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Differential regulation of bradykinin receptor density, intracellular Ca^{2+}, and prostanoid release in skin and foreskin fibroblasts. Effects of cell density and interleukin-1α

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1 Bradykinin (BK) receptors, cytosolic Ca^{2+}, and prostanoids were studied in human skin and foreskin fibroblasts.

2 B_{max} values of BK receptors were higher in foreskin than in skin fibroblasts, increasing with cell densities in both cell types. IL-1α-dependent receptor induction was blocked by cycloheximide.

3 BK-stimulated cytosolic Ca^{2+} elevation was higher in confluent than in non-confluent cultures and larger in foreskin than in skin fibroblasts. Responses were not enhanced after IL-1-α-induced up-regulation of BK receptors.

4 Intrinsic prostanoid production was higher in foreskin than in skin fibroblasts at comparable cell densities. In foreskin, but not in skin fibroblasts, BK stimulation increased the release of PGE_2 10 fold and that of 6-oxo-$PGF_{1\alpha}$ 6–7 fold.

5 Preincubation with IL-1α had a marked effect on prostanoid release in foreskin fibroblasts only. Subsequent BK stimulation increased the release of PGE_2 and 6-oxo-$PGF_{1\alpha}$ 7–10 fold in skin fibroblasts while this increase was only 30% in foreskin fibroblasts. Release of TXA_2 reached values up to one third of the other prostanoids. The IL-1α induced rise in BK-stimulated PGE_2 synthesis was fully abolished by specific inhibition of cyclo-oxygenase 2.

6 IL-1α sensitized BK-stimulated prostanoid synthesis and modulated prostanoid patterns differently in fibroblasts from skin and foreskin. The IL-1α effects on prostanoid release were not related to BK receptor numbers nor to the BK-stimulated Ca^{2+} signal but appear to be due to induction of prostanoid synthesizing enzymes. Foreskin fibroblasts seem to be unique and significantly different from fibroblasts of other skin locations in respect to their response to inflammation-associated kinins and cytokines.

Keywords: Interleukin-1; bradykinin; bradykinin receptors; intracellular Ca^{2+}; prostanoids; cyclo-oxygenases 1 and 2; cultured human cells; skin fibroblasts; foreskin fibroblasts

Abbreviations: AA, arachidonic acid; ASA, acetylsalicyclic acid; BK, bradykinin; B_{max}, maximal specific binding; COX, cyclo-oxygenase; IL, interleukin; K_D, equilibrium dissociation constant; PBS, phosphate buffered saline; PG, prostaglandin; PLA_2, phospholipase A_2; TXA_2, thromboxane A_2

Introduction

Inflammatory responses in tissues are modulated by a number of potent kinins and cytokines. One of them, bradykinin (BK) is an endogenous pro-inflammatory and vasoactive nonapeptide. It stimulates formation and release of prostanoids by a receptor-mediated pathway in synovial fibroblasts (O'Neill *et al*., 1987), gingival fibroblasts (Lerner *et al*., 1992), endothelial cells (McIntyre *et al*., 1985), smooth muscle cells (Zhang *et al*., 1991) and variably in cultured fibroblasts from human skin of different origins (Baciulis *et al*., 1992). BK acts *via* receptor subtypes B_1 and B_2 (Faussner *et al*., 1991). Fibroblasts form the constituent cellular part of the connective tissue. They maintain or modify the extracellular matrix and play a crucial role in tissue homeostasis and in wound healing. Cultured fibroblasts are usually considered cells with uniform properties independent of the origin of the skin biopsy. Foreskin fibroblasts are often used as controls when fibroblasts from patients are studied for inherited metabolic disorders.

Fibroblasts are reported to express mainly the B_2 BK receptor subtype (Roscher *et al*., 1983). The extent of BK-stimulated prostaglandin E_2 (PGE_2) release from cells is greatly enhanced by preincubation with the pro-inflammatory cytokine interleukin-1 (IL-1) (Burch *et al*., 1988; Bathon *et al*., 1989). Mechanisms of interactions between modulators and mediators in different tissues and in cultured cells may involve induction of enzymes of the prostanoid synthesis and/or increases in BK receptor numbers (Bathon *et al*., 1992). In various tissues and cell types these mechanisms are far from being understood and still need further clarification. Therefore, influences of low dose interleukin-1α (IL-1α) on BK-stimulated prostanoid release in human fibroblasts from normal skin and from foreskin were studied with emphasis on the level of BK receptor numbers and of BK-stimulated cytosolic Ca^{2+} signals. The aim of the present study, therefore, was to examine differences in the two types of fibroblasts.

