Journal of Dermatological Science (2005) **38**, 177—188

ELSEVIER

Journal of Dermatological SCIENCE

www.intl.elsevierhealth.com/journals/jods

Distribution of cannabinoid receptor 1 (CB1) and 2 (CB2) on sensory nerve fibers and adnexal structures in human skin

Sonja Ständer [a,*], **Martin Schmelz** [b], **Dieter Metze** [a], **Thomas Luger** [a], **Roman Rukwied** [b]

[a] *Department of Dermatology and Ludwig Boltzmann Institute for Cell Biology and Immunobiology of the Skin, University Hospital Münster, Von-Esmarchstr. 58, D-48149 Münster, Germany*
[b] *Department of Anesthesiology and Intensive Care Medicine, Faculty of Clinical Medicine Mannheim, University of Heidelberg, Germany*

Received 18 September 2004; received in revised form 18 January 2005; accepted 25 January 2005

KEYWORDS
Cannabinoid receptors;
Cutaneous nerve fibers;
Nociception;
Itch;
Human skin

Summary

Background: Cannabinoid receptors mediate the psychopharmacological action of marijuana and have been localized in the central and peripheral nervous system as well as on cells of the immune system.
Objective: Up to now, two cannabinoid receptors (CB1 and CB2) have been cloned and recent studies on animal tissue gave evidence for the presence of cannabinoid receptors in the skin.
Methods: In the present immunohistochemical investigation we determined the precise localization of CB1 and CB2 in sections of human skin and in one case of mastocytosis.
Results: CB1 and CB2 immunoreactivity was observed in cutaneous nerve fiber bundles, mast cells, macrophages, epidermal keratinocytes, and the epithelial cells of hair follicles, sebocytes and eccrine sweat glands. In epidermal keratinocytes, hair follicle and sebaceous glands, CB1 and CB2 were distributed in a complementary fashion. Double-immunostaining with an anti-CGRP antibody suggested the presence of cannabinoid receptors on small afferent peptidergic nerves.
Conclusion: The abundant distribution of cannabinoid receptors on skin nerve fibers and mast cells provides implications for an anti-inflammatory, anti-nociceptive action of cannabinoid receptor agonists and suggests their putatively broad therapeutic potential.

Abbreviations: CB, cannabinoid receptor; CGRP, calcitonin gene-related peptide; FITC, fluorescein-isothiocyanate; NF, neurofilament; PGP, protein gene product

* Corresponding author. Tel.: +49 251 835 6504; fax: +49 251 835 2559.
E-mail address: sonja.staender@uni-muenster.de (S. Ständer).

0923-1811/$30.00
doi:10.1016/j.jdermsci.2005.01.007

1. Introduction

Cannabinoids are the generic term for active compounds of Marijuana, of which Δ^9-tetrahydrocannabinol (THC) is the most common representative. G-protein coupled cannabinoid receptor (CB1) have been identified first in the brain [1,2] and spinal cord [3] of the rat. Administration of the endogenous cannabinoid receptor agonist anandamide [4,5] elicited hypothermia, catalepsy, impaired motor activity and antinociception [6—10] and a central mode of action at CB1 has been discussed. Also, peripherally administered cannabinoids revealed anti-nociceptive and anti-hyperalgesic effects in rats [11] and humans [12], even though competitive binding studies with capsaicin (TRP-V1 receptor) [13], mustard oil (TRP-ANKTM1 receptor) [14], the CB1 receptor antagonist SR141716A [15] and researches with CB1 knock out mice [16] yielded that actions of particularly anandamide, an endogenous cannabinoid receptor agonist, are not being mediated by CB1 only.

In peripheral tissue, CB1 have been identified on neuronal and immune cells [17,18]. On the latter — namely macrophages of the spleen and tonsils [19—21] — a particular cannabinoid receptor was determined that revealed about 40% receptor homology to CB1 only and therefore had been termed cannabinoid receptor 2 (CB2) [19]. According to their distribution, an activation of CB2 was associated with a modulation of the immune system [22] and functionally attributed to the treatment of, e.g. multiple sclerosis [23,24] or the immune deficiency syndrome HIV [25]. However, therapeutic efficacy of cannabinoids in such clinical trials are still lacking [26].

In experimental studies on human tissue or cell lines some authors described the expression of CB1 and CB2. For instance, cannabinoid receptors have been determined in human pyramidal cells, T- and B-lymphocytes, keratinocytes, endothelial and epidermal cell lines [17,27—31], but also in the intestine [32,33] and corneal epithelium [34]. So far, no immunohistochemical study demonstrated the distribution of CB1 and CB2 in human cutaneous nerve fibers. Here, we investigated the presence and distribution of cannabinoid receptors in human cutaneous tissue and their localization on primary sensory nerves, appendage epithelial cells, cutaneous macrophages and skin mast cells.

2. Materials and methods

Biopsies of normal healthy skin (n = 10) from different sites of the body (leg, arm, trunk, face, capillitium) and of one case of mastocytosis were obtained from patients undergoing routine diagnostic and therapeutic surgery. Adult frontal rat brain including white and grey substance, cervical and thoracic spinal cord, and spleen served as a positive control (kindly provided by Prof. Dr. K.D. Richter, Dept. of Animal Experimental Research, University Hospital Münster, Germany). Rat tissue and human skin samples were immediately frozen in liquid nitrogen and cut, after mounting in cryo-embedding compound (Microm Int., Walldorf, Germany), with a cryostat in 3—5 μm sections (4—6 slides of each sample). Frozen sections were postfixed with 2% paraformaldehyde/1% picric acid in 0.1 M Tris-buffered saline (TBS) with 0.3% Triton for 20 min and preincubated for 30 min with 2% bovine serum albumin. Specimens were incubated overnight at 4 °C with rabbit polyclonal antibodies against CB1 and CB2 (CB1, 1:200, Cat. No. 216401; CB2, 1:20, Cat. No. 216407, Calbiochem, San Diego, CA, USA). According the suppliers specification, anti-CB1-antibody was directed against the aa 1—14 (MKSILDGLADTTFR) of human CB1 protein and anti-CB2-antibody against the aa 20—33 (NPMKDYMILSGPQK) of human CB2 protein; no cross-reactivity for CB1 and CB2 was found for the two antibodies. Following the incubation period samples were rinsed three times in 0.1 M TBS with 0.3% Triton for 45 min and sections incubated with donkey Texas red-conjugated anti-rabbit antibody (1:100, Dianova, Hamburg, Germany) or swine FITC-labelled anti-rabbit antibody (1:50, DAKO, Hamburg, Germany). For double-immunofluorescence staining, specimens were first incubated overnight with the anti-CB1/CB2-antibodies, followed by an incubation period with different monoclonal antibodies for 4 h, i.e. mouse anti-neurofilament-antibody (NF, subunits 70, 160, and 200 kDa, Clone 2F11, 1:50, Cat. No. M0762, DAKO, Hamburg, Germany), mouse anti-protein gene product 9.5-antibody (PGP 9.5, Clone 31A3, 1:50, Cat. No. 7863-1004, Biotrend, Köln, Germany), mouse anti-calcitonin gene-related protein-antibody (CGRP, 1:30, Cat. No. CA 1135, Biotrend, Köln, Germany), mouse anti-CD68-antibody (Clone KP1, 1:100, Cat. No. M0814, Dako, Hamburg, Germany), and mouse anti-tryptase-antibody (1:1000, Cat. No. MAB1222, Chemicon, Temecula, CA, USA). The antibodies were developed with pork FITC-labelled anti-rabbit antibody (1:50, DAKO, Hamburg, Germany) and goat Texas red-labelled anti-mouse antibody (1:200, Dianova, Hamburg, Germany). To confirm staining, fluorescence antibodies were applied cross-matched, i.e. CB1 and CB2 antibodies were detected with donkey Texas red-conjugated anti-rabbit antibody (1:100, Dianova, Hamburg, Germany), while neuronal markers were visualized with rabbit FITC-labelled anti-mouse antibody (1:50, DAKO, Hamburg, Germany). After final rinsing with TBS, sections were cover slipped in Vectashield (Vector Laboratories, USA)

