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Receptor-independent effects of natural cannabinoids in rat peritoneal mast cells in vitro

Jean-Luc Bueb [a,*], Didier M. Lambert [b], Eric J. Tschirhart [a]

[a] *Neuroimmunology and Inflammation, Centre de Recherche Public-Santé, 120, route d'Arlon, L-1150 Luxembourg, Luxembourg*
[b] *Unité de Chimie Pharmaceutique et de Radiopharmacie, Ecole de Pharmacie, Université Catholique de Louvain, avenue Mounier 73.40, B-1200 Brussels, Belgium*

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Abstract

Cannabinoids can activate CB_1 and CB_2 receptors. Since a CB_2 mRNA has been described in rat peritoneal mast cells (RPMC), we investigated a series of cannabinoids and derivatives for their capacity to stimulate RPMC. Effects of natural cannabinoids Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^8-THC, endocannabinoids (anandamide, palmitoylethanolamide) and related compounds (*N*-decanoyl-, *N*-lauroyl-, *N*-myristoyl-, *N*-stearoyl- and *N*-oleoyl-ethanolamines; *N*-palmitoyl derivatives (-butylamine, -cyclohexylamine, -isopropylamine); and *N*-palmitoyl, *O*-palmitoylethanolamine), and synthetic cannabinoids including WIN 55,212-2, SR141716A and SR144528 were assessed for their capacity to induce histamine release or prime RPMC stimulated by compound 48/80. Only Δ^9-THC and Δ^8-THC could induce non-lytic, energy- and concentration-dependent histamine releases from RPMC (respective EC_{50} values: 23.5 ± 1.2; 53.4 ± 20.6 μM, and maxima: 71.2 ± 5.5; 55.7 ± 2.7% of the total RPMC histamine content). These were not blocked by CB_1 (SR141716A) or CB_2 (SR144528) antagonists, but reduced by pertussis toxin (100 ng/ml). Endocannabinoids and analogues did neither induce histamine secretion, nor prime secretion induced by compound 48/80 (0.2 μg/ml). Δ^9-THC and Δ^8-THC induced in vitro histamine secretion from RPMC through CB receptor-independent interactions, partly involving $G_{i/o}$ protein activation.

Keywords: Rat peritoneal mast cell; Δ^9-Tetrahydrocannabinol; Δ^8-Tetrahydrocannabinol; *N*-Palmitoylethanolamine; Anandamide; Cannabinoid

1. Introduction

Mast cells and basophils are polyfunctional immune cells implicated in immediate hypersensitivity and the initiation and development of the inflammatory reaction. These cells are present in many tissues and their activation results in the release of potent preformed and newly formed mediators, such as histamine, proteases, cytokines or metabolites of arachidonic acid. The direct consequence of this activation is tissue swelling and/or damage, modification of function, leucocyte invasion and pain. Abnormal regulation of mast cell physiology leading to cell secretion may represent a pathophysiological situation leading to chronic inflammation and ultimately to a loss of tissue and/or organ function [1].

Natural cannabinoids, originally extracted from

* Corresponding author. Fax: +352-45-19-03-50; E-mail: jean-luc.bueb@crp-sante.lu

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the leaves of *Cannabis sativa*, were used for over 4000 years both for their medicinal and psychomimetic effects. Δ^9-Tetrahydrocannabinol (Δ^9-THC) was the first active isolated cannabinoid, or marihuana-derived molecule, and was described as highly psychoactive [2]. Recently discovered and cloned receptors [3–5] for cannabinoids have speeded up the understanding of their underlying molecular mechanisms. The two subtypes of these G protein coupled receptors are the CB_1, very abundant in the brain areas and supposed to mediate neurobehavioural effects, and the CB_2, restricted to date on cells of the immune system, and thought to modulate immunological responses.

A potential endogenous lipidic ligand was first discovered in porcine brain [6] and shown to have functional effects in vitro or in vivo in the rat brain [7,8]. This molecule, *N*-arachidonoylethanolamine or anandamide, was also shown to have functional activity in mouse vas deferens [6] and its presence was noticed in peripheral tissues of rat and human beings [9]. The central/peripheral classification may therefore be too elementary. Another endogenous compound, *N*-palmitoylethanolamine (PEA), first described to be present in the rat brain, liver and muscle [10], was reported to be a potent nanomolar agonist for the CB_2 receptor [11] and to display anti-inflammatory effects [12,13]. The absence of binding of this compound to brain membranes suggested a high selectivity for peripheral CB_2 receptor [14,15]. However, several authors failed to find any PEA binding to CB_2 receptors ([16] for review). PEA, as other *N*-acylated glycerophospholipids, has been shown to accumulate in inflammatory tissues [17]. It reduces tissue inflammation in vivo [18], down-modulates mast cell activation in vivo [19] and in contrast to anandamide, inhibits immunogenic activation in RBL-2H3 cells [11]. The capacity of these plant-derived or endogenous molecules to modify mast cell histamine release in vitro is currently unknown. To address this question, we assessed for this function cannabinoids from natural (Δ^9- and Δ^8-THC), endogenous (anandamide and PEA) and synthetic (palmitoylethanolamide analogues, WIN 55,212-2, SR141716A and SR144528) origin. Considering the different pathways of activation of rat peritoneal mast cells (RPMC) and the existence of a modulation by PEA of the immunological one [11], we focused our interest on the effect of these various cannabinoids on the secretion occurring through the alternate, receptor-independent pathway of mast cell stimulation [20–22].

