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Pharmacology

Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide

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ABSTRACT **Mast cells are multifunctional bone marrow-derived cells found in mucosal and connective tissues and in the nervous system, where they play important roles in tissue inflammation and in neuroimmune interactions. Very little is known about endogenous molecules and mechanisms capable of modulating mast cell activation. Palmitoylethanolamide, found in peripheral tissues, has been proposed to behave as a local autacoid capable of downregulating mast cell activation and inflammation. A cognate *N*-acylamide, anandamide, the ethanolamide of arachidonic acid, occurs in brain and is a candidate endogenous agonist for the central cannabinoid receptor (CB1). As a second cannabinoid receptor (CB2) has been found in peripheral tissues, the possible presence of CB2 receptors on mast cells and their interaction with *N*-acylamides was investigated. Here we report that mast cells express both the gene and a functional CB2 receptor protein with negative regulatory effects on mast cell activation. Although both palmitoylethanolamide and anandamide bind to the CB2 receptor, only the former downmodulates mast cell activation *in vitro*. Further, the functional effect of palmitoylethanolamide, as well as that of the active cannabinoids, was efficiently antagonized by anandamide. The results suggest that (*i*) peripheral cannabinoid CB2 receptors control, upon agonist binding, mast cell activation and therefore inflammation; (*ii*) palmitoylethanolamide, unlike anandamide, behaves as an endogenous agonist for the CB2 receptor on mast cells; (*iii*) modulatory activities on mast cells exerted by the naturally occurring molecule strengthen a proposed autacoid local inflammation antagonism (ALIA) mechanism; and (*iv*) palmitoylethanolamide and its derivatives may provide antiinflammatory therapeutic strategies specifically targeted to mast cells ("ALIAmides").**

Tissue mast cells are multifunctional immune cells and have been implicated in immediate hypersensitivity and inflammatory reactions (1). Mast cells are present in connective tissues of various organs, in serosal cavities, in mucosal epithelia, and in the nervous system (2–4). Mast cell activation results in the release of many potent inflammatory mediators, such as histamine, proteases, chemotactic factors, metabolites of arachidonic acid, and cytokines (5–7). In turn, this can lead to tissue swelling and damage, leukocyte recruitment, pain, and, ultimately, loss of function. Inflammatory phenomena of an autoimmune and nonautoimmune nature are involved in a variety of disorders of skin and mucosal tissues and of the nervous system (8, 9). Mast cells also produce the neurotrophin nerve growth factor (10), which appears to play an important role in a number of these conditions (8). Unregulated mast cell degranulation thus clearly represents a considerable risk to the organism. Unfortunately, almost nothing is known about endogenous molecules and mechanisms capable of modulating mast cell activation.

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Tissue accumulation of N-acylated glycerophospholipids and free *N*-acylamides, such as palmitoylethanolamide, occurs in some pathological conditions (11) known to be associated with inflammatory reactions. Interestingly, palmitoylethanolamide downmodulates mast cell activation *in vivo* (12) and decreases tissue inflammation (13, 14). Taken together, this suggests that palmitoylethanolamide may exert, via mast cells, a local autacoid antiinflammatory function—hence the acronym autacoid local inflammation antagonism (ALIA) (12, 14).

Other free *N*-acylamides, such as arachidonoylethanolamide (anandamide), isolated from porcine brain have been shown to bind to cannabinoid receptors (15, 16), thus suggesting that long-chain unsaturated *N*-acylamides might behave as endogenous ligands for cannabinoid receptors (16). The psychotropic effects of cannabinoids are presumably mediated via activation of a brain cannabinoid receptor (17), which has been cloned and shown to be a typical member of the G-protein-coupled superfamily of receptors (18). Moreover, peripheral effects of cannabinoids, including antiinflammatory actions, have been reported (19, 20). A cannabinoid receptor binding protein (designated CB2), different from the brain receptor (CB1), that occurs only in peripheral tissues has been cloned (21). *In situ* hybridization demonstrated the CB2 mRNA to be concentrated in the marginal zones of the spleen (21). A similar distribution of cannabinoid binding sites was found by autoradiography and included mainly B-cell-enriched lymphoid tissues (22). The peripheral cannabinoid receptor CB2 thus appears to occur in the immune system and might be involved in some nonpsychotropic actions of cannabinoids.