and immediately investigated and photographed with a fluorescence microscope (Olympus BX61, Hamburg, Germany; MetaMorph Software, Visitron-Systems, Puchheim, Germany). Replacement of the primary antibody by mouse or rabbit serum in a concentration corresponding to the antibodies used and preabsorption of the first antibody with the corresponding blocking peptides (MKSILDGLADTTFR for CB1; NPMKDYMILSGPQK for CB2; Cayman Chemicals, Ann Arbor, MI, USA), served as a negative control, which was performed in rat brain, rat spinal cord, rat spleen and human healthy skin.

3. Results

3.1. Immunoreactivity of CB1 in human skin (Table 1)

3.1.1. Small nerve fibers

CB1 was determined in cutaneous nerve fiber bundles. The staining protocol revealed strong reactivity for both Texas Red- and FITC-labelled secondary antibodies, and specificity of CB1 antibody was evidenced by replacement of the antibody with rabbit IgG serum and pre-absorption of the first

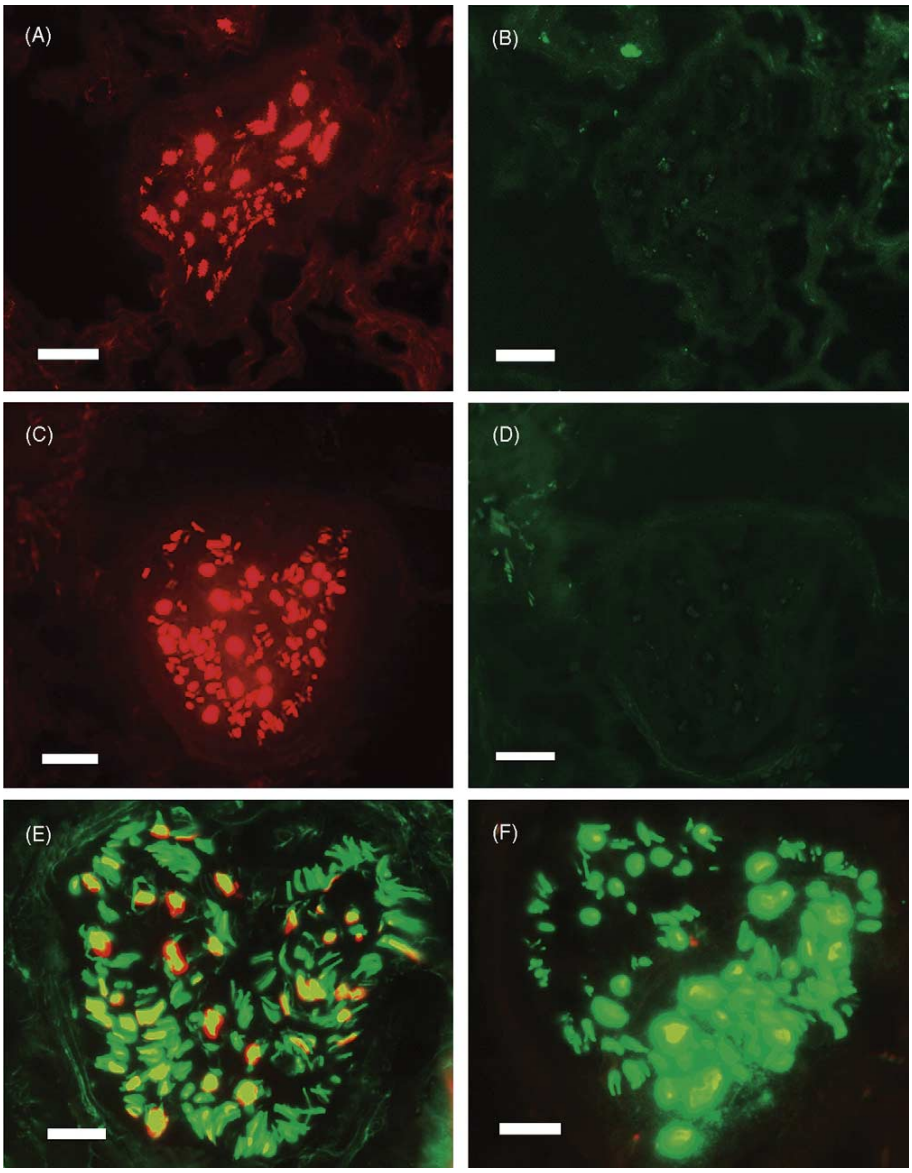

Fig. 1 Controls for CB1 and CB2. Neurofilament staining in a small nerve fiber of the face (A and C). Preabsorption of the anti-CB1-antibody with the corresponding blocking peptide (MKSILDGLADTTFR) (B) and replacement of the anti-CB-antibodies by rabbit serum (D) did not result in a specific immunostaining on adjacent skin specimen. Double labeling of neurofilament and CB1 (E) or CB2 (F) revealed colocalisation (yellow areas). Scale bar: A—D, 14 μm; E—F, 7.5 μm.

antibody with the corresponding blocking peptide. Regardless the investigated tissue, i.e. rat brain, spinal cord, spleen and human skin, these control experiments did not result in any specific immunostaining (Fig. 1(A)–(F)). The distribution and expression of CB1 on skin nerves could be characterized as follows. In superficial and deep reticular dermis, immunoreactivity for CB1 was detected in large myelinated nerve fiber bundles (Figs. 1(E) and 2(A)–(C)), small unmyelinated nerves of the papillary dermis (Fig. 2(D)–(F)) at dermal–epidermal junction, as well as, sporadically, within the epidermis. Staining for CB1 was also observed in single small nerve fibers that were associated with hair follicles. In each skin biopsy of specimens of different sites of the body, such as face, head, trunk and extremities, the distribution of CB1 immunoreactivity was identical. Double-immunofluorescence staining for CB1 with neurofilament (NF) or protein gene product (PGP) 9.5 respectively demonstrated the presence of CB1 on axons. NF and PGP 9.5 stained intensely for axons of nerve fiber bundles in superficial and deep dermis, the epidermal–dermal junction, the epidermis, and around hair follicles, whereas the perineurium of large nerve fibers showed only weak immunoreactivity (Fig. 3). Calcitonin gene-related peptide (CGRP)-positive sensory small nerve fibers showed co-localized immunoreac-

