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Changes in morphology of human skin fibroblasts induced by local anaesthetics: role of actomyosin contraction

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Abstract

Local anaesthetics block action potentials in the membranes of excitable cells but their effects on non-excitable cells are less well known. Some local anaesthetics are applied directly onto the skin, and for this reason the effect of procaine (*p*-aminobenzoic acid diethylamino-etyl ester hydrochloride) and tetracaine (4-[butylamino]benzoic acid 2-[dimethylamino]ethyl ester) upon the morphology and cytoskeleton organisation of human skin fibroblasts was investigated. The time lapse video recording of fibroblasts cultured in serum-enriched medium revealed that the cells rapidly change shape after the addition of the anaesthetic. These effects were fully reversible. The microscopic observations were confirmed by quantitative analysis of projected cell area and cell shape parameters. Local anaesthetics significantly changed the actin cytoskeleton organisation, inducing total disappearance of stress fibres. Serum-starvation or myosin light chain kinase inhibitors, KT 5926 inhibitor (8*R**,9*S**,11*S**)-(−)-9-hydroxy-9-methoxycarbonyl-8-methyl-14-*n*-propoxy-2,3,9,10-tetrahydro-8,11-epoxy,1*H*,8*H*,11*H*-2,7*b*,11*a*-triazadibenzo[*a*,*g*]cycloocta[*cde*]trinden-1-one or wortmannin, which induce the 'relaxed' morphology of the cells, prevent both the anaesthetic-induced changes in cell shape and the disassembly of stress fibres. Together, the observations suggest that local anaesthetics affect the actomyosin system, inducing contraction.

Keywords: Local anaesthetic; Cell contraction; Actin cytoskeleton; Fibroblast, human skin

1. Introduction

Local anaesthetics are commonly used to provide regional analgesia. Anaesthetic agents may be injected near a peripheral nerve, around the spinal cord, or applied topically onto the skin. Despite the years of constant clinical use of local anaesthetics, the molecular mechanism of their action on the cells is not fully understood.

The effects of local anaesthetics have been investigated with respect to both membrane lipids and membrane proteins. It is generally accepted that they interact with the polar head groups of the membrane phospholipids (Barghouthi and Eftink, 1993; Barghouthi et al., 1993) and increase membrane fluidity (Shimooka et al., 1992). In neurones, local anaesthetics act through inhibition of voltage-gated Na^+ channels (Butterworth and Strichartz, 1990; Ragsdale et al., 1994). Recent studies have shown that other cell functions are also affected by local anaesthetics. They act on membrane enzymes, such as phospholipases (Higuchi et al., 1983) and protein kinases (Slater et al., 1993; Oda et al., 1995), α-subunits of G-proteins (Hagelüken et al., 1994) and block ligand-dependent ion channels (Cuevas and Adams, 1994; Fan and Weight, 1994; Li et al., 1995). These agents are also known to alter calcium homeostasis by inhibiting the ryanodine-receptor/Ca^{2+} release channel in endoplasmic reticulum (McPherson and Campbell, 1993). It is not surprising that local anaesthetics exert a variety of effects on both excitable and non-excitable cells. It was reported that they alter cell shape and morphology (Nicolson et al., 1976; Nishiguchi et al., 1995a,b), adhesion (Ohsaka et al., 1990), migration (Moudgil et al., 1977; Hammer et al., 1985), phagocytosis (Okuno et al., 1996) and proliferation (Martinsson et al., 1993). They are known to change mitochondrial function (Tarba and Cracium, 1990), lysosomal enzyme release (Cederholm et al., 1994) and intracellular transport (Garcia-Sierra and Frixione, 1993).

Although local anaesthetics are often applied directly onto the skin, to our knowledge no reports have appeared

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about the effect of anaesthetics on human skin cells. Therefore this study focused on the effect of some of them on the morphology and cytoskeleton organisation of human skin fibroblasts in culture.

2. Materials and methods

2.1. Materials

Dulbecco's Modified Eagle Medium (DMEM) and foetal calf serum were obtained from Gibco (San Diego, CA, USA). Procaine hydrochloride (*p*-aminobenzoic acid diethylamino-etyl ester hydrochloride), tetracaine hydrochloride (4-[butylamino]benzoic acid 2-[dimethylamino]ethyl ester), wortmannin, phalloidin conjugated tetramethylrhodamine B isothiocyanate (TRITC-phalloidin), trypsin, paraformaldehyde, triton X-100, EGTA, 2-[*N*-morpholino] ethanesulfonic acid (MES), Tris were obtained from Sigma St. Louis, USA. The following chemicals were purchased from the sources indicated: KT 5926 inhibitor (8*R*∗,9*S*∗,11*S*∗)-(−)-9-hydroxy-9-methoxycarbonyl-8-methyl-14-*n*-propoxy-2,3,9,10-tetrahydro-8,11-epoxy,1*H*,8*H*,11*H*-2,7*b*,11*a*-triazadibenzo[*a*,*g*]cycloocta[*cde*]trinden-1-one (Calbiochem, San Diego, CA, USA), Coomasie brillant blue R-250 (Merck, Darmstadt, Germany), penicillin and streptomycin (Polfa-Tarchomin, Poland), phosphate-buffered saline (PBS) (Wytwórnia Surowic i Szczepionek, Lublin, Poland). Culture flasks and 12-well plates were purchased from Corning NY, UK.

