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# Specific modifications of the membrane fatty acid composition of human myotubes and their effects on the muscular sodium channels

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The fatty acid (FA) composition of human myotube primary cultures was varied by modifications of the contents of FA in the culture medium. An incubation time of 18 h with a defined FA mixture resulted in the most effective alteration of the original FA pattern of the cells. The increases reached for the relative amounts of palmitic acid (16:0), linoleic acid (18:2) or arachidonic acid (20:4) were 3–5-fold. More than 50% of the extra FA were incorporated in the phospholipid fraction, the remaining share in the triglyceride fraction. Shorter incubation times resulted in less FA incorporation, longer incubation times raised the uptake of FA into the triacylglycerol fraction. For a study of the influence of the membrane modification on the function of the sodium channels, the myotubes were converted into myoballs. The sodium channel properties were then determined using the whole-cell clamp technique. The modified cultures showed no significant alterations in the time constants of activation and inactivation, in the voltage dependence of inactivation ($h_\infty$ curves) or in the average amplitudes of the sodium currents.

## Introduction

Phospholipids are major constituents of biological membranes. The component phospholipids and the fatty acids (FA) bound by them determine to a great extend the chemical and physical properties of a biological membrane or membrane system. Effects of the phospholipid composition on the thickness [1], the fluidity [1,2] and on the properties of specific membrane proteins, such as enzymes, transport systems and ion channels [3] have been described.

In some human muscle diseases, altered compositions of the membrane lipids were observed, and this was discussed as a reason for muscle dysfunctions [4–7]. In neuroblastoma cells, the external application of poly-unsaturated FA has led to their integration into phospholipids and to a decreased excitability of the cells [8].

Apart from direct interactions of membrane lipids and membrane proteins, the lipid composition influences the cellular metabolism, since FA serve as precursors of second messengers [9,10]. Further, phosphatidylserine is thought to be essential for the function of protein kinase C [10], an enzyme which is important for many cellular reactions.

In the present study we have tried to modify the lipid composition of cultured human skeletal muscle cells by the application of specific FA to the culture medium. We tried to optimize the conditions to reach a maximum uptake of FA into the phospholipid fraction and investigated whether certain membrane lipid modifications alter the properties of muscular sodium channels.

## Methods

### *Cell-culture procedure and fatty acid incubation*

The culture methods are based on earlier experiences with rat [11] and human [12] muscle in our

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Abbreviations: BSA, bovine serum albumin; EGTA, ethylene bis(oxoethylenenitrilo)tetraacetate; Hepes, 2,4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; FA, fatty acids; FAMEs, fatty acid methyl esters; FCS, fetal calf serum; HPLC, high performance liquid chromatography; HS, horse serum; $h_\infty$ curve, voltage dependence of inactivation; PBS, phosphate-buffered saline; TLC, thin-layer chromatography.

laboratory. Primary human skeletal muscle cultures were prepared from surgical waste in accordance with the regulations of the Ethics Commission of the University of Ulm. Samples of 300–600 mg skeletal muscle were either stored overnight at 4°C in Hank's solution (Biochrom KG, Berlin, Germany) or directly used for the preparation of mononucleated cells. The samples were washed with Hank's solution several times for the removal of the coagulated erythrocytes. Then they were cut in small pieces, connective and fatty tissue were dissected off as much as possible, and the pieces were minced into fragments of about 1–10 $mm^3$ using fine needles. For enzymatic dissociation, the fragments were transferred into 10 ml of a solution composed of $Ca^{2+}$ and $Mg^{2+}$-free phosphate-buffered saline (PBS) (Biochrom) containing 5.4 mM KCl, 25 mM glucose, 25 mM sucrose, 50 $\mu$g/ml gentamycin (Biochrom), 0.1% bovine serum albumin (#11930, Serva, Heidelberg, Germany), 250 U/ml collagenase (Type II, Sigma, Deisenhofen, Germany) and a 3% solution of bactotrypsin (Difco, Augsburg, Germany). The enzymatic dissociation was performed at 37°C in a shaking water bath or a rotating incubator-shaker (80 rpm) for a total of 60 min by four successive treatments with the trypsin/collagenase solution. After each step the supernatant was filled up with 10 ml of Hank's solution containing 10% FCS, to terminate further protease activity. The suspension was then several times moved back and forth in a pipette to liberate more cells from remaining small tissue fragments and then filtered through a nylon gauze (2 layers pore size 50 $\mu$M, 1 layer pore size, 20 $\mu$M) and centrifuged at $100 \times g$ for 10 min. The pellet was resuspended in culture medium.