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Methods

Fibroblast cultures

Human dermal fibroblasts were grown according to standard procedures (Luethy *et al*., 1983) in Eagle's minimum essential medium (MEM Earl's salts), supplemented with 200 U ml^{-1} penicillin G; 50 $\mu g\ ml^{-1}$ chlortetracycline-HCl; 0.21 mg ml^{-1} L(+)-glutamine; 2 mg ml^{-1} $NaHCO_3$ and 10% foetal calf serum (FCS). Cell strains were prepared from genital skin of healthy male children operated for phimosis (foreskin fibroblasts, $n=5$), or for prominent ears and for hernia etc. (skin fibroblasts, $n=5$) (Luethy *et al*., 1983). The age of the donors ranged from 2.5–12 years. All experiments were performed at low passage numbers (5th–10th). Fibroblasts from skin and from foreskin did not differ in the number of passages, rate of growth, morphological aspects (examined by phase contrast microscopy), or in known biochemical properties except for the expression of higher androgen receptor numbers in foreskin fibroblasts (personal communication by A. Roscher, Munich).

Prostanoid release

Cultures were grown in 24-well multidishes. Medium was removed and cultures were washed once with warmed Hanks' balanced salt solution (HBSS). Fibroblasts were incubated at 37°C in HBSS with and without 100 nM BK for 5 min. The supernatants were collected immediately. Cellular protein was determined according to Lowry *et al*. (1951). Prostanoid synthesis was also measured in cultures after pre-exposure to 15 U ml^{-1} of IL-1α for 24 h (specific activity of IL-1α: 3×10^8 U mg^{-1} according to the D_{10} bioassay) (Hopkins & Humphreys, 1989). Prostanoids (PGE_2, 6-oxo-$PGF_{1\alpha}$, TXA_2) were determined in the supernatants by ELISA techniques. Total cyclo-oxygenase (COX) was maximally inactivated by 50 μM acetylsalicylic acid (ASA) while cyclo-oxygenase 2 (COX-2) activity was totally inhibited by 10 μM of the specific inhibitor NS 398.

BK receptor binding studies

[^{3}H]-BK equilibrium binding studies [2,3-prolyl-3,4-^{3}H(N)]-BK was purchased from DuPont NEN. Specific radioactivities varied between 82 and 114 Ci $mmol^{-1}$. The following solutions were prepared one day before use and stored at 2–4°C: Buffer A (in mM) = NaCl 20, KCl 5.4, KH_2PO_4 4.4, Na_2HPO_4 0.7, $NaHCO_3$ 4.2, glucose 5, N-methyl-D-glucamine 120, HEPES 10 and 0.05% bovine serum albumin, pH 7.3. Buffer B (standard incubation buffer) = buffer A plus (in mM) $MgCl_2$ 0.25, $MgSO_4$ 0.2, $CaCl_2$ 0.48, bacitracin 2, and 10 μM phosphoramidon, pH 7.3. Buffer C = buffer B minus bacitracin and minus phosphoramidon (Roscher *et al*., 1983). Phosphate buffered saline (PBS) (in mM) = NaCl 137, KCl 2.7, Na_2HPO_4 10.6, KH_2PO_4 1.5 pH 7.4. Binding studies were performed at 4°C with cell monolayers in 6-well multidishes. The growth medium was removed and the cultures were washed twice with 2 ml of buffer A. The cells were then preincubated for 30 min with 0.75 ml of buffer B. Three culture dishes each were incubated with 0.2, 0.4, 0.8, 1.2, 2, 4, 6 and 8 nM [^{3}H]-BK, respectively, in 0.5 ml of buffer B with and without unlabelled BK (3 μM). This amount was sufficient to fully displace specific binding without interfering with nonspecific binding. After 2 h of incubation the supernatants were removed and the cultures rinsed three times with 2 ml of buffer C and twice with 2 ml of PBS. Cells were scraped off in 1 ml H_2O and transferred to counter vials for liquid scintillation. Protein contents were determined in corresponding culture dishes. Specific binding of [^{3}H]-BK was calculated by subtracting nonspecific from total binding and expressed as fmol mg^{-1} cell protein. Saturation kinetics were established for several cell lines with and without pre-exposure to 15 U ml^{-1} of IL-1α for 24 h. Equilibrium dissociation constants (K_D) and maximum binding (B_{max}) of the ligand were calculated using a computer curve-fit program.

[^{3}H]-BK binding studies at B_{max} conditions Once the conditions for maximum equilibrium binding (6–8 nM [^{3}H]-BK) were known, receptor binding was studied in all cell lines using the saturating concentration of 8 nM [^{3}H]-BK.

Intracellular Ca^{2+} measurements

Cytosolic free Ca^{2+} was measured with fura-2 in single cells with a calibrated video imaging system (Reber *et al*., 1990).