2.2. Cell culture

Human skin fibroblasts were obtained from skin grafts of adult, healthy donors and were cultured in DMEM supplemented with 100 i.U./ml penicillin and 10 μg/ml streptomycin in the presence of 10% foetal calf serum. For the experiments, cells from 5 to 15 passages after isolation were used. The cells were plated at an initial cell density of $7 \times 10^3/\text{cm}^2$ in 12-well plates with or without glass coverslips inserted into each well, 24 h before each experiment. The cells were cultured in a humidified atmosphere with 5% CO_2 at 37°C. For some experiments, cells were starved in serum-free DMEM.

2.3. Anaesthetic treatment

Procaine hydrochloride and tetracaine hydrochloride were diluted in the culture medium from stock solution in water to the working concentrations of 2.5 mM and 0.1 mM respectively. In order to study the inhibition of anaesthetic-induced changes in cell shape, anaesthetic-containing medium was added after 24-h preincubation of cells in medium containing 1 μM KT5926 or 1 μM wortmannin.

2.4. Time-lapse video films

Cells growing on glass coverslips in Rose's perfusion chambers were observed with an inverted Olympus IMT-2 microscope using phase contrast optics, at a temperature of 37°C. The cell images were recorded with a Hitachi KP-161 CCD camera attached to a PC computer, and digitized using the VCR 099 program written by Dr. P. Jochym. Frames were collected at 30-s intervals for 3 h.

2.5. Measurements of cell size and shape

After 30 min, 1 h, 3 h, or 24 h incubation of fibroblasts in DMEM supplemented with 10% foetal calf serum with or without anaesthetics, the cultures were rinsed twice with PBS and the cells were fixed with 3.7% formaldehyde in PBS and stained with Coomasie brillant blue R-250 according to the method described by Penn (1980).

Stained fibroblasts were observed in the inverted Olympus IMT-2 microscope with bright-field optics. The cell images were recorded with the Hitachi KP-161 CCD camera, digitized and processed with the CYTOCYCLE 2.0 program written by R. Tokarski. The projected surface area and shape parameters of the cells were analysed with the image analysis system after image binarization. To characterise quantitatively the anaesthetic-induced changes in shape and size of fibroblasts the following parameters were measured by computer-aided methods: (1) the surface area of cell projection [in μm^2]; (2) cell extension, (3) cell dispersion, and (4) cell elongation. The cell extension, dispersion, and elongation were defined and calculated as described by Dunn and Brown (1986) (cf. Wójciak-Stothard et al., 1995; Korohoda and Madeja, 1997).

2.6. Statistical analysis

At least 70 cells were analysed for each value measured. Mean and standard deviation for each parameter were calculated. Statistical significance was determined with the non-parametric Mann-Whitney U-test, with $P < 0.05$ considered significant.

2.7. Staining of actin cytoskeleton

For the visualisation of actin cytoskeleton cells were grown on glass coverslips. The cells were washed twice in tris-buffered saline (TBS: 20 mM Tris, 154 mM NaCl, 5 mM EGTA, 2 mM $MgCl_2$, pH 7.5) and fixed in 3.7% paraformaldehyde in cytoskeleton buffer (CB: 10 mM MES, 150 mM NaCl, 5 mM EGTA, 5 mM $MgCl_2$, 5 mM glucose, pH 6.1) for 15 min, and then washed 3 times with cytoskeleton buffer (CB) and permeabilized in 0.1% Triton X-100 in PBS for 1 min at room temperature. The preparations were washed twice with CB and stained with TRITC-phalloidin at a concentration of 500 ng/ml for 45 min and then washed 5 times with TBS.

2.8. Microphotography

Microphotographs were taken under a Leitz Orthoplan microscope with an epifluorescence and phase contrast optics, equipped with the Nikon FX-35DX camera or under an inverted Olympus IMT-2 microscope with bright-field optics equipped with the Olympus OM-4 Ti camera. High-sensitivity Kodak TMAX 3200 films or Fomapan 100 films were used.

3. Results

3.1. Effect of anaesthetics on human skin fibroblasts morphology

A time lapse video recording of human skin fibroblasts incubated in medium with foetal calf serum revealed that the cells changed shape after addition of the anaesthetic. As shown in Fig. 1, 10 min of incubation in the procaine-containing medium (2.5 mM) caused retraction of the cell tails which transformed to a few long retraction fibres. During the next 20 min the cells retracted their leading edge, became rounded, and started to produce the blebs. They formed numerous blebs for up to 1 h and then began to respread. The respreading was slow and resulted in a return to the normal morphology of fibroblasts after 24 h (data not shown).