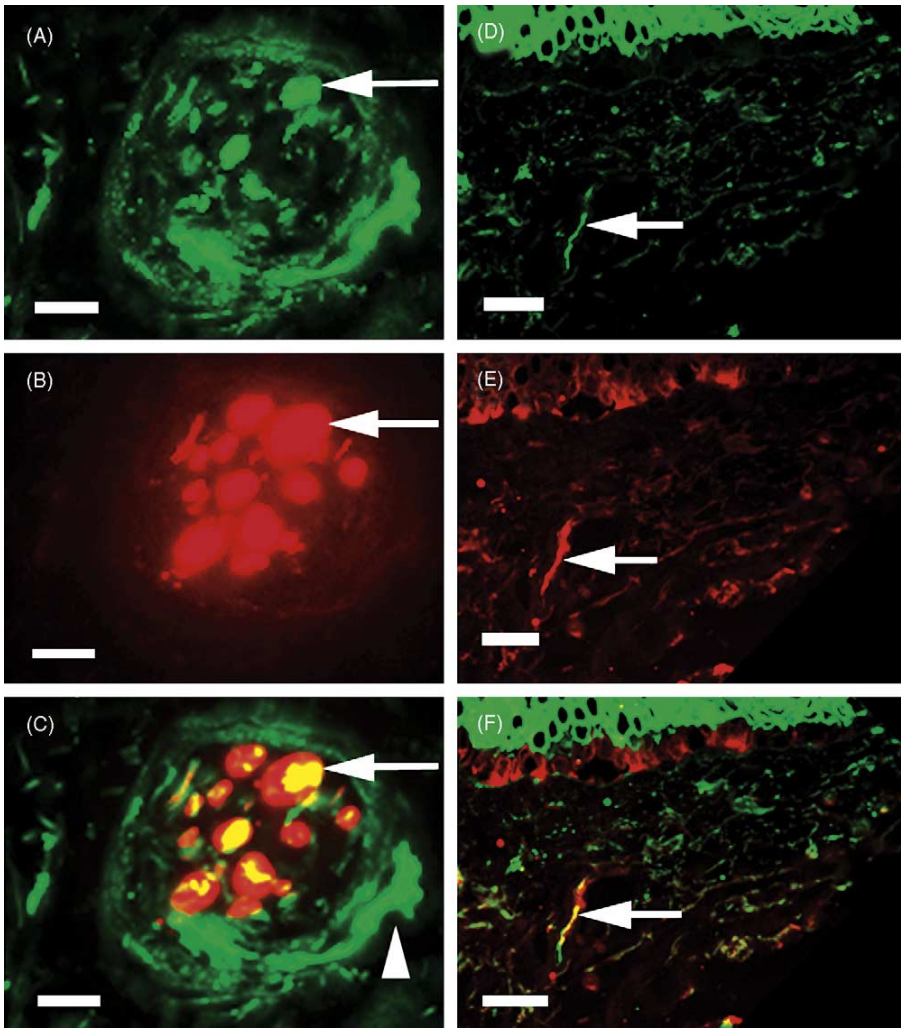

Fig. 2 Immunoreactivity for CB1 on nerve fiber bundles in normal human skin from the face. Positive immunoreactivity for CB1 in a large (A, arrow) and a small subepidermal (D, arrow) nerve fiber. The neuronal markers neurofilament (B, arrow) and protein gene product 9.5 (PGP 9.5, E, arrow) show intense immunoreactivity in axons. In the overlay, the presence of CB1 on several axons in a large myelinated nerve fiber bundle (C, arrow), a mast cell next to the nerve fiber (arrowhead) and a subepidermal small nerve fiber (F, arrow) can be seen. Interestingly, basal keratinocytes stain intensely for PGP 9.5 (E) while CB1 stains suprabasal keratinocytes (D). Bar: A–C, 7 μm; D–F, 34 μm.

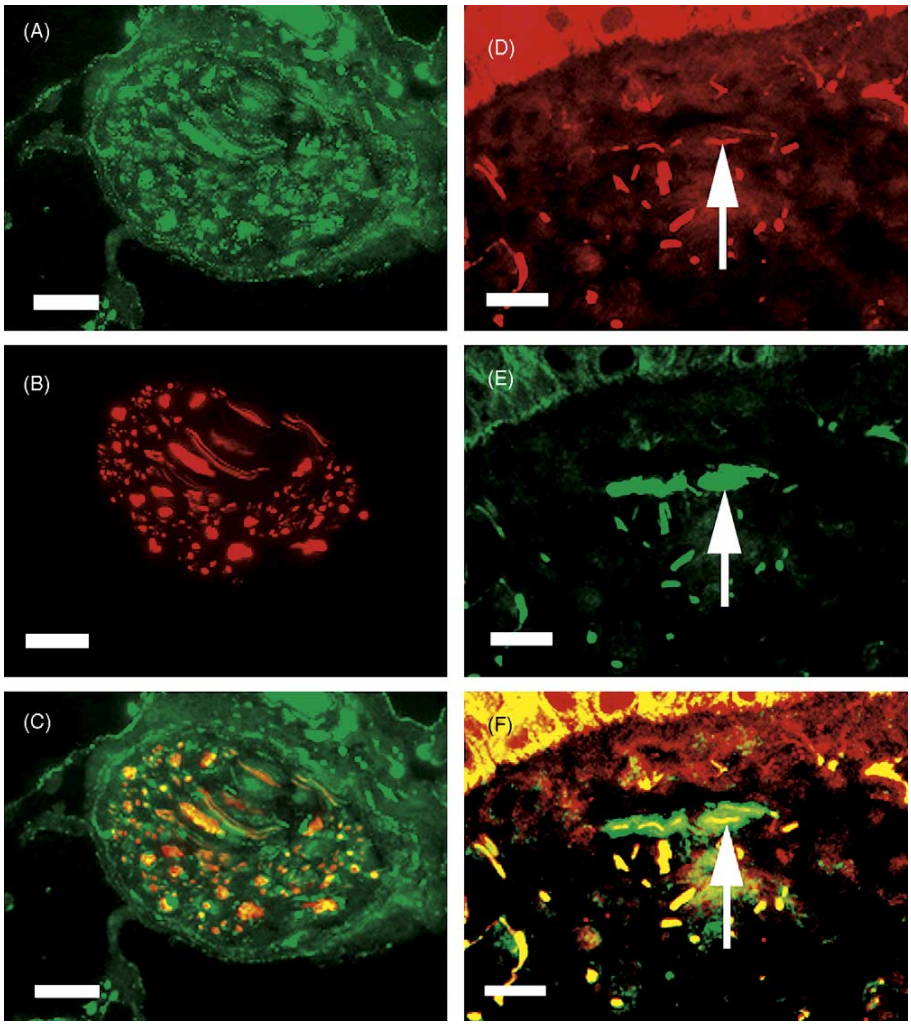

Fig. 3 Immunoreactivity for CB2 on a dermal and subepidermal small nerve fiber in normal human skin from the arm. Positive immunoreactivity for CB2 in a large dermal myelinated nerve fiber bundle (A) which showed abundant axonal immunoreactivity for NF (B). The overlay of A and B revels co-localisation of CB2 mainly on axons (C). Weak immunoreactivity of CB2 in a small subepidermal nerve fiber (D, arrow). NF is intensely expressed in the small unmyelinated subepidermal nerve fiber (E, arrow). Overlay of D and E shows the presence of CB2 on the small subepidermal nerve fiber (F, arrow). Bar: A—C, 14 μm; D—F, 14 μm.

tivity with CB1 (Fig. 4(A)—(C)). As a positive control, rat frontal brain, cervical and thoracic spinal cord was stained for CB1, in which immunoreactivity was determined in several cell bodies and small dendritic axons (data not shown) (Table 1).