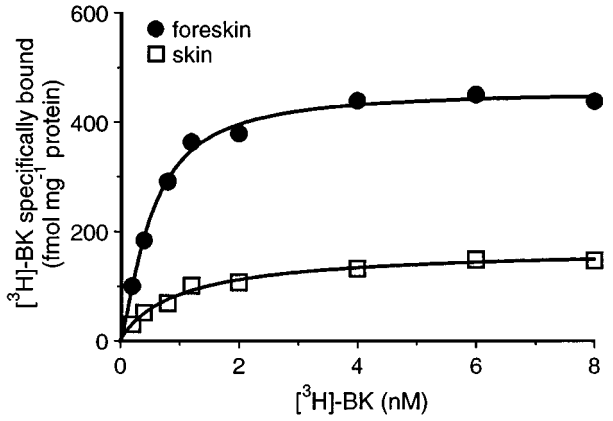

Figure 1 Equilibrium binding of [^{3}H]-BK to cultured human skin and foreskin fibroblasts. Confluent monolayers of one skin and foreskin fibroblast strain were cultured in 6-well multidishes and grown to equal protein contents (139 and 135 μg per well, respectively). Cultures were incubated *in situ* with increasing concentrations of [^{3}H]-BK in the absence and in the presence of 3 μM nonlabelled BK for 2 h at 4°C. Mean values are expressed in fmol specifically bound BK per mg of cell protein.

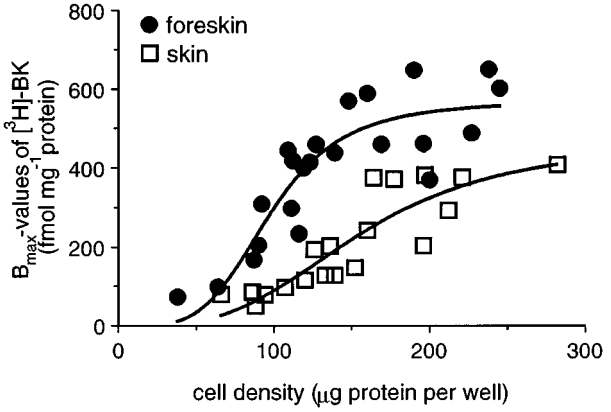

Figure 2 BK receptor numbers per mg of cellular protein in relation to cell density. B_{max} values from skin and foreskin fibroblasts, including five cell strains of each cell type, are plotted against protein contents per well (9.4 cm^2) as a measure for cell density. Curves were drawn according to a least square fit program.

Cells were grown on coverslides, loaded with dye by incubation with 3 μM fura-2-acetoxymethylester for 45 min at 37°C. Changes in the intensity of fura-2 fluorescence were obtained by exciting at 340 and 380 nm and recording the ratio at an emission of 510 nm.

Statistical analysis

All numerical data are expressed as means ± s.d. Data were analysed statistically by Student's *t*-test or by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test (Tables 1 and 2). A value of $P<0.05$ was considered statistically significant.

Materials

Chemicals and cell culture materials were obtained from the following sources: MEM Earl's salts and PBS, Seromed, Biochrom GmbH (Berlin, Germany); penicillin G, Hoechst (Frankfurt, Germany); foetal calf serum (FCS), Sebak (Aidenbach, Germany); chlortetracycline-HCl, cycloheximide, N-methyl-D-glucamine, HEPES (N-[2-hydroxy ethyl] piperazine-N′-[2-ethane sulphonic acid]), phosphoramidon (N-(α-rhamno-pyranosyl-oxyhydroxy-phosphinyl)-Leu-TRP) and bovine serum albumin, Sigma Chemical Co (St. Louis, MO, U.S.A.); acetylsalicylic acid, Fluka (Buchs, Switzerland); NS-398, a selective cyclo-oxygenase-2 inhibitor, Biomol, Anawa Trading SA (Wangen, Switzerland); $NaHCO_3$, NaCl, KCl, KH_2PO_4, Na_2HPO_4, D(+)-glucose, $MgCl_2$, $MgSO_4$ and $CaCl_2$ of highest analytical grades,

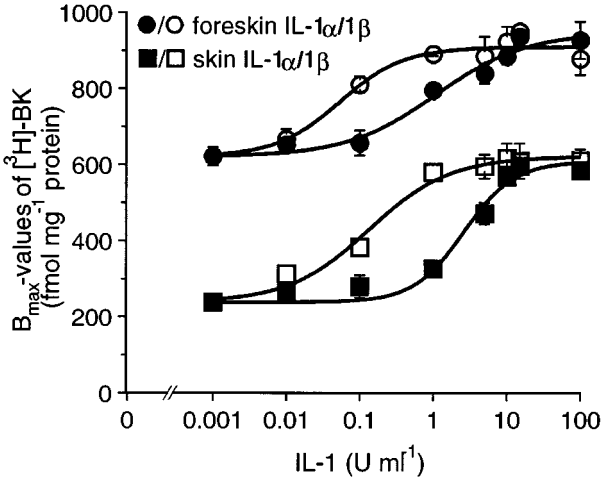

Figure 3 Induction of specific BK binding in skin and foreskin fibroblasts by IL-1α and IL-1β. Confluent skin and foreskin fibroblast cultures of equal cell density were exposed to increasing doses of IL-1α (closed symbols) and of IL-1β (open symbols) for 24 h at 37°C. For each condition triplicate cultures of one skin and one foreskin strain were analysed. Cellular B_{max} values are expressed in fmol specifically bound BK per mg of cell protein.