To determine whether the procaine-induced shape changes observed in the individual fibroblasts were relevant to the whole population of the anaesthetic-treated cells quantitative analysis of the cell size was used. The average projected surface areas of fibroblasts cultured under various experimental conditions are shown in Fig. 2. After 30-min incubation in the procaine-containing medium the average projected cell area decreased by approximately 40% in comparison with that of the control cells. This effect was not so pronounced after 1 h and 3 h of incubation. Regardless of whether or not the culture medium was changed to the medium without anaesthetic no changes were observed in the measured surface area of the cells after the next 24 h of incubation. The fibroblasts incubated in the tetracaine-containing medium reacted similarly to the procaine-treated cells but rounded more strongly at the same time and started to respread considerably earlier (Table 1, Fig. 2).

3.2. Effect of anaesthetics on actin cytoskeleton of human skin fibroblasts

Examination of the actin cytoskeleton organisation by fluorescence microscopy revealed striking differences be-

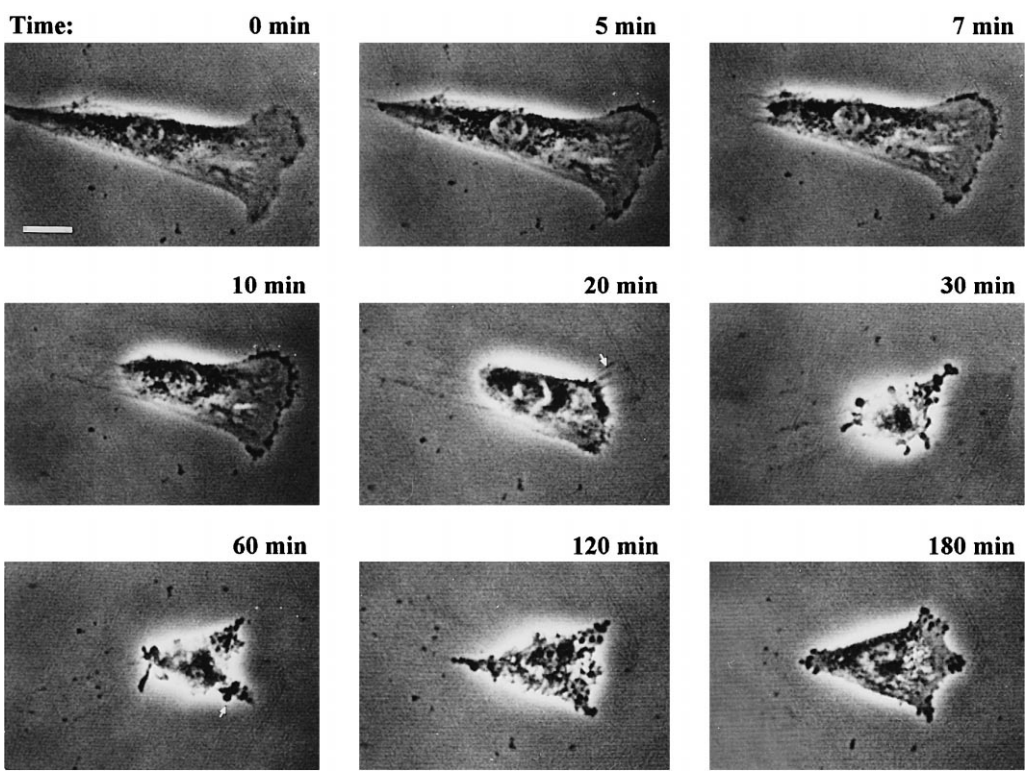

Fig. 1. Effects of procaine on the cell shape of human skin fibroblasts. Cells were grown on glass coverslips in a Rose's chamber in DMEM supplemented with 10% foetal calf serum. Time-lapse video films were recorded for 3 h after addition of 2.5 mM procaine. Arrows indicate retraction fibers formed 20 min and blebs formed 60 min after addition of procaine. Bar = 10 μm.

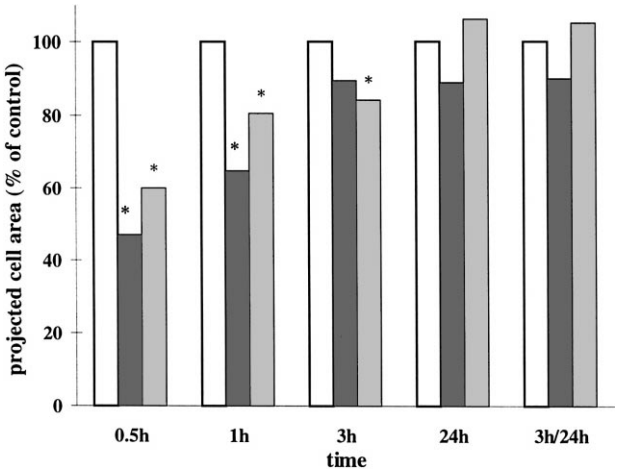

Fig. 2. Anaesthetics induced changes in projected area of skin fibroblasts. Cells were cultured in DMEM supplemented with 10% foetal calf serum (open columns) or in the same medium containing 0.1 mM tetracaine (dark grey columns) or 2.5 mM procaine (light grey columns) for 30 min, 1 h, 3 h, 24 h or 3 h in anesthetic and then for 24 h in control medium (3 h/24 h). The surface area of cell projection for each of the experimental conditions was determined and is presented as percentage of control. At least 150 cells were calculated for each experimental point. The results presented are the means of at least five separate experiments. The values significantly different from control are indicated by (*).