3.1.2. Epithelial cells, macrophages and mast cells

In the epidermis, CB1 immunoreactivtiy was observed in keratinocytes of the stratum spinosum and stratum granulosum (Fig. 5(A)). Additionally, hair follicles, the differentiated epithelial cells of the infundibulum and the inner hair root sheet stained for CB1 (Fig. 5(B)). In contrast, the outer root sheet, the bulge, the bulb of the hair follicles and the musculi arectores pili were negative. In sebaceous glands, CB1 was found in the central differentiated sebaceous cells (Fig. 5(C)) while the peripheral undifferentiated cells were negative. The secretory portion of sweat glands showed strong immunoreactivity in myoepithelial cells but not secretory cells. Epithelial cells of the dermal duct of sweat glands revealed only weak staining. In the dermis, single but not all CD68-positive macrophages showed cytoplasmatical immunoreactivity for CB1 (Fig. 6(A) and (B)). All mast cells in normal skin and identically in mastocytosis stained for CB1, which was confirmed by double-immunostaining

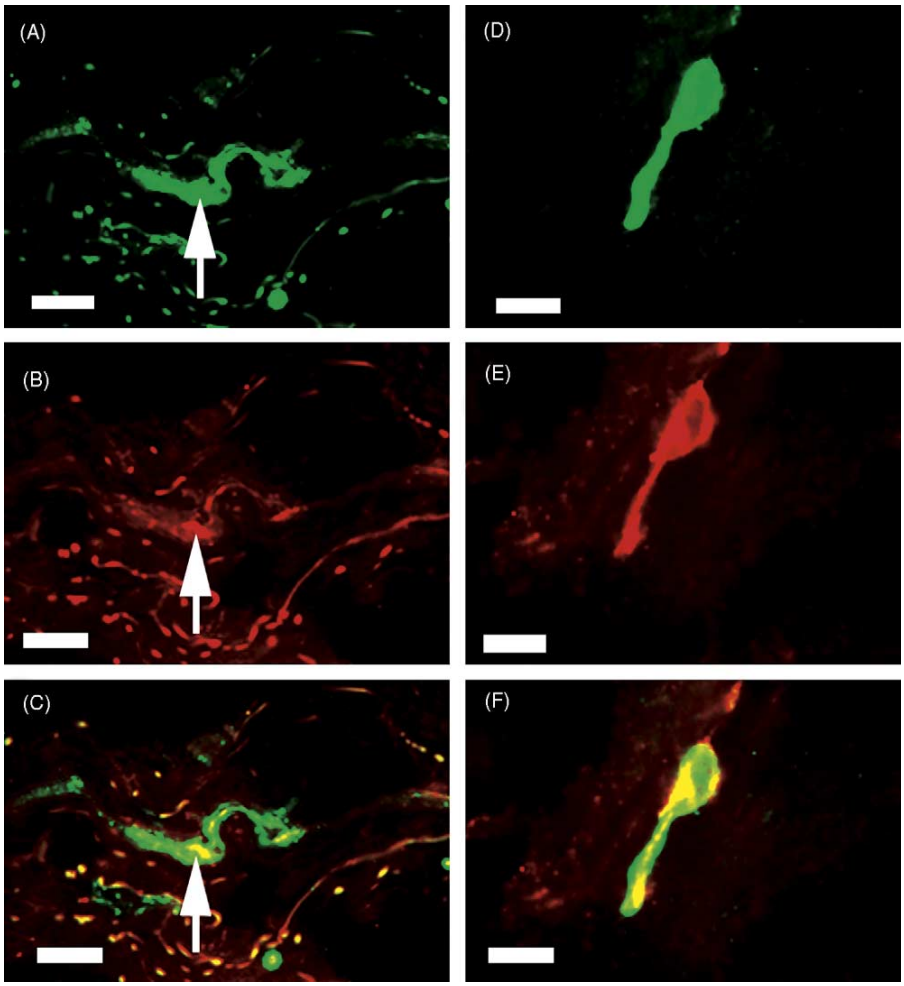

Fig. 4 CB1 and CB2 are present on sensory dermal nerve fiber bundles. A small subepidermal nerve fiber of the arm (A—C) and dermal nerve fiber of the trunk (D—F) revealed positive immunoreactivity for CB1 (A, arrow), CB2 (D) and the neuropeptide CGRP (B and E). C and F are overlay images of A, B and D, E respectively. Bar: A—F, 14 μm.

with the mast cell marker tryptase (Fig. 6(E) and (F)). These findings were constant in specimens from different areas of the body.

3.2. Immunoreactivity of CB2 in human skin (Table 1)

3.2.1. Small nerve fibers

Immunoreactivity for CB2 was detected in cutaneous small nerve fibers with both staining protocols, i.e. detection of CB2 by Texas Red- and FITC-labelled secondary antibodies. Similar to the determination of CB1, immunoreactivity for CB2 was identified in large myelinated nerve fiber bundles of the superficial and deep reticular dermis (Figs. 1(F) and 3(A)—(C)), in small unmyelinated nerves of the papillary dermis (Fig. 3(D)—(F)), at the dermal—epidermal junction, and occasionally within the epidermis and small nerve fibers associated with hair follicles. In skin specimens of different body sites (face, head, trunk and extremities) distribution of CB2 immunoreactivity was identical. Double-immunofluorescence staining for CB2 with NF or PGP 9.5 revealed distinct co-localization of CB2 with both neuronal markers. Most nerve fibers showed a moderate staining along the axon. Receptor staining was co-localized with the sensory neuropeptide calcitonin gene-related peptide (CGRP) (Fig. 4(D)—(F)). Specificity of CB2 antibodies was evidenced by replacement of the antibody with rabbit IgG serum and pre-absorption of the antibody with the corresponding peptide. No specific immunostaining was obtained in the investigated tissue, i.e. rat brain, spinal cord, spleen and human skin (Fig. 1). As a positive control rat spleen was stained with anti-CB2. Many but not all macrophages showed an intense cytoplasmic immunoreactivity (data not shown).

Table 1 Positive immunoreactivity of cannabinoid receptors in different skin cells.

Epidermis	Nerve fibers	Adnexal structures, mast cells, Macrophages
CB1		
Keratinocytes of the stratum spinosum and granulosum	Single epidermal nerve fibers, small unmyelinated subepidermal nerves, large dermal myelinated nerves	Differentiated sebaceous cells, differentiated epithelial cells of the infundibulum and inner root sheath of hair follicles, myoepithelial cells of eccrine sweat glands, sweat gland duct, mast cells, macrophages
CB2		
Basal keratinocytes	Single epidermal nerve fibers, small unmyelinated subepidermal nerves, large dermal myelinated nerves	Undifferentiated sebaceous cells, undifferentiated infundibular hair follicle cells, myoepithelial cells of eccrine sweat glands, sweat gland duct, mast cells, macrophages