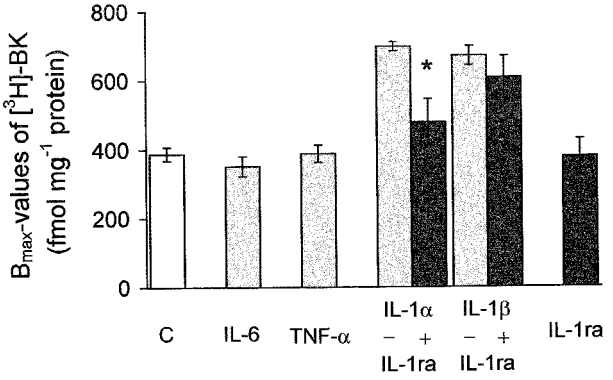

Figure 4 Specificity of the induction of BK receptor sites by different interleukins and by TNF-α. Effects of an IL-1 antagonist (IL-1ra). BK receptor binding sites were determined in confluent skin fibroblast cultures after exposure to IL-1α and IL-1β (15 U ml^{-1} of culture medium) for 24 h in the absence and in the presence of IL-1ra (300 ng ml^{-1}). The putative inducers, IL-6 (15 U ml^{-1}) and TNF-α (10 ng ml^{-1}) were also used. In addition, cells were incubated with IL-1ra alone as a control. For each condition triplicate cultures of one skin fibroblast strain were analysed. Specific BK binding is expressed as mean ± s.d. in fmol BK per mg of cell protein.

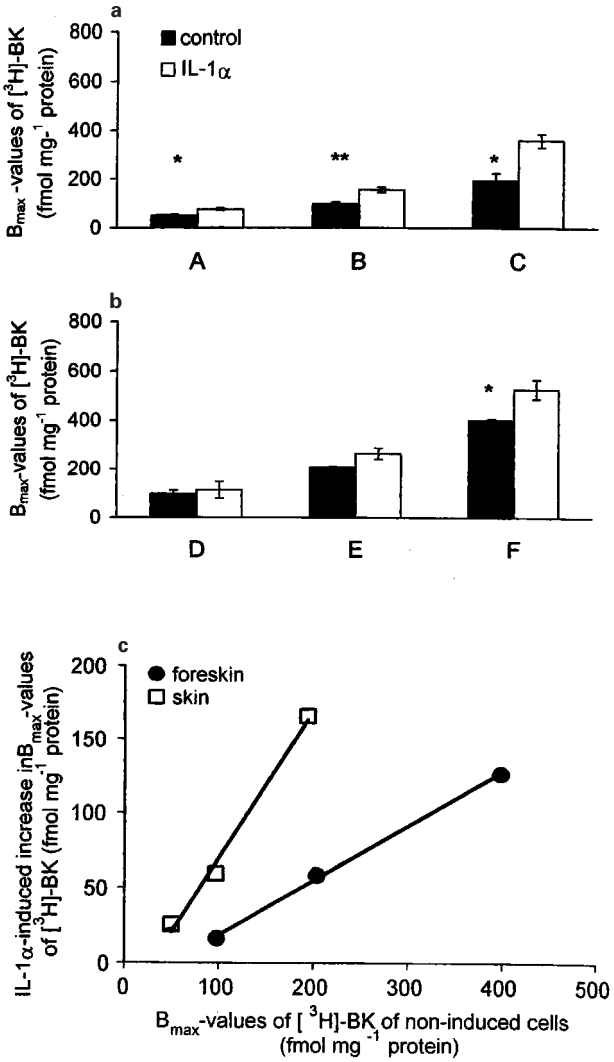

Figure 5 IL-1α-induced increase in BK binding in relation to cell density in skin and foreskin fibroblast cultures. Cultures of three different degrees of confluency with skin fibroblasts (A–C) or with foreskin fibroblasts (D–F) were exposed to IL-1α (15 U ml^{-1}) for 24 h at 37°C. Protein values per plate varied in skin fibroblasts (a) between A, 88; B, 107; and C, 126 μg and in foreskin fibroblasts (b) between D, 64; E, 90; and F, 119 μg per well respectively. Mean BK binding values ± s.d. of triplicate cultures of one skin and one foreskin fibroblast strain were analysed for each condition. (c) Relationship between the extent of induced increase in BK binding and the non-induced B_{max} values in skin and in foreskin fibroblasts; $^{*}P<0.05$, $^{**}P<0.005$.