tween the anaesthetic-treated and the control cells. Human skin fibroblasts grown in the serum-containing medium displayed well developed bundles of F-actin (stress fibres), arranged in parallel to the long axis of the cell (Fig. 4A and Fig. 6A). Incubation of cells in the procaine-containing medium for 10 min induced a rapid disappearance of stress fibres and the reorganisation of the F-actin network to intensely fluorescent patches which were randomly distributed throughout the cytoplasm (Fig. 3A). After 30 min the cells were rounded and F-actin had accumulated around the nucleus as well as in blebs on cell surfaces (Fig. 3B and Fig. 4B). The cells treated with procaine for 1 h or longer expressed some stress fibres on the periphery and the pattern of F-actin distribution returned slowly to that in the control cells (Fig. 3C,D,E). The anaesthetic-induced effect on F-actin organisation appeared fully reversible after 24 h of incubation in the control medium (Fig. 3F). The time course of reappearance of stress fibres in the procaine-treated fibroblasts correlates well with the respreading of these cells (cf. Figs. 2 and 3).

3.3. Effects of serum starvation on anaesthetic-induced F-actin reorganisation

In contrast to the strong effects of the anaesthetics upon the morphology and the actin cytoskeleton of fibroblasts grown in medium supplemented with serum, procaine did not induce cell shape changes in the serum-starved fibroblasts in culture (Fig. 4D). Serum starvation caused a relaxation of the control cells and the pattern of F-actin distribution was altered, only a few thin bundles of F-actin being visible in their cytoplasm (cf. Fig. 4A and C). In contrast to the complete disruption of the microfilament organisation in the procaine-treated cells in the presence of serum (Fig. 4B), incubation of the serum-starved cells with procaine induced a significant increase in the number and thickness of actin bundles (Fig. 4D). Essentially identical results were obtained with tetracaine (data not shown).

3.4. Effects of myosin light chain kinase inhibitors on anaesthetic-induced changes in cell shape and actin cytoskeleton

To determine whether the procaine-induced shape changes were related to the actomyosin contraction two myosin light chain kinase inhibitors were used: KT5926 and wortmannin. KT5926 and wortmannin inhibit myosin light chain kinase phosphorylation of the myosin regulatory light chain, which in turn inhibits actomyosin contractility (Hosoya, 1994; Burridge and Chrzanowska-Wodnicka, 1996). Neither compound had any effect on the shape of cells cultured in the control medium

Table 1
Inhibition of anesthetic-induced changes in the shape of human skin fibroblasts by the myosin light chain kinase inhibitor KT5926

	Control	Procaine	Tetracaine	KT5926	Procaine +KT5926	Tetracaine +KT5926
surface area (μm^2)	5371 ± 298^{a}	3259 ± 140^{a}	2543 ± 140^{a}	6588 ± 378^{a}	5202 ± 305^{c}	$3352 \pm 168^{a,b,c}$
extension	2.5 ± 0.12^{a}	1.58 ± 0.06^{a}	1.89 ± 0.09^{a}	2.44 ± 0.09	$1.98 \pm 0.08^{a,b,c}$	2.48 ± 0.06^{c}
dispersion	0.49 ± 0.05^{a}	0.19 ± 0.02^{a}	0.19 ± 0.02^{a}	0.55 ± 0.05	$0.38 \pm 0.03^{b,c}$	$0.32 \pm 0.03^{a,b,c}$
elongation	2.02 ± 0.1^{a}	1.38 ± 0.06^{a}	1.7 ± 0.08^{a}	1.89 ± 0.09	$1.63 \pm 0.08^{a,c}$	2.16 ± 0.07^{c}

Cells were cultured in DMEM with 10% foetal calf serum with or without 1 μM KT5926 inhibitor for 24 h. Procaine or tetracaine was added for 30 min to the concentration of 2.5 mM or 0.1 mM respectively. The surface area of cell projection, cell extension, dispersion and elongation were determined. Values are means ± S.D. for at least 70 cells for each experiments. All values were compared to those for control untreated cells (values significantly different from control are indicated by [a]). We also compared cells treated with anaesthetics in the presence of inhibitor to cells treated with inhibitor alone (values significantly different from the value for KT5926-treated cells are indicated by [b]) and to cells treated with the given anaesthetic (values significantly different from the value for anaesthetic-treated cells are indicated by [c]).