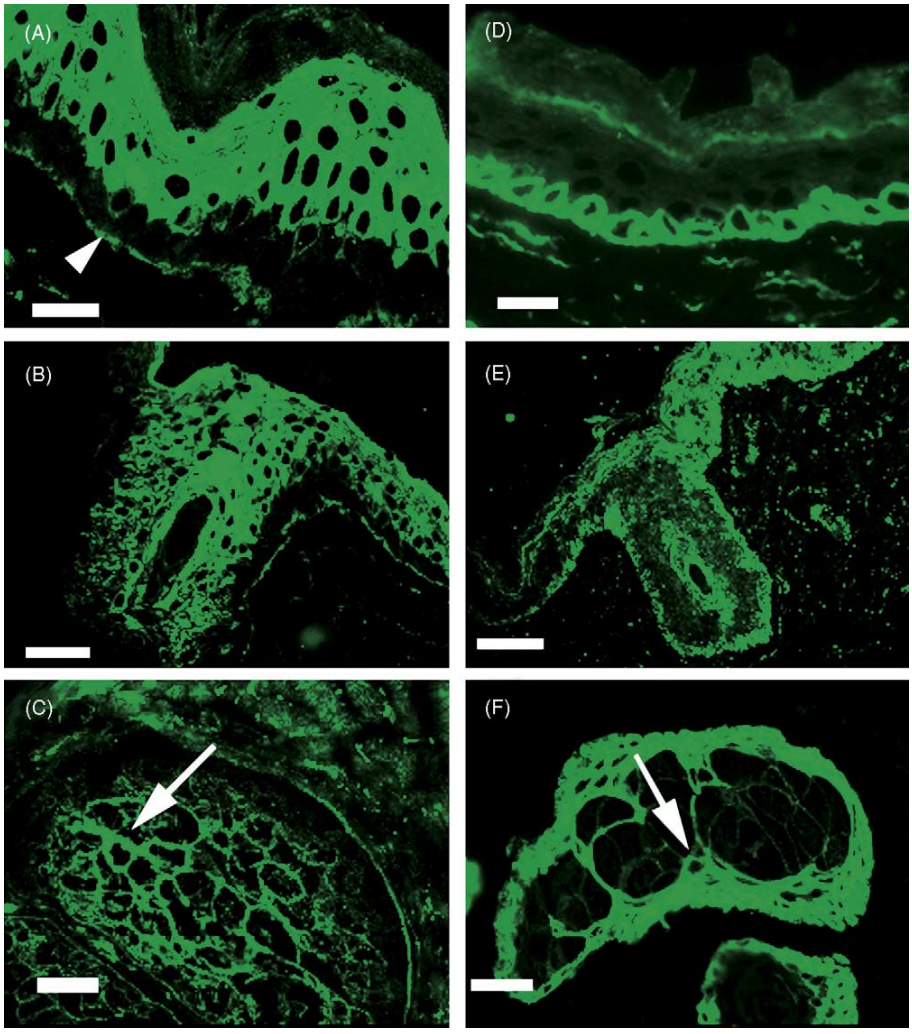

Fig. 5 CB1 and CB2 are present on epithelial cells. Suprabasal keratinocytes of the epidermal stratum spinosum and stratum granulosum revealed immunoreactivtiy for CB1 (A, arrowhead: basal lamina) as well as the differentiated epithelial cells of the hair follicle infundibulum (B), and the central differentiated cells of sebaceous glands (C, arrow). CB2 stains undifferentiated cells such as the basal epidermal keratinocytes (D), the outer infundibular cells of hair follicles (E) and the peripheral sebaceous cells (F, arrow). Bar: A, 29 μm; B, 57 μm; C, 40 μm; D, 29 μm; E, 43 μm; F, 40 μm.

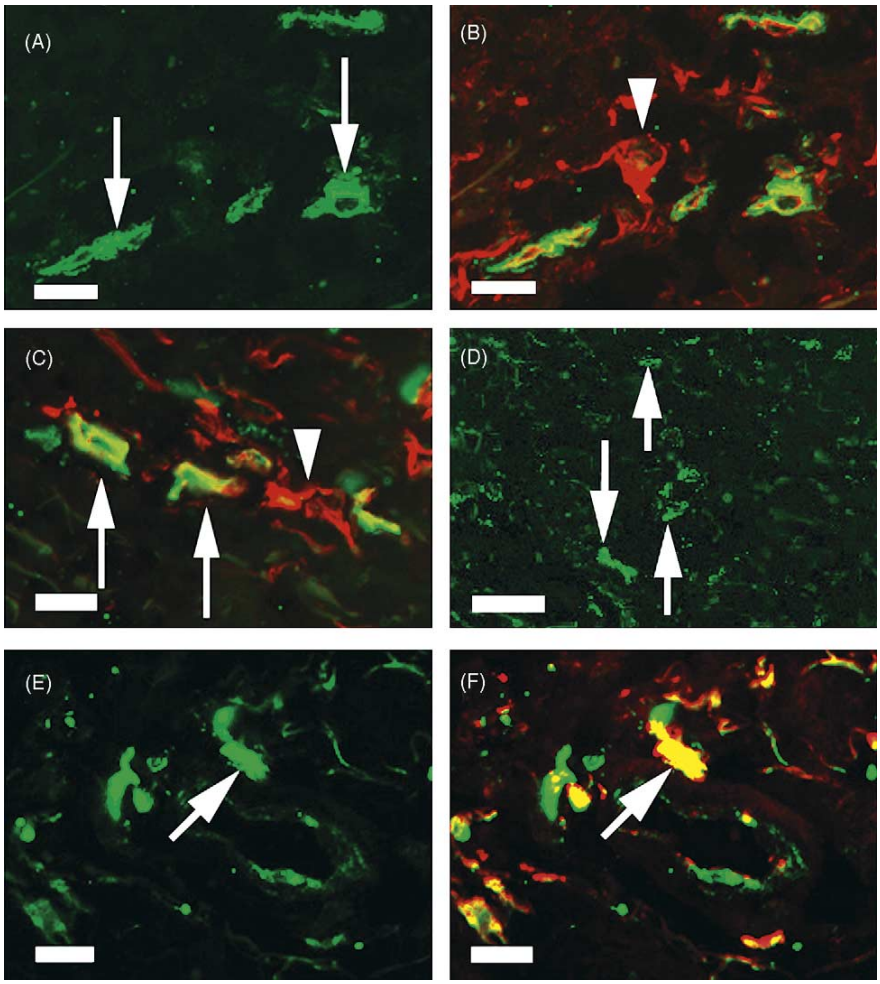

Fig. 6 Expression of CB in macrophages and mast cells. Single (A, C, arrows) but not all (B, C, arrowhead) CD68-positive cutaneous macrophages stain for CB1 (B: overlay of CB1- and CD68-staining) and CB2 (C, overlay of CB2- and CD68-staining). Mast cells in mastocytosis stain intensively for CB2 (D, arrows) and CB1 (E, arrow), which was confirmed by double-immunostaining with the mast cell marker tryptase (F, arrow, overlay with E). Bar: A and B, 14 μm; C, 9 μm; D, 20 μm; E and F, 14 μm.

3.2.2. Epithelial cells, macrophages and mast cells

Major CB2 immunoreactivtiy was observed in basal keratinocytes of the epidermis (Fig. 5(D)). In contrast to the recorded CB1 staining, undifferentiated cells of the infundibulum (Fig. 5(E)), the outer hair root sheet and the bulb of hair follicles stained for CB2. The inner root sheet, the bulge of the hair follicles and the musculi arectores pili were negative. These immunoreactivity patterns were constant throughout the observed specimens from different body areas. In sebaceous glands, CB2 was identified in peripheral undifferentiated cells (Fig. 5(F)), whereas central differentiated sebaceous cells were, however, negative. In sweat glands, the myoepithelial cells of the secretory portion and epithelial cells of the duct showed positive immunoreactivity. Most but not all CD68-positive macrophages were positive for anti-CB2-staining (Fig. 6(C)). Furthermore, all mast cells stained for CB2 in both normal skin and also identically in mastocytosis (Fig. 6(D)), as confirmed by double-staining with the mast cell marker tryptase.