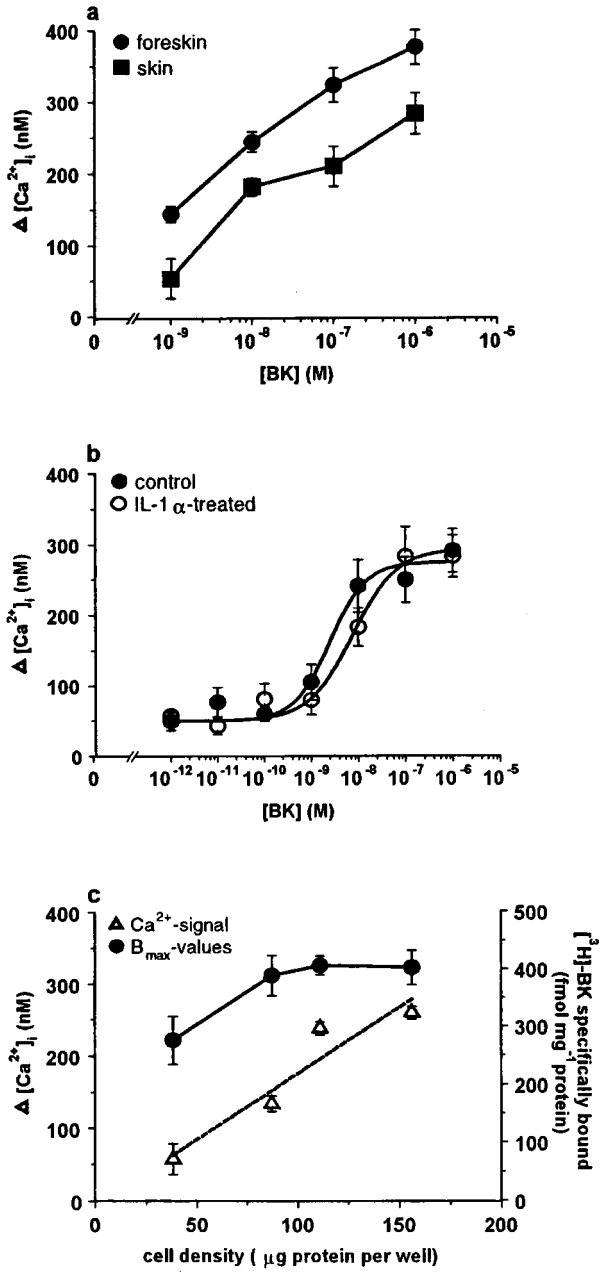

Figure 6 BK-stimulated cellular Ca^{2+} signals. (a) Transient increases in BK-stimulated cytosolic Ca^{2+}, released from intracellular pools, are plotted against BK concentrations. Ca^{2+} was measured by means of the fluorescent dye fura-2 in one skin and foreskin fibroblast strain. (b) Transient increases in BK-stimulated cytosolic Ca^{2+} were also measured in one foreskin fibroblast strain without and after IL-1α induction for 24 h. (c) Cytosolic Ca^{2+} signals, stimulated with 100 nM BK, and BK receptor binding were measured in cultures of one foreskin fibroblast strain of different confluences. Ca^{2+} and B_{max} values are plotted against cell density. All Ca^{2+} values in the figure express stimulated Ca^{2+} increases and were calculated from Ca^{2+} levels after BK-stimulation minus basal Ca^{2+} levels.

Merck AG (Darmstadt, Germany); [2,3-prolyl-3,4-^{3}H(N)]-BK, DuPont NEN Research Products (Regensdorf, Switzerland); L(+)-glutamine p.a. and bacitracin, Fluka Chemie AG (Buchs, Switzerland); BK-triacetate and trypsin 1:250 from bovine pancreas, Serva GmbH (Heidelberg, Germany); Falcon® cell culture material, Becton Dickinson (Basle, Switzerland); IL-1α and IL-1β, Boehringer AG (Mannheim, Germany); IL-1 receptor antagonist, R+D (Minneapolis, MN, U.S.A.); fura-2 AM, Molecular Probes (Leiden, The Netherlands).

Table 1 Prostanoid release in response to BK and/or IL-1α-stimulation in cultured fibroblasts

	Release (ng mg^{-1} protein)		
	PGE_2	6-oxo-$PGF_{1\alpha}$	TXA_2
Skin fibroblasts ($n=5$)			
Control	21.9±10.4	56.7±24.7	n.d.
BK	26.3±8.1	62.7±21.6	n.d.
IL-1α	50.0±9.7	68.8±25.7	n.d.
IL-1α+BK	327.0±87.0*	406.0±92.3*	117.2±25.6
Foreskin fibroblasts ($n=5$)			
Control	110.3±32.1	205.1±134.1	n.d.
BK	1034.6±308.1	1339.3±485.9	n.d
IL-1α	7544.9±1781.1*	3374.9±919.1*	196.0±96.2
IL-1α+BK	9338.1±1823.7*	5068.9±1200.7*	219.2±67.1

Control cultures and cultures after pre-exposure to 15 U ml^{-1} IL-1α for 24 h were stimulated for 5 min with 100 nM BK. Intrinsic, non-stimulated prostanoid production was also measured in cultures without (controls) and after pre-exposure to IL-1α (IL-1α). Prostanoids were determined in the supernatants by means of ELISA test kits. Values are expressed in ng prostanoid mg^{-1} cell protein and represent means±s.d. of cultures from five different donors. *$P<0.01$ in comparison to control values. n.d., not detectable.

