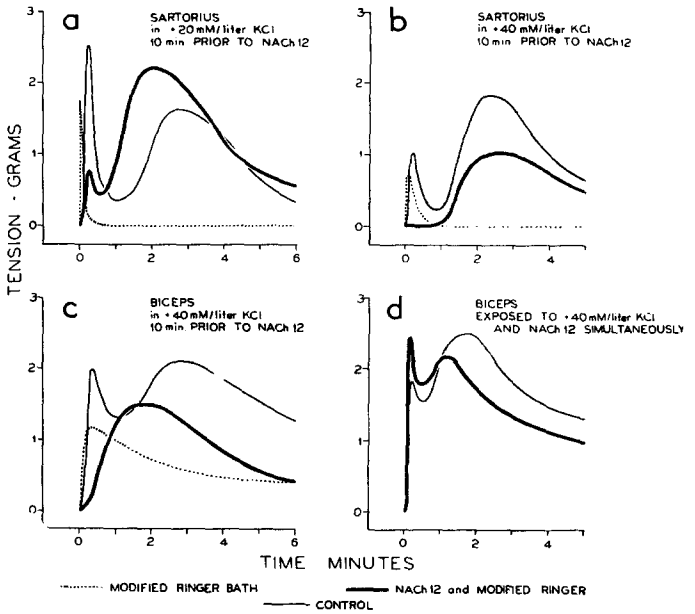

Fig. 4. Effect of high potassium concentration on the response of muscle to $2.3 \cdot 10^{-3}$ NACh 12: (a) sartorius bathed in Ringer with plus 20 mmoles/l KCl for 10 min and (b) plus 40 mmoles/l KCl for 10 min; (c) biceps bathed in Ringer with plus 40 mmoles/KCl for 10 min prior to exposure to stimulating compound; (d) biceps exposed to plus 40 mmoles/l KCl Ringer and the stimulating compound simultaneously. Controls obtained with paired muscles from the same animal.

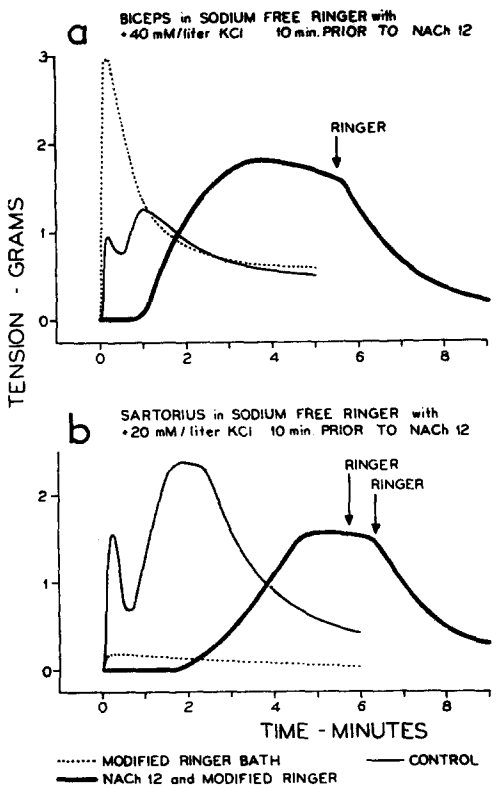

Fig. 5. Combined effect of high potassium and sodium-free Ringer on the response of (a) biceps and (b) sartorius muscles to $2.3 \cdot 10^{-3}$ NACh 12. Controls obtained with paired muscles from the same animal.

kept in such a bath for 10 min with a concentration of KCl of plus 20 mmoles/l, the first response of the muscle to noracetylcholine 12 is diminished and the second increased* (Fig. 4a). If the concentration of KCl is plus 40 mmoles/l, then the first response to noracetylcholine 12 is abolished and the second diminished (Fig. 4b, c). If the muscle is not bathed in high potassium solution in advance but is exposed to the stimulating compound and the modified Ringer solution at the same time, the first response is not abolished (Fig. 4d).

When high potassium is combined with sodium-free Ringer, the first twitch again is abolished and the second contraction is increased and delayed. Again, as with sodium-free Ringer alone, no relaxation occurs unless plain Ringer is substituted with or without additional potassium. Potassium-free Ringer does not produce any effect on the tension when used alone. The first response to noracetylcholine 12 is not affected appreciably, but the second contraction is not readily discernible and the muscle gradually relaxes instead (Fig. 5).

Physostigmine ($7 \cdot 10^{-3}$) did not produce a change in tension or only a minor increase within the bathing period of 20–30 min. Only one twitch was produced by noracetylcholine 12 in a muscle so prepared, reaching peak within 30–60 sec with prolonged falling phase (Fig. 6). When bathing and testing solutions were kept below 10° both twitches were considerably prolonged but not otherwise changed.