4. Discussion

Numerous studies described the expression of cannabinoid 1 receptors in animal and human brain tissue [2,35—39]. Their regional distribution had been associated with the psycho-active attributes of cannabinoids and their influence on emotion [40,41]. In addition, identification of CB1 and CB2 in dorsal root ganglia cells of the rat [42—45] sug-

gested a cannabinoid receptor expression on primary afferent neurons [7,46—50]. Even peripheral administration of cannabinoid receptor agonists suppressed pain associated behaviour in animals [11,51—53] as well as nociception in humans [12]. These findings indicated the expression of cannabinoid receptors on nociceptive units, as already demonstrated in cultured primary sensory afferents of the rat [18,32,44].

The present study describes for the first time the distribution pattern of cannabinoid receptors on human cutaneous nerve fibers. CB1 and CB2 have been identified on large (myelinated) and thin (unmyelinated) calcitonin gene-related peptide (CGRP) positive nerve fibers, as evidenced by double-immunofluorescence staining with neuronal markers (neurofilament, PGP 9.5) and anti-CGRP antibodies. These immunohistological data underline previous functional studies, showing that an activation of cutaneous cannabinoid receptors by topical administration of selective receptor agonists attenuated the experimentally induced nociceptor excitation and subsequent pain and itch perception [12], reduced mechanical hyperalgesia [12,50,54] and decreased the release of neuropeptides, particularly CGRP, from terminal afferent units [55,56]. In human skin, CGRP causes vasodilatation [57] and contributes to the maintenance of long lasting flare reactions [58]. The recent observation that topical administration of a CB receptor agonist attenuated the chemically evoked flare reaction in humans [59] can be explained by the present determination of CB receptors on CGRP positive nerve fibers. However, no final conclusion can be made which particular cannabinoid receptor sub-type (CB1 and/or CB2) is essentially involved to influence these vascular responses and further studies are currently made to elucidate this issue. It has been considered that cannabinoids may have a putatively huge therapeutic potential, not only when administered peripherally in order to ameliorate inflammatory processes [12,60] or to enhance morphine effects [53], but also following oral administration to treat intractable pain or itch [46—48,52,61,62]. Obviously, systemic drug application suggests a rather central mode of action, whereas peripheral activation of particularly CB2 revealed anti-nociceptive and anti-inflammatory effects via both the central [51] and peripheral nervous system [63—65].

These presence of CB2 on cutaneous nerve fibers are quite intriguing since this receptor has been assumed to be preferably expressed on blood and immune cells, e.g. B-and T-lymphocytes, natural killer cells, and macrophages; the latter could also be shown in this study [19,21,66,67]. Apparently, the effects of activation of CB2 cannot be restricted to immune-regulatory functions, but should also include nociception. The present data on CB2 expression on human skin nerve fibres substantiate this assumption, although their specific functional role remains unclear. Also, recent studies demonstrated cannabinoids in cancer research, in which CB2 agonists considerably contributes to tumour cell angiogenesis and apoptosis [68—70].

In immortalized (HaCaT) and normal (NHEK) human epidermal keratinocytes, the cannabinoid anandamide facilitated epidermal differentiation and skin development via CB1 [31]. Given the identified complex and complementary distribution of CB1 and CB2 on human epidermal keratinocytes, hair follicles and sebaceous glands, our recent findings of cannabinoid receptor immunoreactivity in epithelial cells of appendage structures and epidermal keratinocytes suggest that skin cell differentiation may also be facilitated by activation of these receptors in vivo. However, this has to be confirmed in further studies. Here, epithelial cells revealed intense immunolabeling also in the cytoplasm. A recent study demonstrated that after CB1 transfection in HEK-293 cells, CB1 are present at the plasma membrane but substantial proportions (approximately 85%) of receptors are localized in intracellular vesicles [71]. The authors concluded that due to its natural constitutive activity, CB1 permanently and constitutively cycles between the plasma membrane and endosomes that finally leads to a predominantly intracellular localization at steady-state. This finding and interpretation explains nicely the staining distribution pattern of CB1 and CB2 presented in this study.

Abundant labeling for CB1 and CB2 was identified on the myoepithelial cells of the secretory portion of eccrine sweat glands. It has been reported that CB1 and CB2 agonists attenuate electrically induced smooth muscle contraction of the isolated bladder in mouse and rat, however not in isolated sections of dog, pig, monkey and human [72]. Thus, the present finding of CB1 and CB2 on myoepithelial cells of the secretory portion of eccrine sweat glands may indicate a functional involvement of cannabinoids at sweat glands. Moreover, CB1 expression has been identified in differentiated sebocytes. It was reported that acne, a sebaceous gland derived skin disorder, is initiated or exacerbated by the release of neuropeptides and subsequent neutral endopeptidase expression [73]. Both might be impaired by cannabinoid receptor agonists and therefore could provide new insights into the pathogenesis of sebaceous gland associated skin diseases and its treatment by cannabinoids.

In the present study we further detected immunoreactivity for both CB1 and CB2 on human skin mast cells with identical expression pattern in nor-

mal skin and mastocytosis. This finding is in accordance with a previous report that demonstrated CB2 on rat peritoneal mast cells as well as inhibitory functions of the cannabinoids nabilone, WIN55,212-2 and palmithoylethanolamide on mast cell degranulation [74]. Remarkably, the cannabinoid anandamide partly reversed these effects and thus behaved as an endogenous mediator with negative regulatory properties [74]. It will be of great interest to scrutinize cannabinoids as anti-inflammatory agents, particularly in diseases with mast cell driven inflammatory processes, e.g. mastocytosis, allergic diseases or even urticaria. The identification and wide distribution of CB1 and CB2 in human skin may indicate a variety of their functional role, such as inhibitory effects on sensory nerves as a new tool for pain and itch treatment, differentiation of epithelial cells and appendage structures or mast cell stabilizing anti-inflammatory properties.

Acknowledgement

The authors are grateful to Andrea Wissel for expert technical assistance.