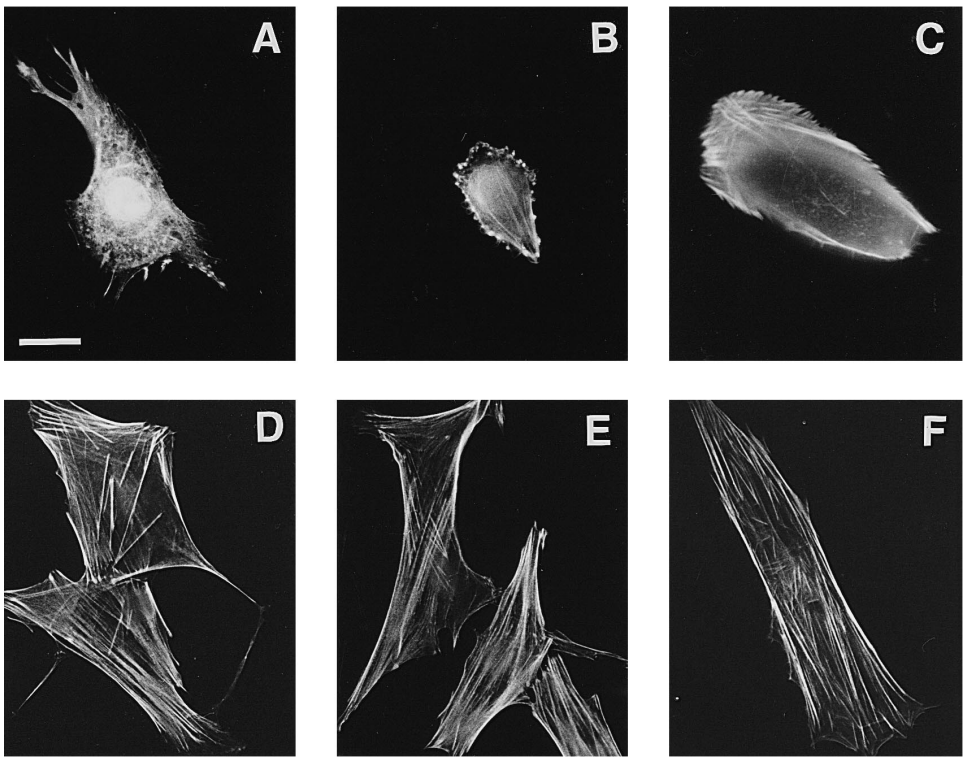

Fig. 3. Procaine induced disintegration of actin cytoskeleton in human skin fibroblasts. Cells were cultured on glass coverslips in DMEM supplemented with 10% foetal calf serum containing 2.5 mM procaine for 10 min (A), 30 min (B), 1 h (C), 3 h (D), 24 h (E) or for 3 h in 2.5 mM procaine and then for 24 h in control medium (F). F-actin was stained with TRITC-phalloidin. Note the depolymerization of actin stress fibres to strongly fluorescent patches around the nucleus (B) and reappearance of stress fibres after prolonged procaine treatment (D,E) or upon removal of anesthetic (F). Bar = 20 μm.

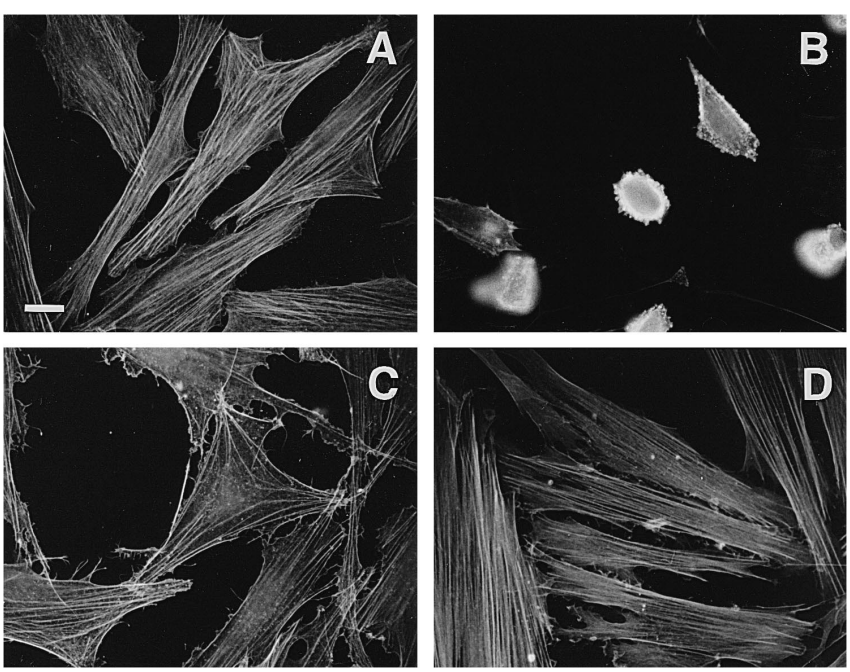

Fig. 4. Procaine induced stress fibres in serum-starved human skin fibroblasts. Cells were cultured in DMEM with (A,B) or without foetal calf serum (C,D) for 24 h. Cells were incubated in procaine-containing medium for 30 min (B,D). F-actin was stained with TRITC-phalloidin. Note the presence of stress fibres in cells treated with procaine in serum-free medium. Bar = 20 μm.