The yield of muscle cells from a biopsy of 0.5–1 g was usually between 3 and $5 \cdot 10^6$ mononucleated cells per g muscle tissue, of which 10–50% were viable and proliferative cells. This was sufficient for many electrophysiological and biochemical experiments. At least four 250 ml flasks of confluent muscle cultures were normally obtained from one biopsy. This is equivalent to 16 mg protein and 3–4 mg lipid.

The culture medium used consisted of a 1:1 (v/v) mixture of Ham's F-12 medium and CMRL medium (both Biochrom) with 5% FCS, 5% HS (both Gibco, Eggenstein, Germany) and 2.5 mg/ml glucose, 0.3 mg/ml glutamine (Biochrom) and 1.2 g/l $NaHCO_3$. The cultures were kept in an atmosphere with 5% $CO_2$. 1 or 2 days (d) after seeding in conventional tissue culture flasks (#690160 or #658170, Greiner, Frickenhausen, Germany) the serum content of the medium was reduced to 2% FCS and 2% HS. Under this condition the cultures became confluent within 10–14 d and the formation of myotubes occurred. 5 to 10 d after the first detection of myotubes the muscle cells were in an optimum condition for the measurement of sodium currents. During the following week, the remaining non-fusing cells, probably fibroblasts, continued to proliferate slightly, so that the myotubes were packed in a dense network of mononucleated cells. To avoid spurious results attributable to various amounts of fibroblasts in cultures from different muscles, all comparisons between modifications and controls were carried out with cultures grown in parallel from one biopsy.

For the incubation with FA, the cultures were slightly rinsed with serum-free medium and incubated in serum-free culture medium supplemented with 1.44% FA-free bovine serum albumin (Sigma) and different concentrations of a certain FA. Palmitic acid (16:0) and linoleic acid (18:2) were used in a concentration of 0.4 mM whereas arachidonic acid (20:4) and docosahexaenoic acid (22:6) (all Sigma) were applied in a concentration of 0.2 mM. The free concentrations of FA were calculated to be 0.5 $\mu$M for a total FA concentration of 0.4 mM, and 0.15 $\mu$M for a total FA concentration of 0.2 mM [13]. Control cultures were incubated with the corresponding media containing only albumin and ethanol which was used as a solvent for the 100 mM FA in the stock solutions. The incubation time was varied between 4 and 60 h to find maximum incorporation.

*Incorporation of [$^{14}$C]linoleic acid into phospholipids and triacylglycerols*

The localization of the incorporated FA was determined using radioactive linoleic acid. 1 $\mu$Ci per ml, representing 20 nmol $^{14}$C-labelled linoleic acid, was added to the culture medium, the cells were then incubated for 18 h or 60 h, to study short and long-term incorporation. For harvesting the cells the medium was discarded and the cultures were washed three times with 5 ml PBS (Gibco). The cells were first scraped off the culture dish with 2 ml PBS and methanol (1:1, v/v), then the culture dish was washed twice with 1 ml methanol. Then, 3 ml chloroform was added to the harvested cells combined with the methanolic washes. This mixture was homogenized with a Dounce grinder and centrifuged at $4000 \times g$ for 5 min. Then the chloroform layer was collected. This procedure was repeated twice. The combined chloroform phases were evaporated under nitrogen at 35°C and resuspended in 0.5 ml chloroform. Then 100 $\mu$l aliquots were spotted on thin layer chromatography (TLC) plates (silica gel $200 \times 200 \times 0.25$ mm, Merck, Darmstadt, Germany) together with phospholipid, triacylglycerol and free FA standards. A mixture of petrolether, diethylether and acetic acid (70:30:1, v/v) was used as mobile phase. After developing, the plates were automatically scanned for radioactivity using a TLC radio scanner, and the detected hot spots were identified by the standard substances. Subsequently, the silica gel spots containing the radioactive lipids were scraped off the TLC

plate and the lipids were extracted by chloroform/methanol [14]. They were then available for FA analysis by HPLC [15]. The HPLC runs were fractionated in 30 s intervals and the radioactivity in these fractions was determined by liquid scintillation counting.

