Biochimica et Biophysica Acta 855 (1986) 197–199
Elsevier

BBA 70265

BBA Report

Liposome-delivered phosphatidylcholine enhances the acetylcholine sensitivity of dystrophic mouse myotubes

F. Eusebi *, G. Cossu, M. Molinaro and D. Giacomoni **

Dipartimento di Fisica and Istituto di Istologia ed Embriologia generale, University of Rome, via A. Scarpa, 14-00161 Roma (Italy)

(Received September 16th, 1985)

Key words: Liposome; Muscular dystrophy; Myotube; Acetylcholine sensitivity; Phosphatidylcholine

Myotubes were obtained in vitro from satellite cells of normal and dystrophic C57BL/6J/dydy mice. The acetylcholine sensitivity (mV/nC) of dystrophic myotubes determined with conventional electrophysiological techniques, was lower than that of normal myotubes. Incubation of dystrophic myotubes with liposomes containing phosphatidylcholine (a lipid present in higher amounts in normal adult muscle) significantly increased their acetylcholine sensitivity.

Murine muscular dystrophy, which shows a recessive mode of inheritance, is characterized by a gradual degeneration of muscle fibers and is associated with leaky membranes. Normal and dystrophic murine muscles differ in the activity of some enzymes, in lipid metabolism and in the level of membrane bound proteins [1]. Most of the abnormalities of the dystrophic muscle are very probably secondary manifestations of the disease whose primary etiology is unknown. These abnormalities point to the possibility of a membrane defect [1], a conclusions reached also for the human Duchenne dystrophy [2]. In agreement with this possibility is the observation that the phospholipid composition of the dystrophic muscle membranes is different from that of the normal muscle. For instance, phosphatidylcholine (PC) is present in lower amounts in dystrophic murine muscle membranes while phosphatidylethanolamine (PE) and sphingomyelin (SM) are present in higher amounts [3]. Lower levels of PC have also been observed in patients with Duchenne muscular dystrophy [4] compared to normal individuals, while higher levels of sphingomyelin have been reported in dystrophic chicken [5] and in humans [6]. If indeed lipid alterations play a role in the expression of this disease one would like to see whether some of its abnormalities can be corrected by changing the phospholipid composition of the membranes of dystrophic myotubes. We have shown that muscle satellite cells from normal and dystrophic mice can grow and differentiate in vitro [7] and that dystrophic myotubes have a reduced membrane potential and give a lower electrical response to acetylcholine [8] compared to normal myotubes. On the other hand, it is possible to modulate the phospholipid composition of cells in vitro by incubating them with liposomes of defined composition [9,10]. Purpose of the present work was to investigate whether modulation of membrane phospholipids affects the acetylcholine sensitivity of murine dystrophic myotubes in vitro.

Satellite cells were isolated from C57BL/6J/dydy mice at 4–6 weeks of age (when the recessive clinical aspects of the disease are evident) and

* To whom all correspondence should be addressed.
** Permanent address: Department of Microbiology and Immunology, College of Medicine, University of Illinois at Chicago, Chicago, IL, U.S.A.

cultured as described [7] in minimum essential medium supplemented with 10% horse serum and 3% embryo extract. Under these conditions cells duplicate actively for the first 3–4 days of culture and then fuse into multinucleate myotubes. Satellite cells from C57BL/6J mice were used as normal controls. Liposomes containing PC and cholesterol (C) at a molar ratio 20 : 1 (Lip PC : C) or PE, sphingomyelin and cholesterol at a molar ratio of 10 : 10 : 1 (Lip SM: PE : C) were prepared by the reverse phase evaporation method [11] in 10-fold dilution of phosphate-buffered saline without Ca^{2+} and Mg^{2+} (lipid concentration) 20 mg/ml). Normal and dystrophic myotubes (7–8-days old; 5–10 nuclei) formed in cultures were exposed to either type of liposomes for 6 h or kept as untreated controls. Liposomes were added to the medium with a final concentration of 2 mg lipid/ml, and were removed from the extracellular fluid before electrophysiological determinations. Membrane potential of the myotubes and their acetylcholine sensitivity were assessed with standard electrophysiological techniques as previously described [12], 10–30 min after removal of the liposomes from the medium. Acetylcholine was applied iontophoretically to myotube surface and the local acetylcholine sensitivity was taken as the ratio of depolarization (mV) to the quantity of charge (nC) which flows through the iontophoretic pipette. To allow better comparison of the data all values of chemosensitivity were corrected and referred to a resting potential of −40 mV [13,14].