2. Materials and methods

2.1. Chemistry

N-Acylethanolamines including PEA (Table 1, compounds *1–10*) were prepared as described elsewhere [23], from their respective acyl chlorides and amines. Spectroscopic data (^{1}H- and ^{13}C-nuclear magnetic resonance, IR) and elemental analysis were in accordance with their chemical structures.

2.2. Chemicals

Δ^9-THC, Δ^8-THC, benzalkonium chloride (BAC, a mixture of quaternary benzyldimethylalkylammonium chlorides), pertussis toxin (PTX), dinitrophenol (DNP), 2-deoxy-D-glucose (deoxyGlc), compound 48/80 and bovine serum albumin (BSA) were bought from Sigma-Aldrich (St. Louis, MO, USA) and anandamide from Cayman Chemical (Ann Arbor, MI, USA). The synthetic agonist WIN 55,212-2 was from RBI (Natick, MA, USA) or from NEN (Boston, MA, USA) when tritiated, and the CB_1 antagonists SR141716A and SR144528 were a generous gift from Sanofi Recherche (Montpellier, France). All other chemicals were of analytical grade and purchased from Sigma-Aldrich or from Merck Eurolab (Louvain, Belgium).

2.3. Mast cells

The procedure followed in the care and euthanasia of the animals was in accordance with the European Community standards on the care and use of laboratory animals (Europe Directive 86/609/CEE Nov. 24, 86). RPMC were purified from male albino Wistar rats weighing 300–350 g. The rats were sacrificed by stunning and bleeding. Then, 10 ml of physiological salt solution (PSS) containing (mM): NaCl, 137; KCl, 2.7; $CaCl_2$, 0.3; $MgCl_2$, 1; NaH_2PO_4, 0.4; glucose, 5.6; HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid], 10 and NaOH to pH 7.4, sup-

plemented with 0.2% BSA, were injected intraperitoneally. The peritoneal cavity was opened after a 2 min gentle massage and the peritoneal fluid collected and centrifuged for 2 min at $180\times g$. Mast cells were purified from the resuspended pellet by centrifugation for 10 min at $220\times g$ on a BSA gradient (40 and 30%, w/v).

2.4. [^{3}H]WIN 55,212-2 binding to mast cells

Binding studies with [^{3}H]WIN 55,212-2 (5–100 nM) were conducted in intact cells at 4°C in Hanks' balanced salt solution with fatty acid-free BSA in Maxisorp 96-well plates (Nunc, Roskilde, Denmark). To define non-specific binding, 10 μM unlabelled WIN 55,212-2 was present when required. Compounds were added with the tritiated ligand, and cells (5×10^5) were then pipetted into the well. After 30 min, cell suspension was rapidly filtered on GF/C glass fibre filters (Whatman, Maidstone, UK) using a 12-well Brandel cell harvester. Radioactivity on filters was measured with a Beckman β-counter by liquid scintillation in Aquasol-2 (New England Nuclear, Boston, MA, USA).

2.5. Histamine release from mast cells

After a 5 min equilibration period of the mast cells (15 000–20 000 purified mast cells/assay) at 37°C in PSS, the cells were challenged for 5 min with the various compounds. The reactions were stopped by adding ice cold buffer to the samples. Histamine from supernatants was assayed fluorometrically according to the method of Shore et al. [24] without extraction steps, and expressed as a percentage of the total histamine content of the cells, determined by lysing control cells with trichloroacetic acid. Spontaneous histamine release never exceeded 8% and was subtracted from the induced releases.

2.6. Inhibition of histamine release

Treatment of the mast cells with 5.6 mM 2-deoxy-D-glucose (instead of 5.6 mM glucose, control) and/or 100 μM dinitrophenol was done in PSS for 30 min at 37°C. The effect of pertussis toxin on histamine release was assessed after incubation of the mast cells for 2 h at 37°C in PSS. After either of these treatments, the cells were challenged for 5 min with the various cannabinoids as described above.

The effect of BAC, a mixture of quaternary benzyldimethylalkylammonium chlorides, on histamine release was assayed as described previously [25], allowing BAC and the cannabinoids to act simultaneously for 10 min at 37°C on purified mast cells in PSS.

2.7. Statistics

Values are means ± S.E.M. of the indicated number of duplicate experiments. Statistical analyses of data were established using the two-tail Student's *t*-test (*$P<0.05$, significantly different from control).