*Determination of the FA composition of the cultures*

After the various incubation periods, the cells were washed three times with PBS, detatched from the dishes by trypsin treatment (0.03% trypsin in PBS, Gibco), centrifuged and resuspended in 1 ml PBS and then stored under an argon atmosphere at −20°C until use. Prior to the HPLC analysis, margarinic acid (17:0) was added as an internal standard. The samples were lyophilized and the residue was heated for 1 h at 80°C in 2 ml methanol containing 20% methanolic boron trifluoride complex ($BF_3$-$CH_3OH$, Merck). Subsequently, the so-formed FA methyl esters (FAMEs) were extracted using three times 1 ml hexane. The combined hexane phases were evaporated in a vacuum concentrator and the FAMEs in the residue were transformed into free FA by heating the sample with 1 ml of 1 M methanolic potassium hydroxide for 1 h at 80°C. After neutralizing the sample with hydrochloric acid, the FA were extracted with hexane as described before. Acetone, 1 ml containing 50 μl triethylamine (Sigma) and 2 mg 2-bromoacetophenone (Sigma), was added to the dried residue and the free FA were transformed to their phenacyl esters at 80°C during a 30 min incubation in a water bath (modified after [16]).

A volume of 20 μl of the phenacyl ester mixture was injected directly into the HPLC unit (HPLC Pump Model 480, variable wavelength UV monitor, 250 × 4.6 mm (5 μm) column ODS-Hypersil, Gynkotek Germering, München, Germany). Peaks were identified by comparison with the retention times of standard phenacyl esters and verification was accomplished by co-injection with standards. A solvent system was established that was based on the use of a polarity gradient. This system consisted of several steps: (i) elution with acetonitrile/methanol/water (4:6:1, v/v) for 5 min; (ii) between the 5th and the 45th min 15% of the mobile phase was substituted continuously by water and methanol (1:1, v/v) and (iii) within the next 20 min the solvent system changed completely to methanol and water (1:1, v/v). These changes to the mobile phase were made without interruption of the flow. The

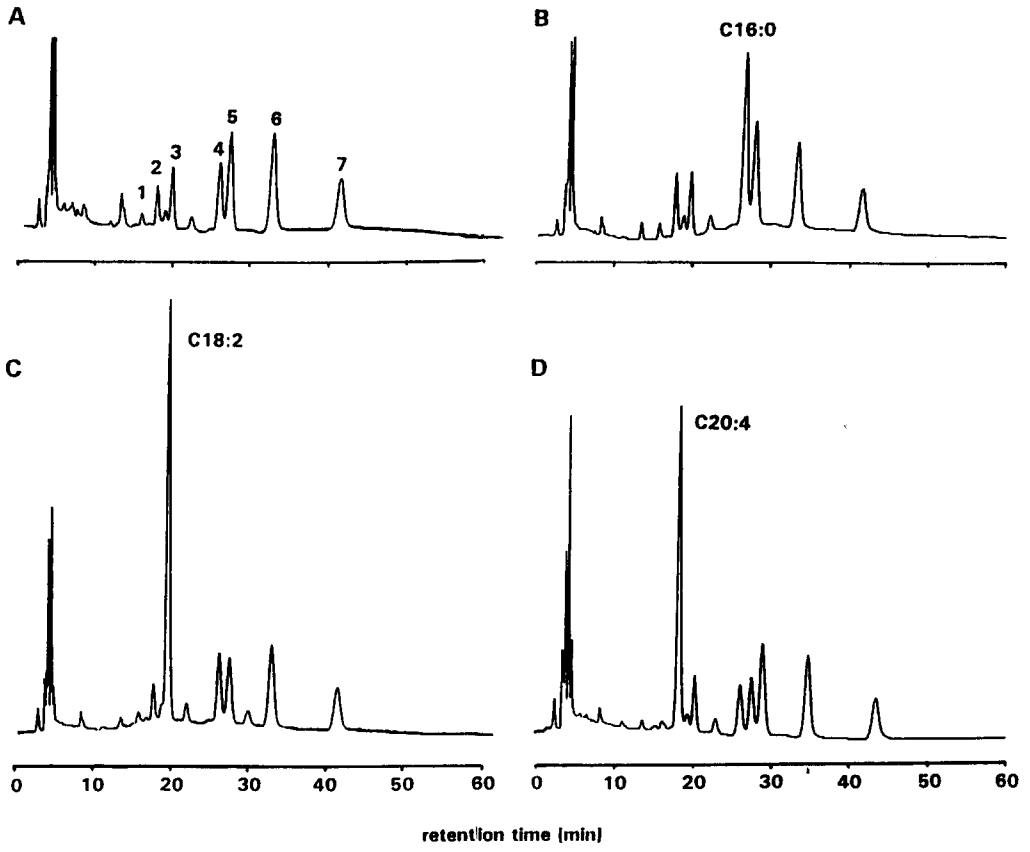


Fig. 1. Separation of fatty acids from the total lipid fraction from myotube cultures. (A) Control. The six major fatty acids 1–5 and 7 are indicated as determined by standards (see also Fig. 2A) (1): 22:6, (2): 20:4, (3) 18:2, (4) 16:0, (5) 18:1, (6) margarinic acid, 17:0; (7) 18:0.; (B–D) Same separation after incubation of cultures for 16–18 h with different fatty acids; B, 16:0; C, 18:2; D, 20:4.

flow-rate was constant at 1 ml/min; the eluted phenacyl esters were detected at 258 nm.