(Fig. 5A,D,G). Fibroblasts preincubated with KT5926 or wortmannin did not change their shape after 30 min of incubation with procaine (Fig. 5E,H) or tetracaine (Fig. 5F,I). Microscopic observations were confirmed by the quantitative analysis. The results are shown in Table 1. The anaesthetic-treated cells had their surface area decreased by approximately 40% in comparison with that of the control cells and also had significantly decreased extension, elongation, and dispersion parameters. KT5926 did not affect the shape parameters of cells incubated in the control medium, but slightly increased the surface area of cell projections. In fibroblasts pretreated with KT5926 procaine did not cause any changes in the surface area of cell projections. In the presence of tetracaine, fibroblasts preincubated with KT 5926 retracted but did so more weakly than non-preincubated cells. The shape parameters of preincubated cells decreased significantly less in the presence of both anaesthetics (Table 1).

Consistent results were obtained when the effect of the anaesthetics on the actin cytoskeleton was studied. Cells treated with myosin light chain kinase inhibitor had fewer numbers of F-actin bundles than did control fibroblasts (cf. Fig. 6A,D). In the presence of the anaesthetics the actin cytoskeleton of KT5926-pretreated cells did not change significantly (cf. Fig. 6A and E,F), in contrast to the complete disorganisation of the actin cytoskeleton ob-

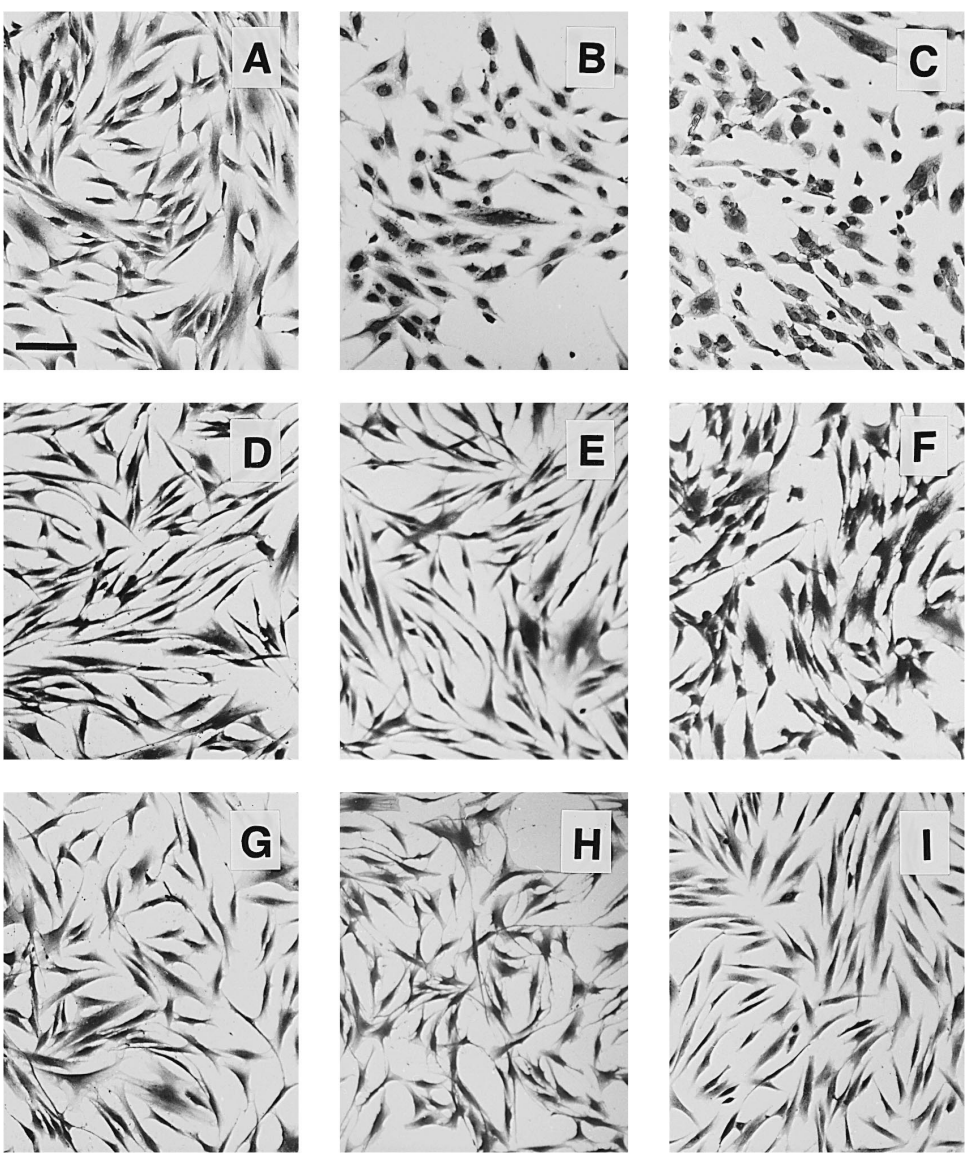

Fig. 5. Inhibition of anesthetic-induced changes in the shape of human skin fibroblasts by the myosin light chain kinase inhibitors KT5926 and wortmannin. Cells were cultured in DMEM with 10% foetal calf serum for 24 h without myosin light chain kinase inhibitors (A,B,C), with 1 μM KT5926 inhibitor (D,E,F), or 1 μM wortmannin (G,H,I). 2.5 mM procaine (B,E,H) or 0.1 mM tetracaine (C,F,I) was present in the culture medium for 30 min. Cells were stained with Coomasie BB and photographed with an Olympus IMT inverted microscope. The morphology of cells preincubated with KT5926 (E,F) or wortmannin (H,I) after addition of anesthetic did not differ from that of the control (A). Bar = 200 μm.