Results

Modulation of bradykinin receptors

BK receptor binding characteristics were determined in foreskin (sexual) and in skin (nonsexual) fibroblasts (Figure 1). At equal cell densities foreskin fibroblasts showed considerably higher receptor numbers than skin fibroblasts (457 versus 148 fmol mg^{-1} protein). K_D-values were not significantly different in the two cell strains (0.72 and 0.76 nM respectively).

Maximum BK receptor densities per mg of cell protein of both types of fibroblasts were strongly dependent on the extent of confluency and increased with increasing cell densities (measured as cellular protein) (Figure 2). Protein content per culture correlated with the respective DNA values (results not shown), thus protein content could be used as a measure for cell number.

K_D values were not modified by cell density (data not shown). B_{max} values expressed per mg of protein plotted against cell protein content per plate showed a sigmoidal characteristic for both cell types.

BK receptor densities in fibroblasts of both types could be enhanced by exposing the cultures to low-dose IL-1α and IL-1β without modification of the K_D values. The extent of induction was related to the dose of IL-1 (Figure 3). A significant increase in receptor number occurred above 0.01 U ml^{-1}. At low dose, IL-1α was one to two orders of magnitude less potent than IL-1β. Both types of IL-1 had the same efficacy and thus achieved the same maximum induction of BK receptor numbers at 10 U ml^{-1} and above. The relative increase in IL-1-induced BK receptor densities was more pronounced in skin fibroblasts than in foreskin fibroblasts. Induction was time-dependent and became maximal after 24 h. B_{max} values of BK receptors in skin fibroblasts increased from 375±23 in non-induced control cells to 671±87 fmol mg^{-1} protein after exposure to 15 U ml^{-1} IL-1α for 24 h. This

Table 2 Non-specific and type 2-specific COX inihibition of BK-stimulated PGE_2-release in control and IL-1α-induced skin and foreskin fibroblasts

PGE_2-release (% of control)			
Non-induced cells		*IL-1α-induced cells*	
Skin fibroblasts			
Control	100±3	Control	100±19
NS 398	113±53	NS 398	5±0.8*
ASA	n.d.	ASA	0.5±0.01*
Foreskin fibroblasts			
Control	100±19	Control	100±26
NS 398	90±13	NS 398	17±1*
ASA	6±0.3*	ASA	4±1*

Non-induced and induced (15 U ml^{-1} IL-1α for 24 h) confluent cultures of skin- and foreskin fibroblasts were stimulated for 5 min with 100 nM BK. PGE_2 formation was inhibited by 50 μM acetylsalicyclic acid (ASA) and 10 μM NS 398, a specific COX-2 inhibitor, respectively. The inhibitory drugs were added for 24 h to the culture media. PGE_2 was determined in the supernatant by means of an ELISA test kit. Values are expressed as per cent (means±s.d.) of the respective non-inhibited controls. n.d., non detectable. For each condition six cultures of one strain of both fibroblast types were analysed.

receptor induction was associated with protein synthesis since coadministration of 200 μM cycloheximide fully abolished receptor up-regulation; in the presence of cycloheximide B_{max} values were 295±14 in non-induced cells and 280±21 fmol mg^{-1} protein in IL-1α exposed cells (results represent mean values±s.d of 12 confluent skin fibroblast cultures for each condition).

The IL-1α-induced increase in BK receptor numbers was blocked by the addition of a 1000 fold excess of a nonspecific IL-1 antagonist (IL-1 ra) while in contrast the induction by IL-1β could not be significantly inhibited (Figure 4). IL-6, (15 U ml^{-1}) TNF-α (10 ng ml^{-1}) or IL-1 ra (300 ng ml^{-1}) did not increase BK receptor binding (Figure 4).

The extent of IL-1α induction increased with increasing cell density in both cell types (Figure 5).

Modulation of intracellular Ca^{2+} release to BK stimulation

The extent of the BK-stimulated transient increase in cytosolic Ca^{2+} (Ca^{2+} signal) was dependent on BK concentrations. Stimulated Ca^{2+} signals in foreskin fibroblasts were consistently higher than in skin fibroblasts (Figure 6a). IL-1α induction did not affect BK-stimulated Ca^{2+} signals as shown in foreskin fibroblasts (Figure 6b). BK-stimulated Ca^{2+} signals in non-confluent cultures were submaximal and were related to cell density and BK receptor numbers. Above 100 μg of protein content the Ca^{2+} signal became maximal, although the BK receptor numbers still increased with higher cell confluency (Figure 6c).