3. Results

3.1. Effects of natural and synthetic cannabinoids on histamine release from mast cells

Natural cannabinoids Δ^9-THC or Δ^8-THC, endogenous molecules anandamide or PEA, PEA derivatives (Table 1, compounds *1–10*) and synthetic molecules like SR141716A, SR144528 (CB_1 and CB_2

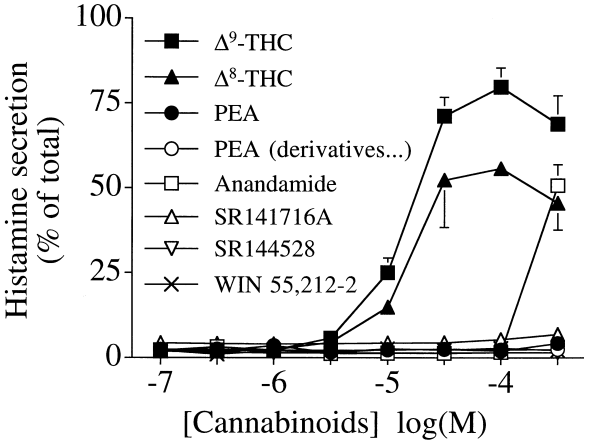


Fig. 1. Effect of various cannabinoid derivatives on histamine release from mast cells. Purified rat peritoneal mast cells in balanced salt solution supplemented with 0.2% BSA were stimulated for 5 min at 37°C with the indicated molecules. All the PEA derivatives induced no histamine release; their results are summarized within the open circle symbol. Values are means ± S.E.M. of three duplicate experiments. S.E.M. not shown are included within the symbols.

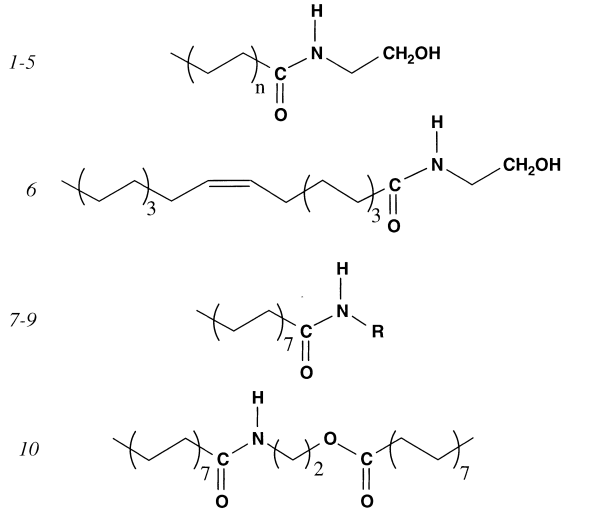
antagonists, respectively) or WIN 55,212-2 were assayed as triggers for histamine release from rat peritoneal mast cells. Fig. 1 shows that only Δ^9-THC and Δ^8-THC were able to induce a concentration-dependent histamine release, with EC_{50} values of 23.5 ± 1.2 and 53.4 ± 20.6 μM, reaching a maximum of 71.2 ± 5.5 and 55.7 ± 2.7% of the total histamine content of the cells, respectively. Anandamide had no effect on histamine release until 3×10^{-4} M (Fig. 1).

3.2. Metabolic dependence of cannabinoid-induced histamine release

Due to the high cannabinoid concentrations needed to induce histamine liberation, experiments were carried out with 2-deoxy-D-glucose (non-hydrolysable glucose substitute) and/or DNP (mitochondrial respiration chain blocker) to assess the functionality of the histamine secretion from mast cells and to verify that histamine was not released through

Table 1
Homologues (*1–6*) and analogues (*7–10*) of *N*-palmitoylethanolamine

1	*N*-decanoyl-ethanolamine	(n=4)
2	*N*-lauroyl-ethanolamine	(n=5)
3	*N*-myristoyl-ethanolamine	(n=6)
4	*N*-palmitoyl-ethanolamine (PEA)	(n=7)
5	*N*-stearoyl-ethanolamine	(n=8)
6	*N*-oleoyl-ethanolamine	
7	*N*-palmitoyl-R-amine	R = butyl
8	*N*-palmitoyl-R-amine	R = cyclohexyl
9	*N*-palmitoyl-R-amine	R = isopropyl
10	*N*-palmitoyl, *O*-palmitoylethanolamine	