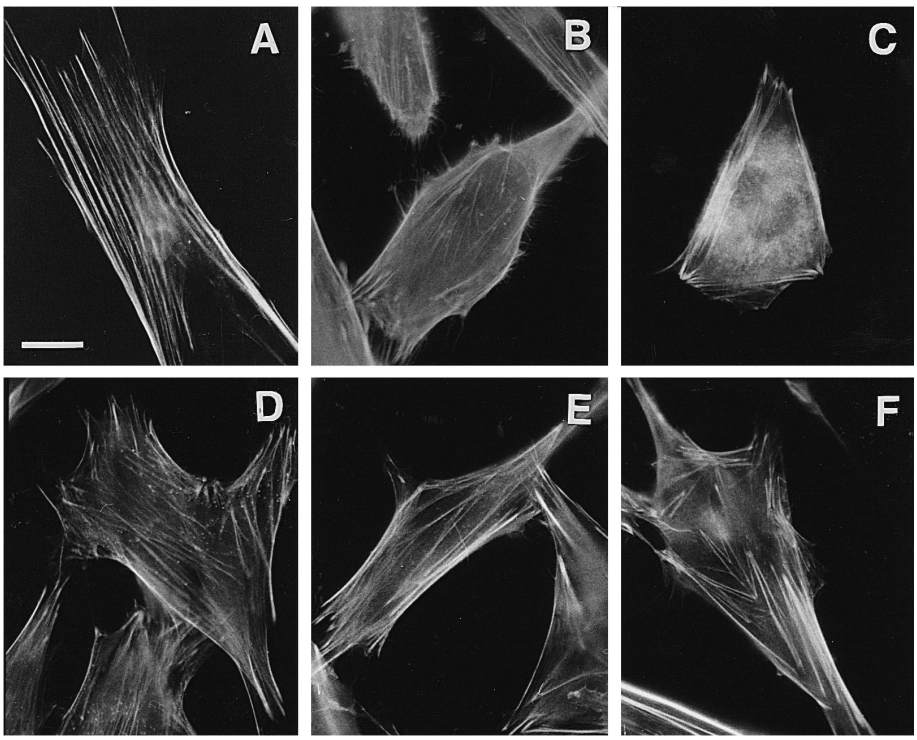

Fig. 6. Inhibition of anesthetic-induced changes in the actin cytoskeleton of human skin fibroblasts by the myosin light chain kinase inhibitor KT5926. Cells were cultured on glass coverslips in DMEM with foetal calf serum without (A,B,C) or with (D,E,F) 1 μM KT5926 inhibitor for 24 h. Procaine (B,E) or tetracaine (C,F) was added for 30 min to the concentration of 2.5 mM or 0.1 mM respectively. F-actin was stained with TRITC-phalloidin. Note the presence of F-actin bundles in cells preincubated with KT5926 and treated with anaesthetics (E,F) Bar = 20 μm.

served in the control fibroblasts after addition of the anaesthetic (Fig. 6B, C).

4. Discussion

It has been demonstrated that the local anaesthetics, procaine and tetracaine, used at concentrations of 2.5 mM and 0.1 mM, respectively, cause extensive rounding of human skin fibroblasts in culture. A number of local anaesthetic formulations (gels or creams) are available for topical anaesthesia in the following doses: procaine 0.5–1% (150–300 mM) and tetracaine 0.25–1% (80–300 mM) (Strichartz and Berde, 1994). Local anaesthetics easily cross the plasma membranes at physiological pH (Dean et al., 1984) so it can be assumed that the skin cells in vivo are influenced by even higher doses of anaesthetics than the doses used in experiments presented here. Transient rounding in culture was previously observed by others in amoebae in the presence of procaine (Hülsmann et al., 1976; Stockem and Kłopocka, 1988). Changes in cell shape as an effect of local anaesthetics were also reported for human red blood cells (Nishiguchi et al., 1995a). The present microscopic observations were confirmed by quantitative analysis of cell size and shape. The average values for projected cell area and cell shape parameters for the control cells are in agreement with those found in previous studies on 3T3 cells, chicken embryo fibroblasts and mouse embryo fibroblasts (Middleton et al., 1989; Pletjushkina et al., 1994). These values decreased greatly after addition of anaesthetics (Table 1, Fig. 2).