*Electrophysiology*

After the myotubes were detached from the bottom of the culture dish with 0.03% trypsin-EDTA (L 2133, Biochrom) they were centrifuged and resuspended in culture medium where they formed spherical syncytia, i.e. myoballs (diameter 40 to 70 $\mu$m) within 6 h. The floating myoballs were transferred into a hydrophobic experimental dish filled with 'external solution' containing (in mM) 140 NaCl; 3.5 KCl; 1.0 $CaCl_2$; 1.4 $MgCl_2$; 10 Hepes (pH 7.4). For whole-cell recording the cells were sucked to patch pipettes pulled from haematokrit capillaries with tip diameters of about 5 $\mu$m, (resistance 300 to 500 k$\Omega$). The pipettes were filled with 'internal solution' containing (in mM) 140 CsCl; 1.4 $MgCl_2$; 10 EGTA; 10 Hepes. The recordings were made with a home-made amplifier. For the investigation of the voltage dependence of inactivation ($h_\infty$ curves) a cyclic pulse program was applied consisting of a 100-ms conditioning pulse to $-135$ mV, followed by a 100-ms or alternatively 8-ms prepulse to a variable potential, and a constant test pulse to $-20$ mV [17]. For the investigation of the voltage dependence of activation a cyclic pulse program was applied with each cycle consisting of a constant 100-ms prepulse to $-135$ mV and an 8-ms test pulse that was varied from $-65$ mV to $+31$ mV in 4-mV steps. The current transients were digitized at 40 kHz. All experiments were performed at room temperature. For data reduction, Boltzmann distributions were fitted to the $h_\infty$ curves and the inflection point and slope of each of these curves were extracted from the analysis. The time constants of activation and inactivation, $\tau_m$ and $\tau_h$, were determined from the sodium current transients as described in Ref. 12.

## Results

*Modification of the FA composition of myotube cultures*

HPLC revealed the 6 major fatty acids 16:0, 18:0, 18:1, 18:2, 20:4 and 22:6, representing more than 90% of the total FA content of the myotube cultures (Fig. 1A, Table I). Changing the standard external medium to serum-free medium supplemented with 1.44% BSA and 0.4% ethanol (control condition) had no significant influence on the FA composition and the viability of the myotube cultures (Table I, line 2). When the medium was enriched with a specific FA, this same FA appeared in significant amounts in the FA spectrum of the culture after 16–18 h of incubation (Fig. 1, Table I). The greatest modification (with respect to control) of the FA pattern was a 7-fold increase of arachidonic acid (Fig. 1D, Table I). A similarly drastic change occurred with 22:6 under the same incubation conditions as used for 20:4 (not shown). Compared to linoleic acid, the cultures did not well tolerate the presence of approximately 0.5 $\mu$M free (total concentration: 0.4 mM) 20:4 or 22:6, since the two latter FA led to granulation of the cells within 2–4 h. Under this condition the myotubes had partially detached from the dish after 16–18 h. After a reduction to a calculated free concentration of about 0.15 $\mu$M FA (total concentration: 0.2 mM), the cells showed no visible cytotoxic effects and looked comparable to those treated with 0.4 mM linoleic acid corresponding to a free concentration of 18:2 of 0.5 $\mu$M.

The incorporation of externally applied FA into the cellular lipids of the muscle cultures was dependent on the incubation time. The application of 18:2 to a culture did not lead to significant changes within the first 4 h. After an 18-h treatment a 6 to 7-fold increase of incorporated linoleic acid was seen in myotube cultures and further incubation up to 72 h led to a

TABLE I

*Fatty acid composition of muscle cultures in dependence of externally applied medium*

The signals of the six major fatty acids were added, defined as 100% and the relative amount of each given in %. Line 1: cultures in standard culture medium. Line 2: control cultures in serum-free medium enriched with 1.44% BSA and 0.4% ethanol for 16 h. Lines 3–5: media enriched with specific fatty acids and corresponding amounts of BSA and ethanol. *n*: number of independent experiments.