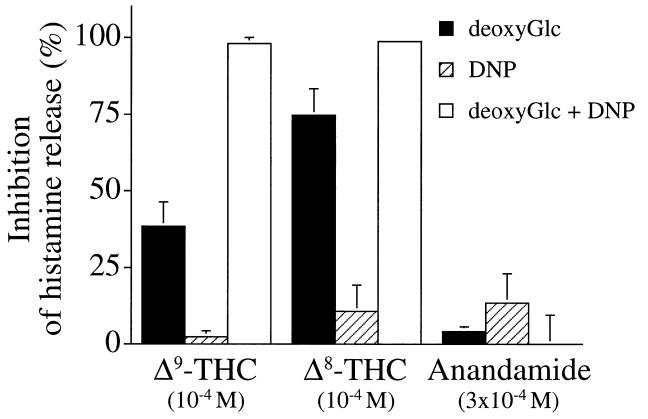


Fig. 2. Inhibition by 2-deoxy-D-glucose and/or dinitrophenol of histamine release induced by Δ^9-THC, Δ^8-THC and anandamide from mast cells. Purified rat peritoneal mast cells were treated in balanced salt solution supplemented with 0.2% BSA for 30 min at 37°C with 2-deoxy-D-glucose (deoxyGlc, 5.6 mM) and/or dinitrophenol (DNP, 100 μM) before being stimulated for 5 min with Δ^9-THC, Δ^8-THC and anandamide at the indicated concentrations. Results are expressed as percentage of inhibition of the corresponding controls (i.e., histamine release in the absence of deoxyGlc and DNP, and in the presence of 5.6 mM glucose). These control values were 79.7 ± 5.7% of the total histamine content for Δ^9-THC, 55.7 ± 2.7% for Δ^8-THC and 50.7 ± 6.2% for anandamide. Values are means ± S.E.M. of three duplicate experiments. S.E.M. not shown are included within the bars.

membrane destruction. Inhibition of the histamine release induced by Δ^9-THC and Δ^8-THC was partial by substituting glucose by 2-deoxy-D-glucose, but total when combined with DNP (Fig. 2), showing the energy dependence, i.e. the physiological process of the mechanism. However, 2-deoxy-D-glucose and/or DNP did not inhibit the histamine release induced by the endogenous cannabinoid anandamide (Fig. 2), suggesting a non-physiological, possibly deleterious effect on mast cell membranes.

3.3. Receptor-independent effects of cannabinoids in rat mast cells

The CB antagonists SR141716A and SR144528, assayed up to 10 μM on histamine secretion induced by Δ^9-THC (Fig. 3A) and Δ^8-THC (Fig. 3B), only showed significant inhibitory effects when used at the highest concentration and for only one concentration (3×10^{-5} M) of both cannabinoids.

PTX was able to inhibit histamine secretion by up to 75% in the case of Δ^8-THC (Fig. 4), the histamine

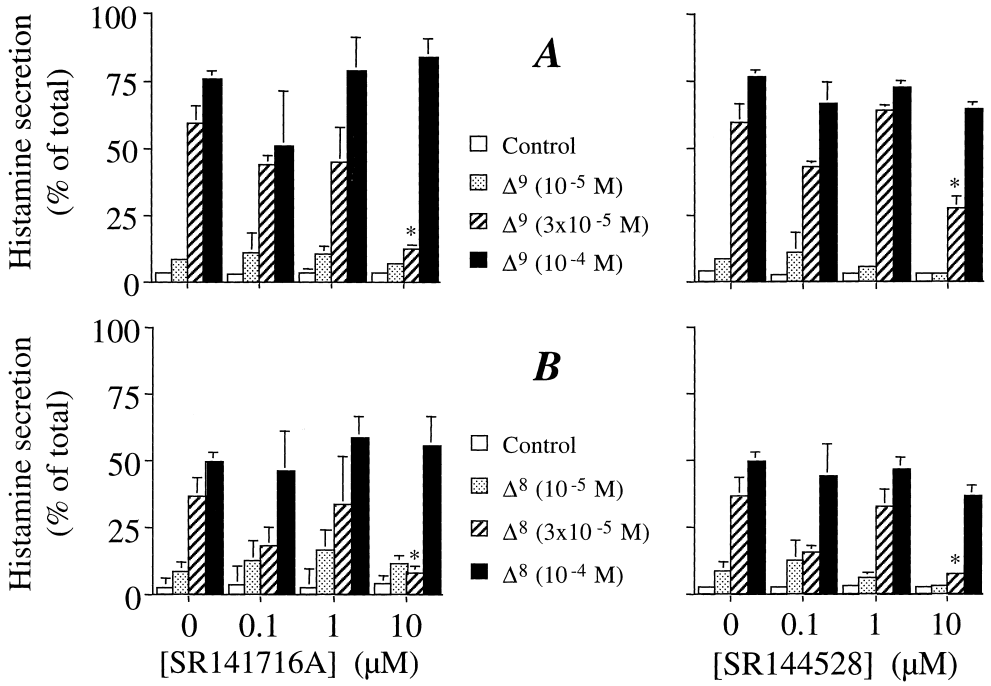
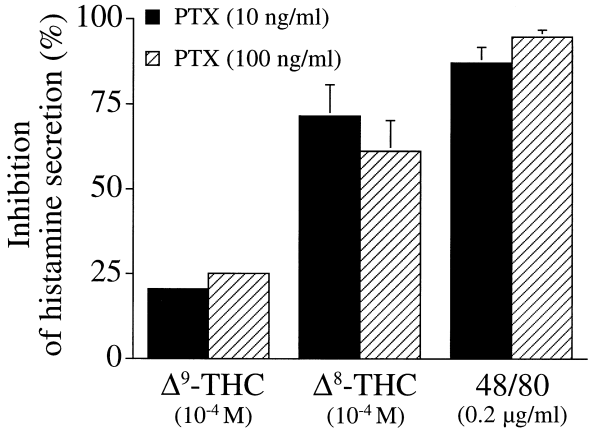