TRITC-phalloidin staining of the anaesthetic-treated cells revealed that the cell shape changes were accompanied by disintegration of the actin cytoskeleton (Fig. 3). The disappearance of actin filament bundles and a decrease in F-actin content after the addition of local anaesthetics were also reported for other cell types: 3T3 fibroblasts, Chinese hamster ovary cells and corneal epithelial cells (Nicolson et al., 1976; Heacock et al., 1984; Dass et al., 1988) or in human skin fibroblasts for benzamide, the other type of anaesthetics (Korohoda et al., 1994). The bundles of F-actin in fibroblasts cultured on solid substrates usually contain, among other proteins, myosin II. These contractile structures are referred to as stress fibres (Small, 1982; Byers et al., 1984). The assembly and disassembly of actin and myosin II containing structures are precisely regulated in living cells, leading to changes in cell shape and cell movement. There are probably two different mechanisms responsible for restructuring actin stress fibres (Kolega and Taylor, 1993). One mechanism involves not contraction but the disassembly of the constituent actin and myosin filaments. Examples of this process were the disappearance of stress fibres in the serum-starved cells (Giuliano et al., 1992), in the cells

treated with kinases inhibitors (Mobley et al., 1994; Volberg et al., 1994; Chrzanowska-Wodnicka and Burridge, 1996) or in the mitotic cells in prophase (Sanger et al., 1990). The second mechanism involves reorganisation of stress fibres as a consequence of their isotonic contraction. Examples of such a pathway include serum and growth factor stimulation of quiescent cells (Giuliano et al., 1992) and tail retraction during amoeboid movement (Taylor and Condeelis, 1979). The same effect was observed in cells treated with cytochalasins and phosphatase inhibitors (Kolega et al., 1991 Hirano et al., 1992). The aim of the present study had been to discover whether local anaesthetics induced actin cytoskeleton reorganisation by stimulating contractility or disassembly of actin and myosin filaments. To discriminate between these two mechanisms the effect of agents that block contractility by inhibiting the myosin light chain kinase on the anaesthetic-induced changes of human skin fibroblasts morphology and cytoskeleton were investigated. Contractility of non-muscle cells is mainly regulated by the phosphorylation of the 20-kD myosin light chain catalysed by a myosin light chain kinase (Citi and Kendrick-Jones, 1986). Two different inhibitors of myosin light chain kinase were used: KT5926 and wortmannin. KT5926 is a relatively specific inhibitor of myosin light chain kinase. It has also been shown to have a much weaker inhibiting activity against Ca^{2+}/calmodulin-dependent kinase II (Nakanishi et al., 1992). The other inhibitor of myosin light chain kinase—wortmannin—is also a potent inhibitor of phosphatidyloinositol-3-kinase (Powis et al., 1994). It was assumed that KT5926 and wortmannin affected the observed reaction of human skin fibroblasts in the same manner. The most probable explanation of this effect would be inhibition of myosin light chain kinase.

It has been shown that both myosin light chain kinase inhibitors strongly prevent the anaesthetic-induced rounding of human skin fibroblasts (Fig. 5, Table 1). Furthermore, 24-h incubation of fibroblasts in medium containing KT5926 or wortmannin stimulated the cells to adopt a relaxed state, which was accompanied by an increase in the projected cell area and in a significant loss of stress fibres (Fig. 5D and Fig. 6D, Table 1). Similar results were obtained for epithelial cells by Volberg et al. (1994) who used [1-(5-isoquinolinesulfonyl)-2 methylpiperazine, HCl] (H7), a broad spectrum inhibitor of protein kinases, and by Chrzanowska-Wodnicka and Burridge (1996) for 3T3 fibroblasts with both H7 and KT5629 inhibitors. In the authors' present experiments, when relaxed cells were treated with procaine or tetracaine, the number of actin bundles not only did not decrease but some new ones were formed (Fig. 6D, F).

In agreement with results of previous experiments with 3T3 fibroblasts (Ridley and Hall, 1992; Barry and Critchley, 1994) the removal of serum from the culture medium resulted in loss of actin stress fibres (Fig. 4C), but in human skin fibroblasts a few thin actin bundles remained even after 24 h incubation in serum-free medium. In tensed cells, incubated in serum containing medium, anaesthetics evoked the isotonic contraction and rounding of cells. In relaxed cells incubated in the serum-free medium or in the presence of myosin light chain kinase inhibitors, anaesthetics evoked the isometric contraction which led to the formation of some stress fibres. The mechanism by which local anaesthetics alter cytoskeleton actin organisation is not known (Begg et al., 1996). The present results strongly support the hypothesis that the local anaesthetic-induced cytoskeleton changes are due to actomyosin contraction. This reaction has not previously been reported. The contraction in non-muscle cells is under dual regulation and is connected to both the activity of myosin and the structure of actin gel (the solation–contraction coupling hypothesis) (Taylor and Fechheimer, 1982). Stress fibres contract when the actin gel is solated by the addition of cytochalasin (Kolega et al., 1991; Giuliano et al., 1992). It is rather unlikely that anaesthetics caused solation of actin gel in human skin fibroblasts and thereby induced contraction because, in other systems, anaesthetics did not depolymerize F-actin. For example, procaine used at milimolar concentrations stimulates cortical actin polymerization in sea urchin eggs (Begg et al., 1996). It has recently been shown that the nocodazol-induced depolymerization of microtubules in fibroblasts result in both cell contraction and myosin light chain phosphorylation (Danowski, 1989; Kolodney and Elson, 1995). In the present experiments, microtubules in human skin fibroblasts showed the normal organisation despite the addition of anaesthetics (data not shown). Therefore it is concluded that anaesthetics change human skin fibroblast cytoskeleton organisation by directly affecting the actomyosin system.

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