Anandamide produces many of the behavioral and physiological responses of cannabinoids attributed to activation of the central receptor (23–25). The C_{16} saturated *N*-acylamide palmitoylethanolamide was reported not to interact with the brain receptor (15, 16). To date, there is no evidence that mast cells express CB2, nor have effects of cannabinoids on mast cells been described. The present study was designed to examine mast cells for their expression of CB2 and responses to *N*-acylamides and cannabinoid compounds. RBL-2H3 (rat basophilic leukemia), a rat mast cell line (26), was used. These cells have high-affinity IgE receptors ($Fc_{\varepsilon}RI$) (27–29) and have been used extensively to study the signaling pathways leading to exocytotic release of inflammatory mediators during antigen stimulation of mast cells (30–34). RBL-2H3 cells were found to express the CB2 gene and cannabinoid-binding protein. Cannabimimetic compounds and palmitoylethanolamide, but not anandamide, inhibited the secretory response of RBL-2H3 cells triggered by an immunogenic stimulus. Palmitoylethanolamide thus displays an agonist-like behavior toward CB2.

Abbreviations: ALIA, autacoid local inflammation antagonism; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; DNP, dinitrophenyl; HSA, human serum albumin; RPMC, rat peritoneal mast cell; THC, tetrahydrocannabinol.

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MATERIALS AND METHODS

RBL-2H3 Cell Culture. Rat basophilic leukemia cells of the secreting subline 2H3 were kindly provided by Pietro Ghezzi (Istituto Mario Negri, Milan), and grown in stationary cultures at 37°C in Eagle's minimal essential medium supplemented with 2 mM L-glutamine, 100 units of penicillin per ml, and 20% (vol/vol) heat-inactivated fetal bovine serum. Cells were passaged twice weekly.

Mast Cell Preparation and Purification. Peritoneal mast cells were collected by peritoneal lavage of male Wistar rats (200–250 g) (Charles River Breeding Laboratories) and isolated on a bovine serum albumin (BSA) gradient (35). Final purity of mast cells was ≥90%, as judged by toluidine blue and safranin staining (36).

[^{3}H]Serotonin Release Assay. A mouse monoclonal IgE which is specific for dinitrophenol (DNP) haptens (clone SPE-7; Sigma) was used. Dinitrophenylated human serum albumin (DNP-HSA) was employed as the triggering agent in these experiments. The level of conjunction was 30–40 mol of DNP per mol of albumin (Sigma). Prior to a release assay, RBL-2H3 cells were detached in 0.5 mM EDTA/phosphate-buffered saline (pH 7.2) (PBS) and replated in 96-well microplates (Falcon), with each 6-mm-diameter well containing 10^5 cells in 100 μl of RPMI 1640 medium supplemented with 50 μg of gentamicin per ml, 10% fetal bovine serum, and 0.1 μCi of [^{3}H]serotonin (5-[1,2-^{3}H(N)]hydroxytryptamine binoxalate) (26.4 Ci/mmol; New England Nuclear; 1 Ci = 37 GBq). After 18 hr at 37°C, this medium was replaced with 100 μl of Pipes buffer (25 mM Pipes/100 mM NaCl/5 mM KCl/0.4 mM $MgCl_2$/1 mM $CaCl_2$/5.6 mM glucose, pH 7.1) containing anti-DNP IgE at 0.3 μg/ml to sensitize the cells. After 1 hr at 37°C, the IgE solution was replaced with 100 μl of prewarmed Pipes buffer containing DNP-HSA at 0.1 μg/ml. Initial studies showed these concentrations to provide for maximal release (15–30% net release) of [^{3}H]serotonin. Cannabinoids and *N*-acylamides were added to the culture wells at this time from 100× or 500× stock solutions in ethanol or dimethyl sulfoxide (DMSO), depending on the supplier's recommendation. The final solvent concentration was therefore 0.2% or 1%. Incubation at 37°C was continued for a further 15 min. The culture supernatants were then collected in Eppendorf tubes and centrifuged for 4 min at 3000 rpm, and 50-μl aliquots were taken for liquid scintillation counting. The cellular contents of each well were solubilized with 100 μl of 1% (vol/vol) Triton X-100 in PBS, and a 50-μl aliquot was taken for counting as above. The percent [^{3}H]serotonin release was calculated as [released dpm/(released dpm + cell-associated dpm)] × 100. Background (spontaneous) release was normally ≤5% of the total radioactivity incorporated and was always subtracted from the stimulated release value ("net" release). IC_{50} values were calculated by the quantal dose–response probits method (37). Neither 0.2% DMSO nor 0.2% ethanol reduced [^{3}H]serotonin release from stimulated cells, whereas 1% solvent produced up to a 40% decline in release.