Fig. 3. Effect of SR141716A and SR144528 on mast cell histamine secretion induced by (A) Δ^9-THC and (B) Δ^8-THC. Purified rat peritoneal mast cells were treated with the CB receptor antagonists for 10 min at 37°C at the indicated concentrations in balanced salt solution supplemented with 0.2% BSA, before being stimulated for 5 min with various concentrations of Δ^9- and Δ^8-THC. Values are means ± S.E.M. of three duplicate experiments. S.E.M. not shown are included within the bars. *Significantly different from control stimulation, in the absence of antagonist with the corresponding concentration of Δ^9- or Δ^8-THC ($P < 0.05$).

releaser compound 48/80 being used as internal control. On the other hand, up to its subtoxic concentrations, BAC, supposed to interact with G-proteins, showed no effect on histamine secretion induced by the natural cannabinoids (Fig. 5). All PEA derivatives were also assayed as potential primers for the histamine secretion induced by compound 48/80 to highlight a possible modulatory effect (Table 2). For all the derivatives and concentrations tested, only *N*-palmitoylcyclohexylamine (*8*) showed a significant inhibition ($P < 0.05$), at 10^{-4} M, of the histamine secretion induced by compound 48/80.



3.4. [^{3}H]WIN 55,212-2 binding to mast cells

In our experimental conditions with intact RPMC, no specific binding of [^{3}H]WIN 55,212-2 (5–100 nM),

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Fig. 4. Inhibition by pertussis toxin of mast cell histamine secretion induced by Δ^9-THC, Δ^8-THC and compound 48/80. Purified rat peritoneal mast cells were treated with 10 or 100 ng/ml of pertussis toxin for 2 h at 37°C in balanced salt solution supplemented with 0.2% BSA before being stimulated for 5 min with the indicated concentrations of Δ^9-THC, Δ^8-THC and compound 48/80. Results are expressed as percentage of inhibition of the corresponding controls (i.e., histamine secretion in the absence of pertussis toxin). These control values were 81.9 ± 3.8% of the total histamine content for Δ^9-THC, 64.7 ± 4.8% for Δ^8-THC and 37.2 ± 2.0% for compound 48/80. Values are means ± S.E.M. of three duplicate experiments. S.E.M. not shown are included within the bars.

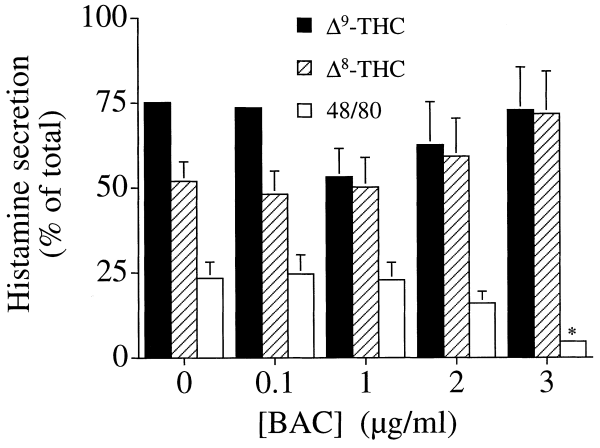

Fig. 5. Effect of benzalkonium chloride on mast cell histamine release induced by Δ^9-THC, Δ^8-THC and compound 48/80. Purified rat peritoneal mast cells were triggered for 10 min at 37°C in balanced salt solution supplemented with 0.2% BSA by a mixture of various concentrations of benzalkonium chloride and 10^{-4} M Δ^9-THC, 10^{-4} M Δ^8-THC or 0.2 μg/ml compound 48/80. Values are means ± S.E.M. of three duplicate experiments. S.E.M. not shown are included within the bars. *Significantly different from control stimulation, without BAC ($P < 0.05$).

a radioligand known to bind to both cannabinoid receptors with a preference for CB_2, was observed.