| Fatty acid | 16:0 | 18:0 | 18:1 | 18:2 | 20:4 | 22:6 | *n* |
|---|---|---|---|---|---|---|---|
| Muscle cultures standard medium | 23.1±4.5 | 32.5±11.8 | 24.3±9.9 | 9.1±4.3 | 6.5± 3.5 | 4.5±3.1 | 10 |
| Muscle cultures serum-free medium | 21.4±6.0 | 26.4± 7.6 | 25.1±8.2 | 9.6±2.5 | 8.2± 2.7 | 9.6±5.3 | 6 |
| Serum free medium +400 $\mu$M 16:0 | 39.9 | 14.7 | 23.9 | 9.9 | 9.4 | 2.1 | 1 |
| Serum free medium +400 $\mu$M 18:2 | 9.0±2.2 | 12.8± 1.7 | 13.2±2.0 | 58.0±4.8 | 4.8± 0.6 | 2.5±1.6 | 4 |
| Serum free medium +200 $\mu$M 20:4 | 10.3±3.3 | 12.6± 4.6 | 18.3±5.2 | 7.3±1.1 | 52.3±11.0 | 2.2±1.3 | 4 |

10–11-fold increase compared to the controls. Similar results of long-term incubation were obtained with the other polyunsaturated FA, 20:4 and 22:6 (not shown).

*Incorporation of externally applied FA into cellular phospholipids*

Changes of the total lipid composition of cells will not automatically lead to a changed lipid composition of the sarcolemma and the internal membrane systems since the externally applied FA may be incorporated into triacylglycerols. In one experimental series we separated phospholipids and triacylglycerols by thin-layer chromatography after incubating the cultures with $^{14}$C-labelled linoleic acid under the same conditions as described above. After 18 h a fraction of 54% of the total incorporated [$^{14}$C]18:2 was found in the phospholipid fraction whereas after 60 h a smaller fraction of only 32% was detected as phospholipid-bound. After 18 h nearly the total amount of $^{14}$C was concentrated in the 18:2 peak (Fig. 2C). The experiment also showed that an incubation with 18:2 results in a FA distribution in the total lipid fraction (Fig. 1C, Table I) which is very similar to that of the phospholipid fraction (Fig. 2B).

*Effects of modified FA compositions on muscular sodium currents*

Sodium currents were recorded from myoballs in the whole-cell configuration each in a control culture and in a culture which had been treated with the FA 18:2 or 20:4 for 16–18 h. After preincubation the myotubes were detached from the culture dishes as described and all cells kept in serum free medium at 37°C for further 2–4 h. All tested myoballs showed inward going sodium currents with an average maximum amplitude of about 20 nA (Table II). Fig. 3A shows a typical set of sodium currents induced by depolarization from a holding potential of −85 mV to potentials between −65 and +31 mV. The current-voltage relation is shown in Fig. 3C. The voltage-dependence of inactivation ($h_\infty$ curves) was not significantly changed in cultures which had been treated with any of the FA. Fig. 3D illustrates the current-voltage relation ($h_\infty$ curve) of the transients of Fig. 3B. Boltz-

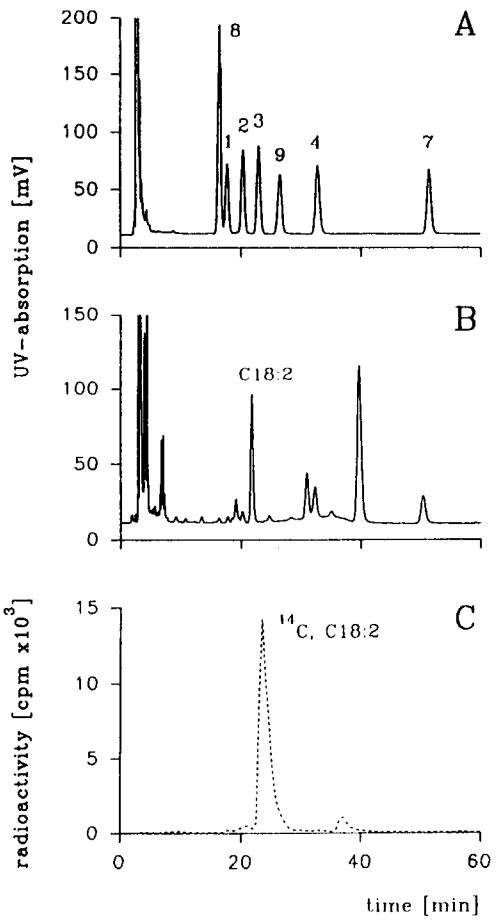


Fig. 2. Separation of fatty acids from the phospholipid fraction of a myotube culture after incorporation of [$^{14}$C]linoleic acid (18:2). (A) Standard run containing from left to right: (8) 18:3; (1) 22:6, (2): 20:4, (3) 18:2, (9) 20:3; (4) 16:0, (7) 18:0; corresponding peaks have the same denomination as in Fig. 1; (B) chromatogram showing the fatty acids from the phospholipid fraction of a muscle culture which was for 18 h incubated with [$^{14}$C]linoleic acid; (C) distribution of the incorporated $^{14}$C-radioactivity as determined by scintillation counting of 50 fractions obtained from the same HPLC run.