Intrinsic, IL-1-induced and BK-stimulated prostanoid syntheses

In the medium of unstimulated skin fibroblasts intrinsic amounts of 6-oxo-$PGF_{1\alpha}$ and PGE_2 could be measured (Table 1). TXA_2 concentrations were below detection limit. Stimulation with 100 nM BK did not significantly increase synthesis of PGE_2 and 6-oxo-$PGF_{1\alpha}$. TXA_2 levels were still not measurable. Pre-treatment of cells with 15 U ml^{-1} of IL-1α for 24 h had no effect on the intrinsic production of 6-oxo-$PGF_{1\alpha}$ but doubled the production of PGE_2. BK stimulation of IL-1α-pre-treated fibroblasts resulted in an 8–10 fold increase in both PGE_2 and 6-oxo-$PGF_{1\alpha}$ compared to BK-stimulated cells not pre-treated with IL-1α. TXA_2 became measurable and concentrations roughly reached one third of those of the other two prostanoids.

Synthesis of the two main prostanoids, PGE_2 and 6-oxo-$PGF_{1\alpha}$, from unstimulated foreskin fibroblasts were two to four times higher than that from skin fibroblasts. Production of TXA_2 was below detection limit (<15 ng ml^{-1} protein) (Table 1).

Stimulation with 100 nM BK markedly increased production of both PGE_2 and 6-oxo-$PGF_{1\alpha}$ five to ten times over intrinsic production. TXA_2 production again could not be measured. BK-stimulated prostanoid synthesis in these fibroblasts was far in excess of that seen in skin fibroblasts.

Pre-treatment of foreskin fibroblasts with IL-1α for 24 h by itself resulted in a very large increase in prostanoid synthesis. The ratio between PGE_2 and 6-oxo-$PGF_{1\alpha}$ thereby became 2:1. This was inverse of that in unstimulated or BK-stimulated fibroblasts. Production of TXA_2 became measurable in four out of five fibroblast strains.

BK stimulation of IL-1α-pretreated foreskin fibroblasts further increased production of both major prostanoids by 30%. Production of TXA_2 did not further increase.

Cyclo-oxygenase (COX) inhibition

In order to distinguish between total COX and COX-2 activities in control and IL-1α-induced skin and foreskin fibroblasts, cells were exposed to ASA, a non-selective and to NS 398, a COX-2 selective enzyme inhibitor, respectively.

In non-induced cultures of both cell types BK-stimulated PGE_2-release was fully abolished by ASA but not affected by NS 398 (Table 2). In IL-1α-induced fibroblasts the selective COX-2 inhibitor reduced PGE_2 release to pre-induction levels while inhibition by ASA was complete (Table 2).

Discussion

BK receptor induction

Fibroblasts form the constituent cellular part of the connective tissue, they interact with immunocompetent cells and represent an important source of cytokines and chemokines. BK is one of the main stimulants for prostanoid release and pain sensation. It interacts with specific cell surface receptors. Fibroblasts mainly express the B_2 BK receptor subtype (Roscher *et al*., 1983). Since skin fibroblasts are considered cells with uniform properties independent of their origin, foreskin fibroblasts are often used as controls in studies with fibroblasts from patients with genetic disorders. It is, however, important to be aware that foreskin fibroblasts, in certain aspects, are significantly different from fibroblasts of other skin locations.

BK receptor densities were much higher in foreskin than in skin fibroblasts while K_D values were similar in both types of fibroblasts and corresponded to the ones described in synovial fibroblasts (Bathon *et al*., 1992). A new and unexpected finding was that unlike other membrane receptors (e.g. cytokine receptors, Waelti *et al*., 1996) BK receptor numbers were up-regulated with increasing cell density in both types of fibroblasts. Nevertheless, differences in receptor numbers between the two types of fibroblasts were maintained

throughout all degrees of cellular densities. Within the range of subcultures used in our experiments, no effect of passage numbers was observed on any of the fibroblast properties and functions. This further supported the evidence that receptor densities were cell type specific. Fibroblasts are solitary in tissue, thus up-regulation of BK receptor numbers by cell density may not have functional consequences *in vivo* except in scar tissue or in keloids where fibroblasts are more dense.

IL-1 has been shown to increase the sensitivity of the prostanoid response to BK in several fibroblast-like cells (Lerner *et al*., 1992; Bathon *et al*., 1992). It was, however, dubious whether this was due to an increase in functional BK receptors and/or due to induction of enzymes of prostanoid synthesis. Up-regulation of the number of BK receptors after IL-1 exposure has been previously observed (Bathon *et al*., 1992). In both types of fibroblasts, BK receptor numbers were increased 24 h after addition of even very small amounts of IL-1 to the cultures. This increase was dose-dependent and reached a maximum above 10 U ml^{-1}. Both forms of IL-1 were equally effective but stimulation with IL-1β was more potent and occurred already at much lower concentrations (>0.01 U ml^{-1}). Increases in BK receptor numbers were due to *de novo* synthesis and thus could be inhibited by cycloheximide. This confirmed a recent publication by Phagoo *et al*. (1997) on IL-1-induced BK receptors in human lung fibroblasts as well as an earlier paper by Sundquist & Lerner (1996). Receptor up-regulation by IL-1α but less that by IL-1β could be inhibited by simultaneous addition of an excess of an IL-1 antagonist. This supported the finding that IL-1β has a higher potency than IL-1α due to its higher affinity for IL-1 receptors (Lerner & Modeer, 1991).