RNA Extraction and Reverse Transcription–PCR Amplification. Total RNA was extracted from RBL-2H3 cells, rat peritoneal mast cells (RPMCs), neuro-2a mouse neuroblastoma cells, adult rat spleen and cerebellum by the guanidinium thiocyanate–phenol/chloroform procedure (38). The RNA was then precipitated with 4 M LiCl at 4°C overnight and collected by centrifugation at 12,000 × *g* for 15 min. PCR primers and internal hybridization probes were synthesized with a Beckman Oligo 1000 DNA synthesizer. The sequences for CB2 were 5′-TTT-CAC-GGT-GTG-GAC-TCC-3′ (5′ primer), 5′-TAG-GTA-GGA-GAT-CAA-GCG-3′ (3′ primer), and 5′-GGT-GAC-GAG-AGC-TTT-GTA-GGT-AGG-TGG-GTA-GCA-CAG-ACA-TAG-GTA-3′ (internal probe), as reported by Munro *et al.* (21). The sequences for CB1 were 5′-CGT-AAA-GAC-AGC-CCC-AAT-3′ (5′ primer), 5′-CTG-GGT-CCC-ACG-CTG-AAT-3′ (3′ primer), and 5′-GGT-GAC-GAT-CCT-CTT-ATA-GGC-CAG-AGG-CCT-GTG-AAT-GGA-TAT-GTA-3′ (internal probe) and corresponded to nt 546–563, 928–945, and 646–690, respectively, of the mature rat brain cannabinoid receptor gene sequence (18). First-strand cDNA synthesis was performed with the 3′ antisense primer and Moloney murine leukemia virus reverse transcriptase (Perkin–Elmer/Cetus). The reaction volume was then increased 5-fold with water and 25 μl of the reverse-transcribed products were used for PCR, in a final volume of 100 μl containing 4 mM $MgCl_2$, 0.5 mM dNTPs, 0.5 μM primer, and 5 units of *Taq* DNA polymerase Stoffel fragment (Perkin-Elmer/Cetus). The reaction cycle consisted of 1 min each at 94°C, 54°C (52°C for CB1), and 72°C. All cDNAs were amplified for 30 cycles. Amplified products were then electrophoresed in a 4% agarose gel and transferred to nylon filters (Hybond-N, Amersham) for hybridization. The internal probes were 3′ end-labeled with [α-^{32}P]dCTP (3000 Ci/mmol; New England Nuclear) by terminal deoxynucleotidyltransferase (Pharmacia). Hybridization was carried out with the internal probes in standard solutions (39) with 10% formamide and 1% SDS at 42°C overnight. The filters were then washed in 1× standard saline citrate at 25°C and exposed to Hyperfilm-MP (Amersham) for 60 min at −80°C.

Radioligand Binding Assays. Pelleted RBL-2H3 cells were routinely stored for up to 1 month at −80°C. For membrane preparation, 3×10^8 cells were suspended in 14 ml of buffer A [50 mM Tris·HCl, pH 7.4/0.1% soybean type II trypsin inhibitor (Sigma)]. All steps were carried out at 4°C unless otherwise noted. The suspension was homogenized in a glass/Teflon homogenizer (20 strokes, 1500 rpm), and then centrifuged at 1500 × *g* for 10 min. The supernatant was retained, the pellet was resuspended in 14 ml of buffer A, and the homogenization step was repeated. This supernatant was pooled with the first and centrifuged at 5000 × *g* for 10 min. The resulting supernatant was centrifuged at 40,000 × *g* for 30 min. The pellet thus obtained was suspended in 14 ml of 50 mM Tris·HCl, pH 7.4/1% fatty acid-free BSA and centrifuged again at 40,000 × *g* for 30 min. This final pellet was rinsed with 50 mM Tris·HCl (pH 7.4) and then suspended in 2 ml of buffer B (50 mM Tris·HCl/3 mM $MgCl_2$/1 mM Tris·EDTA, pH 7.4). This procedure typically yielded 4–8 mg of membrane protein. Freshly prepared membranes were used for each binding experiment.