4. Discussion

Mast cells are polyfunctional immune cells implicated in allergy and in the development of inflammation. As a CB_2 mRNA has been described in mast cells [11], they may represent a potential target for cannabinoids. Cannabinoid receptors recognized a diversity of chemical structures. In this investigation of the effects of cannabinoids to induce or modulate histamine release from rat peritoneal mast cells, we include different cannabinoids differing by their source (natural, endogenous, synthetic) and by their function (agonists, antagonists). We show that rat peritoneal mast cells are able to secrete histamine when stimulated by Δ^9- and Δ^8-THC, two well-known potent natural cannabinoids [26]. This effect is not shared by other cannabinoids used in this study, whatever their function on the receptors: both agonists (WIN 55,212-2) and antagonists (SR141716A and SR144528) as well as analogues of palmitoylethanolamide have no effect on histamine release. The only exception was anandamide. However, this could only be observed at a high concentration, which proved to be deleterious to mast cells, as energy deprivation did not lower anandamide-induced histamine release. Obviously, the ability to modulate histamine secretion strictly depends on the chemical structure: only the cannabinoids containing the benzopyran ring exhibited these properties.

PEA has been reported to be highly effective in reducing antigen-evoked [^{3}H]serotonin release from RPMC [11]. It can moderate mast cell activation

Table 2
Effect on mast cell histamine secretion by compound 48/80 of a pretreatment with non-stimulating concentrations of PEA and its derivatives

48/80 (0.2 μg/ml) preceded by treatment with	[PEA derivatives] log(M)		
	−6	−5	−4
1	50.0 ± 7.0	50.3 ± 8.1	40.2 ± 6.5
2	51.4 ± 8.3	49.1 ± 5.3	45.7 ± 9.1
3	52.6 ± 6.5	49.9 ± 7.7	36.7 ± 6.4
PEA	58.5 ± 2.1	57.0 ± 1.0	52.3 ± 1.9
5	52.0 ± 6.0	49.5 ± 8.5	40.9 ± 7.7
6	53.2 ± 7.0	47.3 ± 7.5	48.0 ± 5.2
7	52.4 ± 6.7	50.7 ± 10.6	43.1 ± 7.5
8	52.0 ± 6.8	49.3 ± 6.6	31.9 ± 8.0*
9	51.4 ± 5.3	52.7 ± 8.5	45.0 ± 5.0
10	52.9 ± 5.8	51.6 ± 6.6	39.2 ± 8.1
48/80 (0.2 μg/ml) alone	54.8 ± 6.0		

Rat peritoneal mast cells were treated or not with PEA and its various derivatives for 10 min at 37°C at the indicated concentrations in PSS before being stimulated for 5 min with 0.2 μg/ml of compound 48/80. Values are histamine secretions (% of total), expressed as means ± S.E.M. of three duplicate experiments. *Significantly different from stimulation by compound 48/80 alone ($P < 0.05$).

induced in vivo by substance P, which would point towards a local autacoid anti-inflammatory function [19]. Substance P triggers rat peritoneal mast cells through a non-immunological transductional pathway, often referred to as peptidergic pathway and common to amphiphilic positively charged molecules (e.g. compound 48/80 or mastoparan [20,21,27,28]). We hence tried to highlight an in vitro modulation by cannabinoids of compound 48/80-stimulated mast cells. Of the various derivatives of PEA, assayed as primers on RPMC in vitro, only *N*-palmitoylcyclohexylamine (*8*) modified histamine secretion induced by compound 48/80. As such, the downmodulating effect of PEA, which has been reported in vivo, may result from a combination of cellular, immunological and/or biochemical signals, and not only from its effects on mast cells.

A dominating peripheral localization for the CB_2 receptor subtype has been proposed, particularly in studies on immune cells [5,29], and the presence of CB_2 mRNA has previously been noticed in rat peritoneal mast cells [11]. However, we did not observe CB_2 receptors in RPMC with [^{3}H]WIN 55,212-2, a preferential CB_2 ligand [30]. Also, SR144528, a CB_2 antagonist [31], did not reduce histamine release induced by Δ^9- or Δ^8-THC. As these are known to be active on both CB_1 and CB_2 receptors, we used the CB_1 antagonist SR141716A [32] to evidence a possible CB_1 receptor-mediated subtype on mast cells. SR141716A did not reduce histamine release induced by Δ^9- or Δ^8-THC, suggesting that no CB_1 receptors are involved in this mechanism. Therefore, our results do not support the hypothesis of the presence of functional CB_1 or CB_2 receptors in rat peritoneal mast cells in our experimental conditions.

PTX-sensitive G_i and G_o proteins have been found in RPMC [20,22,28,33] and are implicated in the peptidergic receptor-independent stimulation pathway. Benzalkonium chloride, a germicide shown to be a selective inhibitor of histamine release induced by compound 48/80 [25] and supposed to act at the G-protein level, revealed no significant concentration-dependent inhibitory effect on histamine release induced by Δ^9-THC or Δ^8-THC. However, PTX partially reduced histamine secretion induced by Δ^9- and Δ^8-THC, indicating that $G_{i/o}$ proteins were only partly responsible for this phenomenon. CB_1 and CB_2 receptors have been shown to couple to PTX-sensitive G-proteins [34,35], but our results in RPMC do not support a classical ligand-receptor interaction. Instead, we wish to propose that Δ^9- and Δ^8-THC are capable of inducing histamine release by using, only to a certain extent, the peptidergic $G_{i/o}$-related secretion pathway.