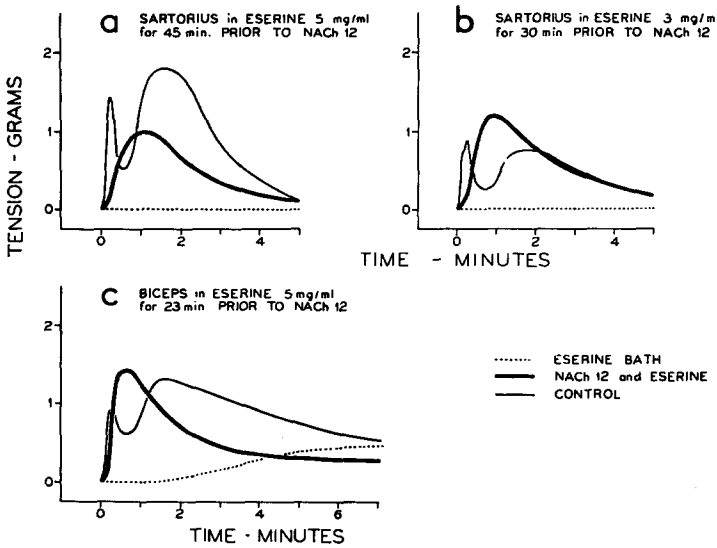

Fig. 6. Effect of physostigmine sulfate on response of muscle to $2.3 \cdot 10^{-3}$ NACh 12. In the experiments shown in *a* and *b* no tension developed with physostigmine alone but in *c* tension did develop and reached constant value after 7 min (dotted lines). Controls were obtained with paired muscles from the same animals.

DISCUSSION

The 2-peak tension response of a muscle under various conditions is not new to the physiological literature. GASSER[8] in his well known review on contracture of skeletal

* A solution of similar osmolarity containing sucrose instead of additional KCl did not give any response nor did it affect the response to noracetylcholine 12.

References p. 380.

muscle mentions a number of chemicals such as acids, alkalies, narcotics and veratrine, which under certain circumstances can produce a two-peaked contraction in an isolated muscle. Particularly pertinent to this discussion is, however, the two-peak response of muscle to acetylcholine. BROWN, DALE AND FELDBERG have described such a response of denervated mammalian muscle to close-arterial injection of acetylcholine[9]. BROWN[10] found a similar although less well differentiated response of normal and denervated frog muscle to close-arterial injection of acetylcholine. The fast response was found to represent an asynchronous tetanus associated with a burst of electrical activity, which in the frog muscle lasted up to 40 sec. This is also the approximate duration of the fast response of muscle to noracetylcholine 12 in our experiments. The second rise in tension was associated with electrical silence fitting GASSER's definition of contracture[8]. BROWN found also that curare depressed both the fast and the slow rise in tension in denervated mammalian and in frog muscle. In our experiments curare failed to antagonize the response to lipid-soluble quaternary amines. The double response described in this report may be similar to that obtained with acetylcholine by BROWN and others, but the curare experiment shows that it is initiated at a different site. It would appear that this site is the conducting membrane and these results would therefore support NACHMANSOHN's theory of conduction[11]. In the rectus abdominis muscle of frog, curare is also unable to inhibit contractures produced by lipid-soluble quaternary amines[2,3].

The mechanism of the double twitch remains to be elucidated. Depolarization produced by noracetylcholine 12 may possibly be responsible for the fast response, since potassium—a depolarizing agent—prevents this first response. Further data about this effect and that of eserine, and the influence of ionic changes will be needed to throw more light on this interesting phenomenon.

ACKNOWLEDGEMENTS

This work was supported in part by the Division of Research Grants and Fellowships of the National Institutes of Health, Grant No. B-573, by the National Science Foundation, Grant No. G-4331, by the Medical Research and Development Board, Department of the Army, Office of the Surgeon General, Contract No. DA-49-007-MD-740, and by the Muscular Dystrophy Associations of America, Inc.

L.P.H. is Research Fellow under Training Grant No. BT-266 from the National Institute of Neurological Diseases and Blindness, National Institutes of Health, U.S. Public Health Service.

REFERENCES

1 E. SCHOFFENIELS, I. B. WILSON AND D. NACHMANSOHN, *Biochim. Biophys. Acta*, 27 (1958) 629.
2 L. P. HINTERBUCHNER, I. B. WILSON AND E. SCHOFFENIELS, *Federation Proc.*, 17, No. 1 (1958).
3 L. P. HINTERBUCHNER AND I. B. WILSON, *Biochim. Biophys. Acta*, 31 (1959) 323.
4 R. STAEMPFLI, *Helv. Physiol. Acta*, 16 (1958) C32, C33.
5 W. D. DETTBARN, *Biochim. Biophys. Acta*, 32 (1959) 381.
6 W. D. DETTBARN, I. B. WILSON AND D. NACHMANSOHN, *Science*, 128 (1958) 1275.
7 W. L. NASTUK AND B. OLSON-ALVING, *Biochemical Pharmacology*, in the press.
8 H. S. GASSER, *Physiol. Revs.*, 10 (1930) 35.
9 G. L. BROWN, H. H. DALE AND W. FELDBERG, *J. Physiol.*, 87 (1936) 394.
10 G. L. BROWN, *J. Physiol.*, 89 (1937) 438.
11 D. NACHMANSOHN, *Harvey Lectures* 1953/54, Academic Press Inc., New York, 1955, p. 57.