[^{3}H]WIN 55,212-2 Binding to RBL-2H3 Cell Membranes. Binding experiments were carried out in siliconized tubes by published procedures (16, 17, 21), with modifications. [^{3}H]WIN 55,212-2 (45.5 Ci/mmol, 22 μM in ethanol; New England Nuclear) was serially diluted in binding buffer (buffer B/0.5% fatty acid-free BSA, and samples (5 μl) of 100-fold concentrated solutions were added to a final volume of 500 μl of binding buffer. Nonradioactive WIN 55,212-2 (1 μM) was used to inhibit specific binding. In these cases, a 5 mM stock of WIN 55,212-2 in DMSO was diluted to 100 μM in buffer B, and 5 μl was added per tube. Binding was initiated by adding 35–40 μg (protein) of membranes and the tubes were incubated for 1 hr at 30°C. The tubes were then centrifuged at 40,000 × *g* for 10 min at 22°C. The pellets were rinsed once with ice-cold 0.5% BSA in PBS, resuspended in 50 μl of 1% Triton X-100, and kept at 37°C for 10 min. Fifty microliters of 95% ethanol was then added and radioactivity was assayed by liquid scintillation counting. All compounds used for competition assays were dissolved as 100× solutions in DMSO, and 5 μl of these solutions was added to the binding mixture (total volume, 500 μl) in the presence of 3–4 nM [^{3}H]WIN 55,212-2. Both total and nonspecific binding were also measured in the presence of 1% DMSO. IC_{50} values for each competitor were calculated according to the logit P transformation analysis (40) and are means of at least three experiments performed in triplicate.

Materials. Cannabidiol was obtained from Sigma. WIN 55,212-2 and Δ^8-tetrahydrocannabinol (Δ^8-THC) were from Research Biochemicals International. Palmitoylethanolamide and anandamide were synthesized in our laboratories, with purity >99.5% as assessed by HPLC. All other reagents, unless specified otherwise, were from Sigma.

RESULTS

RBL-2H3 Cells and RPMCs Express the Gene Encoding the Peripheral Cannabinoid Receptor CB2. Total RNA was reverse-transcribed into cDNA and then primers specific for CB1 or CB2 cDNA were used to selectively amplify the desired product by PCR. With the CB2 primers, the 214-bp predicted amplification product was obtained from RBL-2H3 cells. Hybridization with the specific CB2 internal probe confirmed the presence of the expected receptor mRNA (Fig. 1, lane 3). A hybridizing band of identical length was also detected in amplification products from rat spleen (lane 1), as a positive control, and in RPMCs (lane 2). No hybridizing bands were found in PCR-processed RNA when the reverse transcription step was omitted or when the samples were first treated with DNase I, ruling out a possible contribution by genomic DNA. No hybridizing signal was evident in the PCR amplification products of neuro-2a mouse neuroblastoma cells (lane 4), taken as a negative control.

Reverse transcription–PCR with primers specific for CB1 cDNA, followed by hybridization with the corresponding internal oligonucleotide, was performed on RNA extracted from adult rat cerebellum and RBL-2H3 cells. A 399-bp band was clearly visible in the cerebellum sample, whereas no hybridizing band was detected in RBL-2H3 RNA extracts (Fig. 2). RBL-2H3 cells thus appear to express only the peripheral CB2 type of cannabinoid receptor.

RBL-2H3 Cells Contain Cannabinoid Binding Sites. Radioligand binding experiments revealed specific binding of [^{3}H]WIN 55,212-2 to RBL-2H3 cell membranes. A representative saturation isotherm is shown in Fig. 3. Scatchard transformation of the data gave a K_d of 33.5 ± 6.0 nM and a B_{max} of 4.4 ± 0.6 pmol/mg of protein (mean ± SEM, n = 9 membrane preparations). A K_d of 3.7 nM was reported for WIN 55,212-2 binding to membranes from COS cells transfected with CB2 cDNA (21).