The acetylcholine sensitivity (σ) of untreated normal and dystrophic myotubes was tested in several experiments and found to vary from culture to culture. The average sensitivity was 360 for normal and 261 for dystrophic myotubes (see Table I). The difference between normal and dystrophic myotubes is less marked than that determined before [8]. This is most probably due to progressive improvement of culture conditions and different batches of horse serum we used, resulting in an overall better differentiation of dystrophic cells; and to normalization of the sensitivity to −40 mV membrane potential (see above). (Lip PC : C) or (Lip SM : PE : C) did not affect σ of normal myotubes. Conversely, dystrophic myotubes treated with (Lip PC : C) showed a 20% higher σ while treatment with (Lip SM : PE : C) had no effect (Table I). The results were substantially equivalent for 4 to 7 h liposome-treatments. To investigate whether the 6 h incubation with different liposomes affected the density of myotube acetylcholine receptors (AChRs) we measured the binding of ^{125}I-labelled α-bungarotoxin [15] to normal and dystrophic myotubes after incubation with liposomes of different composition. Data obtained in two different experiments and reported

TABLE I

ACETYLCHOLINE SENSITIVITY (σ) AND α-BUNGAROTOXIN (α-BuTX) BINDING IN NORMAL AND DYSTROPHIC LIPID-TREATED MYOTUBES (5–10 NUCLEI)

RP, membrane resting potential. All values of α are referred to a RP of −40 mV. Values in brackets indicate the number of determinations. Each value represents mean ± S.E., except for αBuTX binding, which indicates the average. P was calculated by Student's t-test.

	RP (mV)	σ (mV/nC)	P	α-BuTX binding (%) per mg protein
Normal myotubes control	−38.1 ± 1.2 (44)	360 ± 12 (31)		100 (9)
			> 0.05	
PE : SM-treated	−39.1 ± 1.4 (25)	358 ± 12 (25)		101 (9)
			> 0.05	
PC-treated	−37.4 ± 0.9 (27)	353 ± 10 (27)		97 (9)
Dystrophic myotubes control	−28.4 ± 2.1 (44)	261 ± 12 (67)		100 (9)
			> 0.05	
PE : SM-treated	−28.7 ± 1.9 (29)	264 ± 14 (43)		103 (9)
			< 0.001	
PC-treated	−29.4 ± 1.2 (46)	330 ± 11 (44)		98 (9)

in Table I, indicated that the 6 h exposure to liposomes of different composition did not alter the level of α-bungarotoxin binding in either dystrophic or normal myotubes. In other experiments we investigated the effect of various liposomes on σ of dystrophic myotubes. Similarly prepared liposomes (Lip SM : C) and (Lip phosphoinositol (PI) : C) had no effect on acetylcholine-induced responses of dystrophic myotubes; while (Lip phosphatidylserine (PS) : C) caused massive degeneration of the cells so that σ could not be determined (data not shown). To rule out the possibility that the enhanced σ was due to liposomes still present in the medium because of insufficient washing, σ of dystrophic myotubes was assessed before and immediately after the addition of 100 μg/ml of (Lip PC : C). No change in σ was observed in dystrophic myotubes within the first 10 min of exposure to (Lip PC : C).