Natural cannabinoids may exert immunosuppressive effects, at high concentrations, by inhibiting the B lymphocyte proliferation [36,37]. This effect was not blocked by pertussis toxin pretreatment, indicating the involvement of cannabinoid-induced membrane effects on lymphocytes [38] in addition to their low nanomolar immunostimulant receptor-mediated effects [38]. Cannabinoids can indeed affect enzymes or ion channels [39–43]. Membrane structure seems to be modified by cannabinoids: they can position themselves within or near the membrane [44,45], or change lipid order [46] and diffusion [47–49]. Some effects were also found not to be correlated with pharmacological potency [46].

Considering (i) the particularity of RPMC, which can secrete histamine following the classical immunological or the peptidergic receptor-independent activation, and (ii) the highly hydrophobic structure of Δ^9-THC, Δ^8-THC, PEA and the other cannabinoids described above, it may be assumed that all the effects observed in these cells may result from CB receptor-independent interactions. These interactions, deleterious in the case of anandamide, remain physiological even at high concentrations for the two active molecules, the plant-derived Δ^9-THC and Δ^8-THC. Moreover, they partly involve $G_{i/o}$ protein activation.

Acknowledgements

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References

[1] S.J. Galli, A.M. Dvorak, H.F. Dvorak, Prog. Allergy 34 (1984) 1–141.