Anandamide, a candidate agonist for the brain cannabinoid receptor (15, 16), inhibited [^{3}H]WIN 55,212-2 binding to RBL-2H3 membranes with an IC_{50} of 33 ± 29 nM (Table 1) in the presence of 1% DMSO. Previously, in the apparent absence of solvent a K_i of 1.6 ± 0.4 μM for anandamide was observed for membranes prepared from COS cells transfected with the CB2 cDNA (21). In the present binding system it was difficult to assess the concentration–response behavior of anandamide in the absence of DMSO, most likely due to its poor aqueous solubility. Palmitoylethanolamide likewise proved very efficacious in radioligand displacement from RBL-2H3 membranes with added 1% DMSO, giving an IC_{50} of 1.0 ± 0.6 nM (Table 1). Under the same conditions,

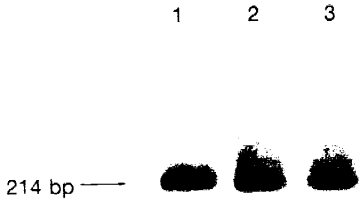

FIG. 1. Expression of CB2 transcripts detected by oligonucleotide probe hybridization of PCR-amplified products from rat spleen (lane 1), RPMCs (lane 2), RBL-2H3 cells (lane 3), and neuro-2a mouse neuroblastoma cells (lane 4).

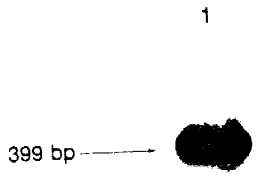

FIG. 2. Expression of CB1 transcripts detected by oligonucleotide hybridization of PCR-amplified products from cerebellum (lane 1) and RBL-2H3 cells (lane 2).

nabilone and Δ^8-THC displaced bound [^{3}H]WIN 55,212-2 with IC_{50} values of 2.6 ± 1.4 nM and 223 ± 120 nM, respectively (Table 1). A nonpsychoactive cannabinoid, cannabidiol, failed to show concentration-dependent displacement activity up to 1 μM, in keeping with its weak affinity for cannabinoid receptors (18, 21).

Cannabinoid- and *N*-Acylamide-Mediated Modulation of Mast Cell Activation. Given the expression of CB2 on both RBL-2H3 cells and RPMCs, it was of interest to examine possible modulatory effects of cannabinoids and the *N*-acylamides on mast cell activation. [^{3}H]Serotonin release from RBL-2H3 cells stimulated with anti-DNP IgE/DNP-HSA is a widely used mast cell model for studying exocytotic release of inflammatory mediators. Two synthetic cannabinoids, nabilone and WIN 55,212-2, strongly inhibited [^{3}H]-serotonin release from DNP-HSA-stimulated RBL-2H3 cells when applied in cotreatment (Table 2). In keeping with the binding data, Δ^8-THC was much less efficacious, and cannabidiol was inactive (up to 60 μM) (Table 2). The rank order of potency of these cannabimimetics was independent of the solvent concentration (0.2% or 1%) used (data not shown). Spontaneous release from resting cells was not altered by any cannabinoid.

In contrast to its radioligand displacement activity, anandamide (in either 0.2% or 1% solvent) failed to block antigen-triggered RBL-2H3 cell degranulation when used up to 50 μM (Table 3). When added together with an active cannabinoid, anandamide antagonized its downmodulatory effect on RBL-2H3 cell [^{3}H]serotonin release, thus effectively shifting the cannabinoid concentration–response curve (irrespective of the final solvent concentration). In the experiments reported here, comparisons were made with respect to cannabinoid EC_{50}

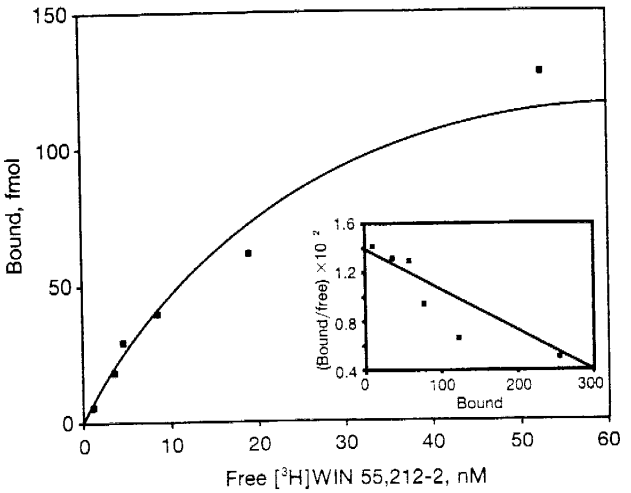

FIG. 3. Saturation isotherm of specific [^{3}H]WIN 55,212-2 binding to RBL-2H3 cell membranes. Specific binding was defined as the difference between the binding that occurred in the presence and in the absence of 1 μM nonradioactive ligand. (*Inset*) Scatchard plot. A representative experiment is shown. Similar results were obtained in eight other experiments with different membrane preparations.