Functionality of BK receptors

Binding of BK to BK receptors results in a temporary increase in the concentration of cytosolic Ca^{2+}, released by an IP_3-mediated pathway from intracellular stores. The increase in intracellular Ca^{2+} concentrations was related to the stimulatory BK concentrations. It was always higher in foreskin than in skin fibroblasts reflecting not only a numerical but also a functional consequence of the difference in BK receptor expression.

Density-upregulated BK receptors were functional but their effectiveness was limited by the maximal extent of the Ca^{2+} signal. Similarly, the limited maximal size of the Ca^{2+} signal may explain the lack of further enhanced BK-stimulated Ca^{2+} response to an IL-1-induced increase in BK receptors. Consequently the IL-1-induced BK-stimulated prostanoid secretion was not related to the size of the Ca^{2+} signal.

Prostanoid secretion

Prostanoids are formed from arachidonic acid (AA) through interrelated enzymatic pathways. They are not stored in the cells and their synthesis is limited by the availability of free AA, liberated by phospholipase A_2 (PLA_2) from membrane phospholipids. It is generally assumed that vascular endothelial cells synthesize primarily PGE_2 and PGI_2 (the latter can be measured in its stable metabolic form: 6-oxo-$PGF_{1\alpha}$), while platelets predominantly form TXA_2. All these three prostanoids can be released from fibroblasts. They are formed by three individual synthases from a common transient precursor, a product of the cyclo-oxygenase-peroxidase complex. Thus the production of the individual prostanoids is regulated by the activities of the cyclo-oxygenase (COX) complex and the individual synthases.

Using commercially available ELISA kit for quantitative determination, intrinsic secretion of both fibroblast types contained 6-oxo-$PGF_{1\alpha}$ as the predominant form followed by PGE_2, while TXA_2 was below detection limit. In skin as well as in synovial fibroblasts (Bathon *et al*., 1992) but not in foreskin fibroblasts BK was only a weak stimulus for prostanoid secretion. In IL-1α pre-treated cells BK stimulation significantly increased the release of all three prostanoids. IL-1α by itself had little effect on intrinsic, non BK-stimulated prostanoid production in skin fibroblasts while it greatly enhanced it in foreskin fibroblasts.

The pro-inflammatory cytokine IL-1 has been shown to increase BK-stimulated prostanoid secretion of several fibroblast-like cells (gingival fibroblasts, synovial cells) (Lerner *et al*., 1992; Bathon *et al*., 1989, 1992). The mechanism by which IL-1 induced the responsiveness of fibroblasts to BK appears to be multifactorial. It has been attributed to an up-regulation of COX activity alone (Lerner *et al*., 1992; Bathon *et al*., 1996) or to a combined induction of PLA_2 and COX (Burch *et al*., 1988; Endo *et al*., 1995; Hulkower *et al*., 1993). *De novo* protein synthesis seems to be involved since the stimulatory effect of IL-1 could not only be abolished by indomethacin and dexamethasone but also by cycloheximide. In non-induced cells COX activity appears to be rate-limiting (Bathon *et al*., 1989), and prostanoid synthesis could not be inhibited by the COX-2 specific inhibitor NS 398. In IL-1-induced cells the increased BK-stimulated prostanoid production could be quantitatively and specifically inhibited by NS 398 suggesting that the enhanced formation was exclusively mediated by induction of the COX-2 type enzyme, which is not expressed in non-induced fibroblasts of both strains. In addition changes in the pattern of secreted prostanoids found after combined IL-1 and BK stimulation seemed to result from activation or induction of the individual synthases. Prostanoid synthesis in foreskin fibroblasts greatly differed from that in skin fibroblasts suggesting that the respective synthases are differently regulated in the two cell types.

Increases in prostanoid secretion in IL-1 pre-treated, BK-stimulated skin fibroblasts were neither a result of higher Ca^{2+} signals nor of enhanced BK receptor numbers but were well explained by IL-1-induced enzyme activities in the pathway of prostanoid synthesis.

PLA_2 is activated by specific as well as by non-specific increases in cytosolic Ca^{2+} concentrations (Baciulis *et al*., 1992). IL-1 by itself stimulated prostanoid release in foreskin fibroblasts only. This may be related to differences in intrinsic PLA_2 activities being higher in foreskin than in skin fibroblasts and responding to non-specific stimuli. The fact that prostanoid synthesis in skin fibroblasts was not significantly enhanced by BK stimulation may be explained by a limited activity of the COX complex.

In vivo, BK stimulation of skin and of foreskin fibroblasts in inflammatory states would, according to our *in vitro* findings, result in different prostanoid patterns and may induce a different pathophysiology compared to the non-inflammatory state. Direct stimulation of prostanoid synthesis by the pro-inflammatory cytokine IL-1 in foreskin fibroblasts may have an implication *in vivo* and indicate that these cells are unique and significantly different from fibroblasts of other skin locations with respect to the number of BK receptors and to their response to kinins and cytokines.

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