[2] Y. Gaoni, R. Mechoulam, J. Am. Chem. Soc. 86 (1964) 1646–1647.
[3] C.M. Gerard, C. Mollereau, G. Vassart, M. Parmentier, Biochem. J. 279 (1991) 129–134.
[4] L.A. Matsuda, S.J. Lolait, M.J. Brownstein, A.C. Young, T.I. Bonner, Nature 346 (1990) 561–564.
[5] S. Munro, K.L. Thomas, M. Abu-Shaar, Nature 365 (1993) 61–65.
[6] W.A. Devane, L. Hanus, A. Breuer, R.G. Pertwee, L.A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger, R. Mechoulam, Science 258 (1992) 1946–1949.
[7] P.B. Smith, D.R. Compton, S.P. Welch, R.K. Razdan, R. Mechoulam, B.R. Martin, J. Pharmacol. Exp. Ther. 270 (1994) 219–227.
[8] J.M. Walker, S.M. Huang, N.M. Strangman, K. Tsou, M.C. Sanudo-Pena, Proc. Natl. Acad. Sci. USA 96 (1999) 12198–12203.
[9] C.C. Felder, A. Nielsen, E.M. Briley, M. Palkovits, J. Priller, J. Axelrod, D.N. Nguyen, J.M. Richardson, R.M. Riggin, G.A. Koppel, S.M. Paul, G.W. Becker, FEBS Lett. 393 (1996) 231–235.
[10] N.R. Bachur, K. Masek, K.L. Melmon, S. Udenfriend, J. Biol. Chem. 240 (1965) 1019–1024.
[11] L. Facci, R. Faltoso, S. Romanello, A. Buriani, S.D. Skaper, A. Leon, Proc. Natl. Acad. Sci. USA 92 (1995) 3376–3380.
[12] S. Mazzari, R. Canella, L. Petrelli, G. Marcolongo, A. Leon, Eur. J. Pharmacol. 300 (1996) 227–236.
[13] S.I. Jaggar, F.S. Hasnie, S. Sellaturay, A.S. Rice, Pain 76 (1998) 189–199.
[14] C.C. Felder, E.M. Briley, J. Axelrod, J.T. Simpson, K. Mackie, W.A. Dewane, Proc. Natl. Acad. Sci. USA 90 (1993) 7656–7660.
[15] T. Sheskin, L. Hanus, J. Slager, Z. Vogel, R. Mechoulam, J. Med. Chem. 40 (1997) 659–667.
[16] D.M. Lambert, V. DiMarzo, Curr. Med. Chem. 6 (1999) 739–755.
[17] V. Natarajan, P.V. Reddy, P.C. Schmid, H.H. Schmid, Biochim. Biophys. Acta 712 (1982) 342–355.
[18] F. Perlik, H. Raskova, J. Elis, Acta Physiol. Acad. Sci. Hung. 39 (1971) 395–400.
[19] L. Aloe, A. Leon, R. Levi-Montalcini, Agents Actions 39 (1993) C145–C147.
[20] T. Nakamura, M. Ui, J. Biol. Chem. 260 (1985) 3584–3593.
[21] M. Mousli, J.-L. Bueb, C. Bronner, B. Rouot, Y. Landry, Trends Pharmacol. Sci. 11 (1990) 358–362.
[22] J.-L. Bueb, M. Mousli, C. Bronner, B. Rouot, Y. Landry, Mol. Pharmacol. 38 (1990) 816–822.
[23] D.M. Lambert, F.G. DiPaolo, P. Sonveaux, M. Kanyonyo, S.J. Govaerts, E. Hermans, J.-L. Bueb, N.M. Delzenne, E.J. Tschirhart, Biochim. Biophys. Acta 1440 (1999) 266–274.
[24] P.A. Shore, A. Burkhalter, V.H. Cohn, J. Pharmacol. Exp. Ther. 127 (1959) 182–185.
[25] G.W. Read, E.F. Kiefer, J. Pharmacol. Exp. Ther. 211 (1979) 711–715.
[26] R. Mechoulam, Marijuana: Chemistry, Pharmacology, Metabolism and Clinical Effects, Academic Press, New York, 1973.
[27] H. Saito, F. Okajima, T.F. Molski, R.I. Sha'afi, M. Ui, T. Ishizaka, J. Immunol. 138 (1987) 3927–3934.
[28] M. Aridor, L.M. Traub, R. Sagi-Eisenberg, J. Cell Biol. 111 (1990) 909–917.
[29] A.B. Lynn, M. Herkenham, J. Pharmacol. Exp. Ther. 268 (1994) 1612–1622.
[30] C.C. Felder, K.E. Joyce, E.M. Briley, J. Mansouri, K. Mackie, O. Blond, Y. Lai, A.L. Ma, R.L. Mitchell, Mol. Pharmacol. 48 (1995) 443–450.
[31] M. Rinaldi-Carmona, F. Barth, J. Millan, J.M. Derocq, P. Casellas, C. Congy, D. Oustric, M. Sarran, M. Bouaboula, B. Calandra, M. Portier, D. Shire, J.C. Breliere, G.L. Le Fur, J. Pharmacol. Exp. Ther. 284 (1998) 644–650.
[32] M. Rinaldi-Carmona, F. Barth, M. Heaulme, D. Shire, B. Calandra, C. Congy, S. Martinez, J. Maruani, G. Neliat, D. Caput, P. Ferrara, P. Soubrie, J.C. Breliere, G. Lefur, FEBS Lett. 350 (1994) 240–244.
[33] M. Aridor, G. Rajmilevich, M.A. Beaven, R. Sagi-Eisenberg, Science 262 (1993) 1569–1572.
[34] K. Mackie, B. Hille, Proc. Natl. Acad. Sci. USA 89 (1992) 3825–3829.
[35] D.M. Slipetz, G.P. O'Neill, L. Favreau, C. Dufresne, M. Gallant, Y. Gareau, D. Guay, M. Labelle, K.M. Metters, Mol. Pharmacol. 48 (1995) 352–361.
[36] S. Specter, G. Lancz, J. Hazelden, Int. J. Immunopharmacol. 12 (1990) 261–267.
[37] H. Schwarz, F.J. Blanco, M. Lotz, J. Neuroimmunol. 55 (1994) 107–115.
[38] J.M. Derocq, M. Segui, J. Marchand, G. Le Fur, P. Casellas, FEBS Lett. 369 (1995) 177–182.
[39] S. Burstein, S.A. Hunter, J. Clin. Pharmacol. 21 (1981) 240S–248S.
[40] S.L. Dalterio, S.A. Bernard, C.R. Esquivel, Eur. J. Pharmacol. 137 (1987) 91–100.
[41] C.J. Hillard, J.A. Auchampach, Biochim. Biophys. Acta 1220 (1994) 163–170.
[42] J.S. Poling, M.A. Rogawski, N. Salem Jr., S. Vicini, Neuropharmacology 35 (1996) 983–991.
[43] J.M. Derocq, M. Bouaboula, J. Marchand, M. Rinaldi-Carmona, M. Segui, P. Casellas, FEBS Lett. 425 (1998) 419–425.
[44] D.P. Yang, A. Banijamali, A. Charalambous, G. Marciniak, A. Makriyannis, Pharmacol. Biochem. Behav. 40 (1991) 553–557.
[45] P. Martel, A. Makriyannis, T. Mavromoustakos, K. Kelly, K.R. Jeffrey, Biochim. Biophys. Acta 1151 (1993) 51–58.
[46] A.S. Bloom, W.S. Edgemond, J.C. Moldvan, Neurochem. Res. 22 (1997) 563–568.
[47] E.P. Bruggemann, D.L. Melchior, J. Biol. Chem. 258 (1983) 8298–8303.
[48] A. Makriyannis, D.P. Yang, R.G. Griffin, S.K. Das Gupta, Biochim. Biophys. Acta 1028 (1990) 31–42.
[49] D.P. Yang, T. Mavromoustakos, K. Beshah, A. Makriyannis, Biochim. Biophys. Acta 1103 (1992) 25–36.