Table 1. Competitive inhibition of [^{3}H]WIN 55,212-2 binding to RBL-2H3 cell membranes by *N*-acylamides and cannabinoids

Compound	IC_{50}, nM
Palmitoylethanolamide	1.0 ± 0.6 (7)
Anandamide	33 ± 29 (5)
Nabilone	2.6 ± 1.4 (4)
Δ^8-THC	223 ± 120 (6)
Cannabidiol	>1000 (3)

Incubations were carried out in the presence of 1% DMSO. Values are means ± SEM, with the number of experiments shown in parentheses.

values with 0.2% solvent (Table 2). Furthermore, anandamide inhibited the action of 30 μM WIN 55,212-2 with an IC_{50} of 1.2 ± 0.6 μM (n = 3). Arachidonic acid and ethanolamine, either singly or in combination, at the same concentrations as anandamide, were ineffective.

Palmitoylethanolamide, unlike anandamide, was highly effective in reducing antigen-evoked [^{3}H]serotonin release from RBL-2H3 cells (EC_{50} of 0.27 ± 0.19 μM, n = 5) (Table 3). As with the active cannabinoids, anandamide (12.5 μM) reduced by 50–100% in three experiments the downmodulatory activity of palmitoylethanolamide used at its EC_{100}. Palmitoylethanolamide displayed occasional modest inhibitory activity at 50–100 μM with 0.2% DMSO. Using RPMCs and the same immunogenic stimulus on [^{3}H]serotonin release, we found analogous behaviors for the active cannabinoids, palmitoylethanolamide, and anandamide (data not shown).

DISCUSSION

The experiments described here assign a functional correlate to the peripheral cannabinoid receptor CB2. We have demonstrated that (*i*) RPMCs and the cognate cell line RBL-2H3 express the CB2 gene; (*ii*) RBL-2H3 cells display a cannabinoid-binding protein (detected with [^{3}H]-WIN 55,212-2) with kinetic parameters comparable to those of the known peripheral receptor; and (*iii*) cannabinoid compounds and a saturated *N*-acylamide, palmitoylethanolamide, but not an unsaturated one, anandamide, downmodulate the immunogenic activation of RBL-2H3 cells. These findings strongly suggest that peripheral cannabinoid receptors exert, upon agonist binding, a negative regulatory effect on mast cell activation and therefore inflammation. The demonstration that palmitoylethanolamide displays an agonistic activity on CB2 suggests that such naturally occurring long-chain saturated fatty acid amides are potential endogenous functional ligands for the receptor.

We examined the agonistic ability of cannabimimetic compounds. A number of such molecules inhibited, in a concentration-dependent fashion, RBL-2H3 cell degranulation induced by IgE receptor crosslinking. Radioligand binding occurred at somewhat lower concentrations of cannabinoid than

Table 2. Cannabinoids downmodulate the immunogenic activation of RBL-2H3 cells: Antagonism by anandamide

Cannabinoid	EC_{50}, μM	
	Control	+ anandamide
Nabilone	2.8 ± 0.2 (9)	4.5 ± 0.6* (3)
WIN 55,212-2	13 ± 2 (9)	112 ± 83* (3)
Δ8-THC	5–30 (7)	>30 (3)
Cannabidiol	>60 (3)	ND

The indicated cannabinoids (0.2% solvent) were present during the 15-min release period. EC_{50} is the concentration inhibiting by 50% the percent net release of [^{3}H]serotonin from DNP-HSA-activated RBL-2H3 cells. Values are means ± SEM with the number of experiments shown in parentheses. Anandamide was used at 12.5 μM. ND, not determined.
*$P < 0.05$ vs. control (Student's t test).