TABLE II

*Parameters of $Na^+$ currents recorded from myoballs, which had been incubated with linoleic acid or arachidonic acid*

Optimum conditions for fatty acid integration into phospholipids were applied. Each fatty acid treated groop was compared to a control group of non-treated cells from the same culture. $I_{max}$ is the $Na^+$ current amplitude induced by a voltage pulse to −20 mV after a prepotential of 100 ms of −135 mV. Holding potential: −85 mV. $\tau_m$ and $\tau_h$ are the Hodgkin–Huxley parameters for activation ($\tau_m$) and inactivation ($\tau_h$) of single $Na^+$ current transients. Both parameters determined from current traces at −20 mV. $U_h$ and $V_h$ represent the characteristics of the voltage-dependence of inactivation of the $Na^+$ currents. A Boltzmann curve was fitted to the data. $U_h$: inflection point of the curve (mV); $V_h$: inverse slope of the curve at $U$ (mV).

| Incubation | $I_{max}$ (nA) | Kinetic parameters | | $h_\infty$ curve parameters | | $n$ |
|---|---|---|---|---|---|---|
| | | $\tau_m$ (ms) | $\tau_h$ (ms) | $U_h$ (mV) | $V_h$ (mV) | |
| Control | 24.0± 8.5 | 0.38±0.05 | 1.03±0.10 | −77.5±3.9 | 7.27±0.69 | 9 |
| 400 μM 18:2 | 22.9±11.7 | 0.45±0.05 | 1.16±0.10 | −75.3±2.4 | 7.17±0.33 | 7 |
| Control | 17.2± 8.9 | 0.60±0.08 | 1.34±0.35 | −73.8±2.4 | 6.80±0.93 | 7 |
| 200 μM 20:4 | 22.5±15.1 | 0.54±0.04 | 1.56±0.23 | −76.9±2.2 | 7.57±0.58 | 9 |

mann distributions, fitted to many of these curves resulted in average inflection points at $-75.3 \pm 2.4$ mV for incubation with 18:2 and $-76.9$ mV for incubation with 20:4. The controls yielded $-77.5 \pm 3.9$ mV and $-73.8 \pm 2.4$ mV, respectively, i.e., the values with modified cultures were not significantly different. Means $\pm$ S.D. are given in each case (Table II). The kinetic parameters $\tau_m$ and $\tau_h$ were also not significantly different for FA-treated cultures and controls (Table II).

## Discussion

Our results demonstrate the possibility of influencing the membrane composition of human muscle cells considerably by specific external addition of FA to the culture medium. The use of albumin as a buffer for FA resulted in a low free FA concentration [13,18] and created a high storage capacity for FA in the culture medium. These two conditions led to a continuous supply of specific FA and avoided cytotoxic effects of high free FA concentrations.

The key reaction of the modification of the membrane lipids is the uptake of externally applied FA into the phospholipid fraction. Hagve and Sprecher [19] found about 90% of the applied FA incorporated into triacylglycerols when they incubated cardiac myocytes in experiments lasting 1 to 2 h. Using our protocol, more than 50% of the incorporated linoleic acid was found in the phospholipid fraction after 18 h. This result is very similar to that obtained by McGee [20] with the neuronal cell line NG108–15. We suggest that the observed changes in the phospholipid fraction of the muscle cultures reflect also changes in the surface membrane composition of the cells. However, we cannot exclude the possibility that considerable changes occur in internal membrane systems.