References

[1] Devane WA, Dysarz III FA, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 1988;34:605—13.
[2] Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 1990;346:561—4.
[3] Farquhar-Smith WP, Egertova M, Bradbury EJ, McMahon SB, Rice ASC, Elphick MR. Cannabinoid CB1 receptor expression in rat spinal cord. Mol Cellular Neurosci 2000;15:510—21.
[4] Mechoulam R, Fride E, Di Marzo V. Endocannabinoids. Eur J Pharmacol 1998;359:1—18.
[5] Di Marzo V, Bisogno T, De Petrocellis L, Melck D, Martin BR. Cannabimimetic fatty acid derivatives: the anandamide family and other endocannabinoids. Curr Med Chem 1999; 6:721—44.
[6] Martin BR, Compton DR, Thomas BF, Prescott WR, Little PJ, Razdan RK, et al. Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs. Pharmacol Biochem Behav 1991;40:471—8.
[7] Harris J, Drew LJ, Chapman V. Spinal anandamide inhibits nociceptive transmission via cannabinoid receptor activation in vivo. NeuroReport 2000;11:2817—9.
[8] Stein EA, Fuller SA, Edgemond WS, Campbell WB. Physiological and behavioural effects of the endogenous cannabinoid, arachidonylethanolamide (anandamide), in the rat. Br J Pharmacol 1996;119:107—14.
[9] Smith PB, Compton DR, Welch SP, Razdan RK, Mechoulam R, Martin BR. The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice. J Pharmacol Exp Ther 1994;270:219—27.
[10] Crawley JN, Corwin RL, Robinson JK, Felder CC, Devane WA, Axelrod J. Anandamide, an endogenous ligand of the cannabinoid receptor, induces hypomotility and hypothermia in vivo in rodents. Pharmacol Biochem Behav 1993;46:967—72.
[11] Johanek LM, Simone DA. Activation of peripheral cannabinoid receptors attenuates cutaneous hyperalgesia produced by a heat injury. Pain 2004;109:432—42.
[12] Rukwied R, Watkinson A, McGlone F, Dvorak M. Cannabinoid agonists attenuate capsaicin-induced responses in human skin. Pain 2003;102:283—8.
[13] Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V, et al. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 1999; 400:452—7.
[14] Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Hogestatt ED, et al. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature 2004;427:260—5.
[15] Adams IB, Compton DR, Martin BR. Assessment of anandamide interaction with the cannabinoid brain receptor: SR 141716A antagonism studies in mice and autoradiographic analysis of receptor binding in rat brain. J Pharmacol Exp Ther 1998;284:1209—17.
[16] Di Marzo V, Breivogel CS, Tao Q, Bridgen DT, Razdan RK, Zimmer AM, et al. Levels, metabolism, and pharmacological activity of anandamide in CB(1) cannabinoid receptor knockout mice: evidence for non-CB(1), non-CB(2) receptor-mediated actions of anandamide in mouse brain. J Neurochem 2000;75:2434—44.
[17] Bouaboula M, Rinaldi M, Carayon P, Carillon C, Delpech B, Shire D, et al. Cannabinoid-receptor expression in human leukocytes. Eur J Biochem 1993;214:173—80.
[18] Pertwee RG. Evidence for the presence of CB1 cannabinoid receptors on peripheral neurones and for the existence of neuronal non-CB1 cannabinoid receptors. Life Sci 1999; 65:597—605.
[19] Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993; 365:61—5.
[20] Matsuda LA. Molecular aspects of cannabinoid receptors. Crit Rev Neurobiol 1997;11:143—66.
[21] Galiegue S, Mary S, Marchand J, Dussossoy D, Carriere D, Carayon P, et al. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem 1995;232:54—61.
[22] Klein TW, Newton C, Larsen K, Lu L, Perkins I, Nong L, et al. The cannabinoid system and immune modulation. J Leukoc Biol 2003;74:486—96.
[23] Arevalo-Martin A, Vela JM, Molina-Holgado E, Borrell J, Guaza C. Therapeutic action of cannabinoids in a murine model of multiple sclerosis. J Neurosci 2003;23:2511—6.
[24] Killestein J, Hoogervorst EL, Reif M, Kalkers NF, Van Loenen AC, Staats PG, et al. Safety, tolerability, and efficacy of orally administered cannabinoids in MS. Neurology 2002; 58:1404—7.
[25] Abrams DI, Hilton JF, Leiser RJ, Shade SB, Elbeik TA, Aweeka FT, et al. Short-term effects of cannabinoids in patients with HIV-1 infection: a randomized, placebo-controlled clinical trial. Ann Intern Med 2003;139:258—66.
[26] Baker D, Pryce G. The therapeutic potential of cannabis in multiple sclerosis. Expert Opin Investig Drugs 2003;12:561—7.
[27] Casanova ML, Blazquez C, Martinez-Palacio J, Villanueva C, Fernandez-Acenero MJ, Huffman JW, et al. Inhibition of skin tumor growth and angiogenesis in vivo by activation of cannabinoid receptors. J Clin Invest 2003;111:43—50.
[28] Liu J, Gao B, Mirshahi F, Sanyal AJ, Khanolkar AD, Makriyannis A, et al. Functional CB1 cannabinoid receptors in human