Table 3. Palmitoylethanolamide, but not anandamide, downmodulates immunogenic activation of RBL-2H3 cells

Compound	EC_{50}, μM	
	0.2% solvent	1% solvent
Anandamide	n.a. (4)	n.a. (4)
Palmitoylethanolamide	n.a. (5)	0.27 ± 0.19 (5)

See Table 2 legend for details. Anandamide was not active (n.a.) up to 50 μM, with either 0.2% or 1% solvent (ethanol). Solvent for palmitoylethanolamide was DMSO. Number of experiments is in parentheses.

did the downmodulatory effect on RBL-2H3 cells; however, this may be due to differences in experimental conditions imposed by each of the procedures (41, 42). Cannabidiol, an extremely poor agonist for cannabinoid receptors (18, 21), failed to inhibit mast cell degranulation. The cannabinoids used, with the exception of cannabidiol, inhibited specific binding of [^{3}H]WIN 55,212-2 to RBL-2H3 cell membranes. As RBL-2H3 cells express only the CB2 receptor it is conceivable that the reported antiinflammatory effects of cannabinoid compounds (19, 20, 43) are mediated, at least in part, through their interaction with this mast cell receptor.

Anandamide, the ethanolamide of arachidonic acid, binds to the brain cannabinoid receptor (CB1) (15, 16), producing many of the behavioral and physiological responses of cannabinoids (23–25). Anandamide also binds to the CB2 receptor on RBL-2H3 cells while remaining functionally inactive, and it antagonizes the ability of other cannabinoids to inhibit serotonin release. This action of anandamide was concentration-dependent and competitive, being blocked by increasing concentrations of cannabinoids. Anandamide inhibited specific [^{3}H]WIN 55,212-2 binding to RBL-2H3 membranes, with an apparent affinity much higher than that reported for the cloned peripheral receptor (21). Apparent receptor affinities of cannabinoids can vary depending on the assay system (17, 44) and conditions used (45). Also, measurement of nonspecific binding with an excess of a cannabinoid having a relatively high K_i (e.g., Δ^9-THC or Δ^8-THC; see refs. 19 and 44) can give misleading results. These observations favor the suggestion that anandamide behaves as a functional antagonist for CB2 on mast cells.

Palmitoylethanolamide, like the active cannabinoids but unlike anandamide, both inhibited [^{3}H]WIN 55,212-2 binding to CB2 and downmodulated mast cell activation. In addition, anandamide antagonized the effect of palmitoylethanolamide. Such opposite behavior is typical of differences in the agonistic ability of receptor ligands and suggests that the two *N*-acylamides have different roles toward CB2. In a complementary fashion, anandamide has been reported to be functionally active at the CB1 receptor, while palmitoylethanolamide failed to even bind the receptor. It is thus tempting to speculate that saturated and unsaturated long-chain fatty acid ethanolamides may be receptor type-specific endogenous agonists, thereby selectively providing for central or peripheral activities.

The mechanisms capable of regulating mast cell properties in normal and pathological conditions remain largely obscure. Increases in mast cell numbers occur in a variety of immunoinflammatory conditions. The potential hazards of unregulated mast cell behavior may have evolved such mechanisms to control immune-mediated inflammatory processes. This could be especially important for neuro-immune interactions, given that mast cells are critical gate cells in the regulation of nervous and immune system communication (7, 10, 46). Tissue accumulation of N-acylated glycerophospholipids and free *N*-acylamides (including palmitoylethanolamide) reportedly occurs in pathological degenerative conditions (11). As such conditions are known to be associated with inflammatory reactions, it is not unreasonable that saturated fatty acid

ethanolamides such as palmitoylethanolamide may behave as local autacoids capable of negatively modulating mast cell activation (ALIA mechanism). In keeping with this hypothesis, palmitoylethanolamide reduces mast cell activation associated with inflammatory processes (12, 14). With these considerations in mind, the described pharmacological effects of palmitoylethanolamide could be mediated by interactions with CB2 receptors on mast cells.

Therapeutic implications of the existence of cannabinoid receptors on mast cells include the development of innovative antiinflammatory drugs. That the human brain and peripheral receptors have relatively low sequence homology (18, 47) suggests the possible synthesis of receptor type-selective molecules. Two additional unsaturated fatty acid ethanolamides that bind to the brain receptor have now been isolated from porcine brain (48). With time, peripheral tissues may be found to elaborate a family of palmitoylethanolamide-related compounds, endowed with agonist-like activity for the peripheral cannabinoid receptor and antiinflammatory behavior ("ALIAmides").

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