Changes in the FA of the composition membrane may have different consequences: first, an enrichment with certain FA that causes changes in membrane properties can be compensated by the cell with changes in the phospholipid pattern [21]. Nevertheless, there are probably changes in the physical, more unspecific membrane parameters like fluidity or thickness that

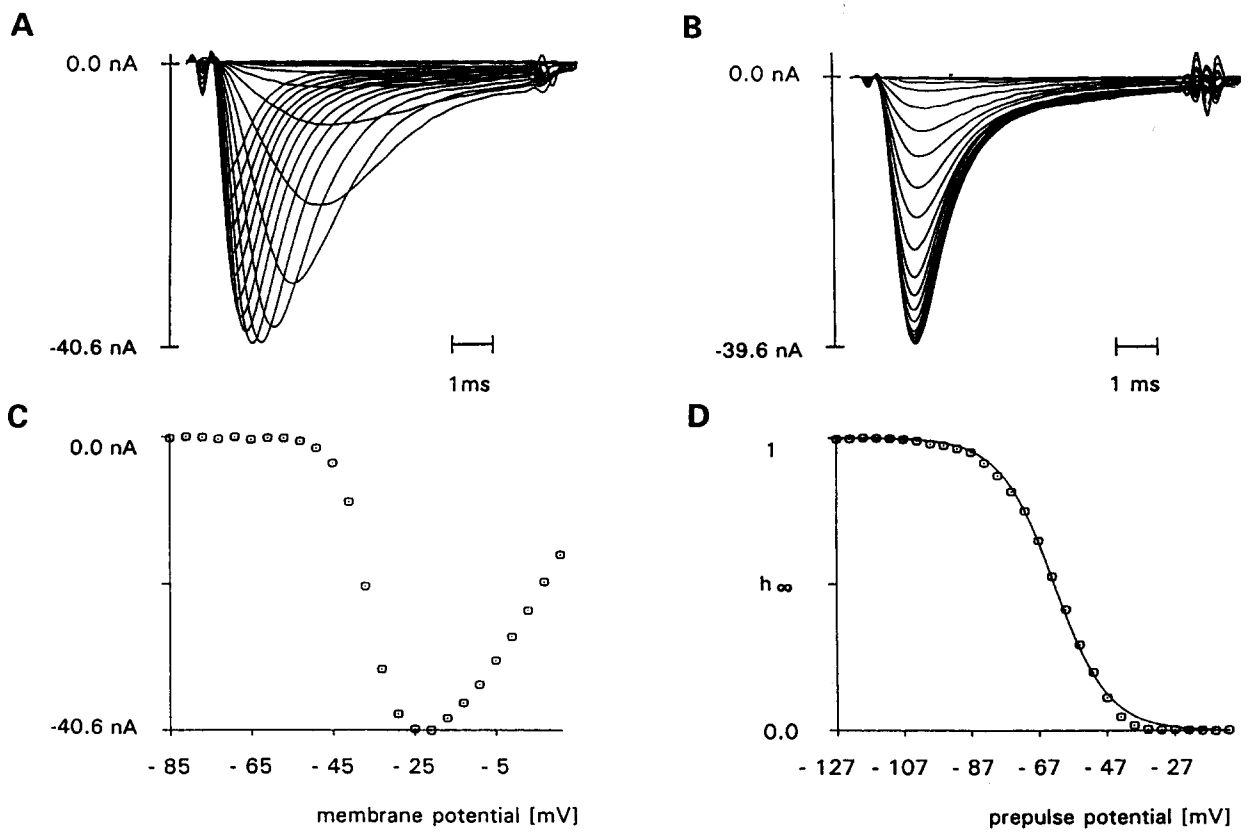


Fig. 3. Sodium currents recorded from a human myoball. The culture was not incubated with fatty acids. (A) Family of sodium inward currents, induced by square voltage pulses going from a holding potential of $-85$ mV to test potentials varying between $-85$ and $+11$ mV in 4 mV steps. (B) Family of sodium inward currents, induced by square voltage pulses of 8 ms duration to a test potential of $-20$ mV. Each test pulse was preceded by a conditioning prepulse of 100 ms duration which varied between $-127$ and $-11$ mV in 4 mV increments. (C) Current–voltage relation of the $Na^+$ currents shown in A; current maxima are plotted against the test potential. (D) Voltage dependence of inactivation of the sodium currents ($h_\infty$ curve). The maxima of the current traces shown in B were plotted against the prepotential. A Boltzmann curve was fitted to the data points.

might have effects on membrane proteins [22]. Second, the enrichment of certain phospholipids carrying specific FA may be important for direct interactions between lipids and membrane proteins [3,23]. Third, the enrichment of the membrane with arachidonic acid and poly-unsaturated FA may have consequences for the release of metabolites serving as second messengers [10,24]. This may be of importance for the study of physiological responses in tissue cultures.

Comparing our results of the FA content in muscle cultures with results obtained with muscle tissue [15,25] we conclude that cultures have lower levels of poly-unsaturated FA. If this is a general feature of cell cultures, many investigations of the physiological effects of metabolites of arachidonic acid may be influenced by a decreased amount of arachidonic acid.