The data reported here show that by delivering PC to murine dystrophic myotubes via liposomes it is possible to enhance the acetylcholine sensitivity of these myotubes in a statistically significant way. The (trivial) possibility that enhancement of σ could be due to liposomes still in the medium was ruled out. Thus, it appears that by increasing the PC content of dystrophic myotubes one can partially reestablish a normal acetylcholine sensitivity. This treatment, however, does not correct the differences in membrane resting potential between normal and dystrophic myotubes. Since we observed no net change in the density of acetylcholine receptors following treatment with (Lip PC : C), it is possible that the acetylcholine receptor channel properties might be modified by the alteration of the lipid environment of the acetylcholine receptor. Alternatively, a number of acetylcholine receptor channels enter an inactivable state by lipid-dependent enzymatic processes and PC remove this inactivation. A candidate for this molecular mechanism underlying the modulation of the transmitter sensitivity is the phospholipid-dependent protein kinase C. In fact, this enzyme (i) phosphorylates the acetylcholine receptor of the electric organ of *Torpedo californica* [16]; (ii) modulates the electrical response to acetylcholine in chick myotubes [12]; (iii) is inhibited in its activity by PC [17]; (iv) is mainly cytosolic (inactive state; Ref. 18) in the normal, and it is mainly membrane-bound (activable state; Ref. 18) in dystrophic myotubes (Cossu, G., Adamo, S., Senni, M.I., Caporale, C. and Molinaro, M., unpublished data).

The role of lipid composition on the functional activity of membrane acetylcholine receptors has been demonstrated in studies using proteoliposomes [19]. We show now that lipid environment may affect acetylcholine receptor functional properties also in a living cell.

This work was supported by a MPI grant (60%) and by a CNR grant special project 'Oncologia', No. 84.00683.44. We thank Dr. S Adamo for critical reading of the manuscript and Mr. M. Coletta for technical assistance in culturing myotubes.

References

1 Strickland, K.P., Hudson, A.J. and Thakar, J.H. (1979) Ann. N.Y. Acad. Sci 317, 187–204
2 Pennington, R.J.I. (1980) Biochem. Soc. Trans. 8, 690–692
3 Pearch, P.H. and Kakulas, B.A. (1980) Aust. J. Exp. Biol. Med. 58, 397–408
4 Kunze, D. and Olthoff, D. (1970) Clin. Chim. Acta 29, 455–462
5 Sunnicht, G.E. and Sabbadini, R.A. (1982) Arch. Biochem. Biophys. 215, 628–637
6 Kunze, D., Reichmann, G., Egger, E., Leuschner, G. and Eckhardt, H. (1973) Clin. Chim. Acta 43, 333, 341
7 Cossu, G., Zani, B., Coletta, M., Bouché, M., Pacifici, M. and Molinaro, M. (1980) Cell Diff. 9, 357–368
8 Cossu, G., Eusebi, F. and Molinaro, M. (1984) Muscle Nerve 7, 73–76
9 Huang, L. (1983) Liposomes (Ostro, M. ed.)pp. 87–124, M. Dekker, New York
10 Clejan, S. and Bittman, S. (1984) J. Biol. Chem. 259, 441–448
11 Szoka, F. and Papahadjopoulos, D. (1978) Proc. Natl. Acad. Sci. USA 75, 4194–4199
12 Eusebi, F., Molinaro, M. and Zani, B.M. (1985) J. Cell Biol. 100, 1339–1342
13 Katz, B. and Tesleff, S. (1957) J. Physiol. 137, 267–278
14 Takeuchi, N. (1963) J. Physiol. 167, 141–155
15 Cossu, G., Pacifici, M., Adamo, S., Bouché, M. and Molinaro, M. (1982) Differentiation 21, 62–65
16 Huganir, R.L., Albert, K.A. and Greengard, P. (1983) Soc. Neurosci. Abstr. 9, 578
17 Kaibuchi, K., Takai, Y. and Nishizuka, Y. (1981) J. Biol. Chem. 256, 7146–7149
18 Nishizuka, Y. (1984) Science 225, 1365–1370
19 Criado, M., Fibl. H. and Barrantes, F.J. (1982) Biochemistry 21, 3622–3629