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

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

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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)

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