Our results did not indicate significant effects of the changed membrane FA composition on the muscular sodium currents. In other studies some electrophysiological effects have been demonstrated. Love et al. [8] described a prolongation of the rise time of the action potential after incubation of the neuronal cell line NG108-15 with poly-unsaturated FA. Binding studies with saxitoxin suggested a reduction of the $Na^+$ channel densitiy as explanation of this observation. In the same cell line, effects on the delayed rectifier $K^+$ channel induced by the enrichment of arachidonic acid in the phospholipidfraction were shown [26]. This modification of the phospholipid-bound FA pattern could reduce the cellular excitability.

Our modifications of the membrane lipids did not alter the $Na^+$ current characteristics which may be explained with a low or a non-interactive influence of 18:2 and 20:4 containing phospholipids on $Na^+$ channels. Furthermore, it is possible that a changed membrane composition has no effets on the core of lipids which are more tightly bound to the $Na^+$ channels [27].

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**References**

1 Morson, L.A. and Clandinin, M.T. (1985) Nutr. Res. 5, 1113–1119.
2 Schwarz, S.M., Ling, S., Hostetler, Draper, J.P. and Watkins, J.B. (1984) Gastroenterology 86, 1544–1551.
3 Deuticke, B. and Haest, C.W.M. (1987) Annu. Rev. Physiol. 49, 221–235.
4 Rabinowitz, J.L. and Cossu, G. (1986) Biochim. Biophys. Acta 879, 394–398.
5 Marx, A., Szymanska, G., Melzner, I. and Rüdel, R. (1988) Muscle Nerve 11, 471–477.
6 Rebello, T. and Watts, D.C. (1985) J. Neurochem. 45, 257–267.
7 Seewald, M., Eichinger, H.M. and Iaizzo, P.A. (1991) Acta Anaesthesiol. Scand. 35, 380–386.
8 Love, J.A., Saum. W.R. and McGee Jr., R. (1985) Cell. Mol. Neurobiol. 5, 333–352.
9 Berridge, M.J. (1984) Biochem. J. 220, 345–360.
10 Rana, R.S. and Hokin, L.E. (1990) Physiol. Rev. 70, 115–164.
11 Boldin, S., Jäger, U., Ruppersberg, J.P., Pentz, S. and Rüdel, R. (1987) Pflügers Arch. 409, 462–467.
12 Pröbstle, T., Rüdel, R. and Ruppersberg, J.P. (1988) Pflügers Arch. 412, 264–269.
13 Spector, A.A., Fletcher, J.E. and Ashbrook, J.D. (1971) Biochemistry 10, 3229–3232.
14 Folch, J., Lees, M., Sloane, S. and Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509.
15 Brinkmeier, H., Siebert-Gulle, K., Mutz, J.V. and Rüdel, R. (1990) J. Neurol. Sci. 98, 225.
16 Borch, R.F. (1975) Anal. Chem. 47, 2437–2439.
17 Fakler, B., Spittelmeister, B., Ruppersberg, J.P. and Rüdel, R. (1990) Pflügers Arch. 415, 693–700.
18 DeGrella, R.F. and Light, R.J. (1980) J. Biol. Chem. 255, 9731–9738.
19 Hagve, T.-A. and Sprecher, H. (1989) Biochim. Biophys. Acta 1001, 338–344.
20 McGee, R. (1981) Biochim. Biophys Acta 663, 314–328.
21 Williams, R.M. and Chapman, D. (1970) Progr. Chem. Fats Other Lipids 11, 33–36.
22 Zakim, D. and Vessey, D.A. (1980) In Principle of Metabolic Control in Mammalian Systems (Herman, R.H., eds.), pp. 337–371, Plenum Press, New York.
23 Atwood, D., Graham, A.B. and Wood, G.C. (1971) Biochem. J. 123, 875–882.
24 Needleman, P., Turk, J., Jakschik, B.A., Morrison, A.R. and Lefkowith, J.B. (1986) Annu. Rev. Biochem. 55, 69–102.
25 Fiehn, W., Peter, J.B., Seiler, D. and Kuhn, E. (1972) In Structure and function of normal and diseased muscle and peripheral nerve, Polish Med. Publ. Proc., 205–209.
26 McGee Jr., R., Sansom, M.S.P. and Usherwood, P.N.R. (1988) J. Membr. Biol. 102, 21–34.
27 Levinson, S.R., Duch, D.S., Urban, B.W. and Recio-Pinto, E.(1986) Ann. NY Acad. Sci. 479, 162–178.