vascular endothelial cells. Biochem J 2000;346(Pt 3):835—40.
[29] Maccarrone M, De Petrocellis L, Bari M, Fezza F, Salvati S, Di MV, et al. Lipopolysaccharide downregulates fatty acid amide hydrolase expression and increases anandamide levels in human peripheral lymphocytes. Arch Biochem Biophys 2001;393:321.
[30] Benito C, Nunez E, Tolon RM, Carrier EJ, Rabano A, Hillard CJ, et al. Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. J Neurosci 2003;23:11136—41.
[31] Maccarrone M, Di Rienzo M, Battista N, Gasperi V, Guerrieri P, Rossi A, et al. The endocannabinoid system in human keratinocytes: Evidence that anandamide inhibits epidermal differentiation through CB1 receptor-dependent inhibition of protein kinase C, activating protein-1, and transglutaminase. J Biol Chem 2003;278:33896—903.
[32] Griffin G, Fernando SR, Ross RA, McKay NG, Ashford ML, Shire D, et al. Evidence for the presence of CB2-like cannabinoid receptors on peripheral nerve terminals. Eur J Pharmacol 1997;339:53—61.
[33] Ruiz-Llorente L, Sanchez MG, Carmena MJ, Prieto JC, Sanchez-Chapado M, Izquierdo A, et al. Expression of functionally active cannabinoid receptor CB1 in the human prostate gland. Prostate 2003;54:95—102.
[34] Straiker AJ, Maguire G, Mackie K, Lindsey J. Localization of cannabinoid CB1 receptors in the human anterior eye and retina. Invest Ophthalmol Vis Sci 1999;40:2442—8.
[35] Katona I, Rancz EA, Acsady L, Ledent C, Mackie K, Hajos N, et al. Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. J Neurosci 2001;21:9506—18.
[36] Egertova M, Elphick MR. Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB. J Comp Neurol 2000;422:159—71.
[37] Marsicano G, Lutz B. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. Eur J Neurosci 1999;11:4213—25.
[38] Westlake TM, Howlett AC, Bonner TI, Matsuda LA, Herkenham M. Cannabinoid receptor binding and messenger RNA expression in human brain: an in vitro receptor autoradiography and in situ hybridization histochemistry study of normal aged and Alzheimer's brains. Neuroscience 1994; 63:637—52.
[39] Herkenham M, Lynn AB, Little MD, Ross Johnson M, Melvin LS, De Costa BR, et al. Cannabinoid receptor localization in brain. Proc Natl Acad Sci USA 1990;87:1932—6.
[40] Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, et al. The endogenous cannabinoid system controls extinction of aversive memories. Nature 2002;418: 530—4.
[41] Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O. Involvement of CB1 cannabinoid receptors in emotional behaviour. Psychopharmacology (Berl) 2002;159:379—87.
[42] Bridges D, Rice AS, Egertova M, Elphick MR, Winter J, Michael GJ. Localisation of cannabinoid receptor 1 in rat dorsal root ganglion using in situ hybridisation and immunohistochemistry. Neuroscience 2003;119:803—12.
[43] Hohmann AG, Briley EM, Herkenham M. Pre- and postsynaptic distribution of cannabinoid and mu opioid receptors in rat spinal cord. Brain Res 1999;822:17—25.
[44] Ahluwalia J, Urban L, Capogna M, Bevan S, Nagy I. Cannabinoid 1 receptors are expressed in nociceptive primary sensory neurons. Neuroscience 2000;100:685—8.
[45] Zhang J, Hoffert C, Vu HK, Groblewski T, Ahmad S, O'Donnell D. Induction of CB2 receptor expression in the rat spinal cord of neuropathic but not inflammatory chronic pain models. Eur J Neurosci 2003;17:2750—4.
[46] Calignano A, La Rana G, Giuffrida A, Piomelli D. Control of pain initiation by endogenous cannabinoids. Nature 1998; 394:277—81.
[47] Dogrul A, Gardell LR, Ma S, Ossipov MH, Porreca F, Lai J. 'Knock-down' of spinal CB1 receptors produces abnormal pain and elevates spinal dynorphin content in mice. Pain 2002;100:203—9.
[48] Drew LJ, Harris J, Millns PJ, Kendall DA, Chapman V. Activation of spinal cannabinoid 1 receptors inhibits C-fibre driven hyperexcitable neuronal responses and increases [35S]GTPgammaS binding in the dorsal horn of the spinal cord of noninflamed and inflamed rats. Eur J Neurosci 2000; 12:2079—86.
[49] Conti S, Costa B, Colleoni M, Parolaro D, Giagnoni G. Anti-inflammatory action of endocannabinoid palmitoylethanolamide and the synthetic cannabinoid nabilone in a model of acute inflammation in the rat. Br J Pharmacol 2002;135: 181—7.
[50] Malan TP, Ibrahim MM, Vanderah TW, Makriyannis A, Porreca F. Inhibition of pain responses by activation of CB(2) cannabinoid receptors. Chem Phys Lipids 2002;121:191—200.
[51] Nackley AG, Makriyannis A, Hohmann AG. Selective activation of cannabinoid CB(2) receptors suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. Neuroscience 2003;119:747—57.
[52] Dogrul A, Gul H, Akar A, Yildiz O, Bilgin F, Guzeldemir E. Topical cannabinoid antinociception: synergy with spinal sites. Pain 2003;105:11—6.
[53] Yesilyurt O, Dogrul A, Gul H, Seyrek M, Kusmez O, Ozkan Y, et al. Topical cannabinoid enhances topical morphine antinociception. Pain 2003;105:303—8.
[54] Bridges D, Ahmad K, Rice AS. The synthetic cannabinoid WIN55,212-2 attenuates hyperalgesia and allodynia in a rat model of neuropathic pain. Br J Pharmacol 2001;133:586—94.
[55] Richardson JD, Kilo S, Hargreaves KM. Cannabinoids reduce hyperalgesia and inflammation via interaction with peripheral CB1 receptors. Pain 1998;75:111—9.
[56] Ellington HC, Cotter MA, Cameron NE, Ross RA. The effect of cannabinoids on capsaicin-evoked calcitonin gene-related peptide (CGRP) release from the isolated paw skin of diabetic and non-diabetic rats. Neuropharmacology 2002;42: 966—75.
[57] Weidner C, Klede M, Rukwied R, Lischetzki G, Neisius U, Skov PS, et al. Acute effects of substance P and calcitonin gene-related peptide in human skin — a microdialysis study. J Invest Dermatol 2000;115:1015—20.
[58] Schmelz M, Luz O, Averbeck B, Bickel A. Plasma extravasation and neuropeptide release in human skin as measured by intradermal microdialysis. Neurosci Lett 1997;230:117—20.
[59] Dvorak M, Watkinson A, McGlone F, Rukwied R. Histamine induced responses are attenuated by a cannabinoid receptor agonist in human skin. Inflamm Res 2003;52:238—45.
[60] Kelly S, Jhaveri MD, Sagar DR, Kendall DA, Chapman V. Activation of peripheral cannabinoid CB1 receptors inhibits mechanically evoked responses of spinal neurons in noninflamed rats and rats with hindpaw inflammation. Eur J Neurosci 2003;18:2239—43.
[61] Neff GW, O'Brien CB, Reddy KR, Bergasa NV, Regev A, Molina E, et al. Preliminary observation with dronabinol in patients with intractable pruritus secondary to cholestatic liver disease. Am J Gastroenterol 2002;97:2117—9.
[62] Kehl LJ, Hamamoto DT, Wacnik PW, Croft DL, Norsted BD, Wilcox GL, et al. A cannabinoid agonist differentially attenuates deep tissue hyperalgesia in animal models of cancer and inflammatory muscle pain. Pain 2003;103:175—86.

[63] Ibrahim MM, Deng H, Zvonok A, Cockayne DA, Kwan J, Mata HP, et al. Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: Pain inhibition by receptors not present in the CNS. Proc Natl Acad Sci USA 2003.
[64] Malan TP, Ibrahim MM, Deng HF, Liu Q, Mata HP, Vanderah T, et al. CB2 cannabinoid receptor-mediated peripheral antinociception. Pain 2001;93:239—45.
[65] Quartilho A, Mata HP, Ibrahim MM, Vanderah TW, Porreca F, Makriyannis A, et al. Inhibition of inflammatory hyperalgesia by activation of peripheral CB2 cannabinoid receptors. Anesthesiology 2003;99:955—60.
[66] Kaminski NE, Abood ME, Kessler FK, Martin BR, Schatz AR. Identification of a functionally relevant cannabinoid receptor on mouse spleen cells that is involved in cannabinoid-mediated immune modulation. Mol Pharmacol 1992;42: 736—42.
[67] Carlisle SJ, Marciano-Cabral F, Staab A, Ludwick C, Cabral GA. Differential expression of the CB2 cannabinoid receptor by rodent macrophages and macrophage-like cells in relation to cell activation. Int Immunopharmacol 2002;2:69—82.
[68] Blazquez C, Casanova ML, Planas A, del Pulgar TG, Villanueva C, Fernandez-Acenero MJ, et al. Inhibition of tumor angiogenesis by cannabinoids. FASEB J 2003;17:529—31.
[69] Galve-Roperh I, Sanchez C, Cortes ML, del Pulgar TG, Izquierdo M, Guzman M. Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. Nat Med 2000;6:313—9.
[70] Sanchez C, de Ceballos ML, del Pulgar TG, Rueda D, Corbacho C, Velasco G, et al. Inhibition of glioma growth in vivo by selective activation of the CB(2) cannabinoid receptor. Cancer Res 2001;61:5784—9.
[71] Leterrier C, Bonnard D, Carrel D, Rossier J, Lenkei Z. Constitutive endocytic cycle of the CB1 cannabinoid receptor. J Biol Chem 2004;279:36013—21.
[72] Martin RS, Luong LA, Welsh NJ, Eglen RM, Martin GR, MacLennan SJ. Effects of cannabinoid receptor agonists on neuronally-evoked contractions of urinary bladder tissues isolated from rat, mouse, pig, dog, monkey and human. Br J Pharmacol 2000;129:1707—15.
[73] Toyoda M, Nakamura M, Morohashi M. Neuropeptides and sebaceous glands. Eur J Dermatol 2002;12:422—7.
[74] Facci L, Dal Toso R, Romanello S, Buriani A, Skaper SD, Leon A. Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. Proc Natl Acad Sci USA 1995;92:3376—80.