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Effects of cannabinoid receptor agonists on immunologically induced histamine release from rat peritoneal mast cells

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Abstract

Immunologic activation of mast cells through the cross-linking of high affinity IgE receptors results in the release of inflammatory mediators which are important in the pathogenesis of allergic reactions. Early studies investigating the effects of palmitoylethanolamide on animal models of inflammation and on rat mast cells led to the hypothesis that endogenous cannabinoids might act as local autacoids which suppressed inflammation by reducing the activation of mast cells. However, more recent studies produced contradicting results. In order to evaluate if cannabinoid receptors are present in mast cells, we studied the effects of endocannabinoids (anandamide and palmitoylethanolamide) and synthetic cannabimimetics (CP 55,940, WIN 55,212-2 and HU-210) on histamine release from rat peritoneal mast cells. When incubated with mast cells alone, only anandamide could induce significant level of histamine release at concentrations higher than 10^{-6} M. When mast cells were activated with anti-IgE, the histamine release induced was not affected by anandamide, palmitoylethanolamide and CP 55,940. In contrast, both WIN 55,212-2 and HU-210 enhanced anti-IgE-induced histamine release at 10^{-5} M and preincubation did not increase the potency. The histamine releasing action of anandamide and the enhancing effects of WIN 55,212-2 and HU-210 on anti-IgE-induced histamine release were not reduced by the cannabinoid receptor antagonists, AM 281 and AM 630. In conclusion, the present study does not support the hypothesis that cannabinoids suppress mast cell activation. Instead, some of the cannabinoid receptor-directed ligands tested enhanced mast cell activation. However, the high concentrations required and the failure of cannabinoid receptor antagonists to reverse such effects also question the existence of functional cannabinoid receptors in mast cells.

Keywords: Cannabinoid receptor; Mast cell; Histamine release, anti-IgE-induced; Anandamide; Palmitoylethanolamide; WIN 55,512-2

1. Introduction

Allergic reactions and the underlying inflammation are often initiated by activation of mast cells (Wasserman, 1983). Although the activation of mast cells immunologically through the cross-linking of high affinity IgE receptors is well documented, little is known about the physiological regulation of inappropriate mast cell mediator release. In this regard, it has been proposed that endogenous cannabinoids might act as local autacoids which suppressed inflammation by reducing the activation of mast cells (Aloe et al., 1993). Cannabinoid receptors are currently categorised into CB_1 and CB_2 subclasses and their amino acid sequences are typical of G-protein-coupled receptors. In fact, these receptors have been reported to couple to pertussis-toxin-sensitive G-proteins to inhibit adenylate cyclase activity (Matsuda, 1997). While the CB_1 receptor exits in both the central nervous system and in peripheral tissues, the CB_2 receptor has so far been located only in peripheral tissues. Hence, it is generally believed that the psychoactive actions of cannabinoids are mediated via the central CB_1 receptors while the immunomodulatory actions of cannabinoids are mediated mainly via the peripheral CB_2 receptors. Various endogenous cannabinoid receptor agonists (endocannabinoids) have been suggested and these include anandamide, 2-arachidonyl glycerol and palmitoylethanolamide (Pertwee, 1997).

Since the characterisation of cannabinoid receptors was only established within the last decade, there is only a limited literature on the interaction of mast cells and cannabinoids. A detailed study by Facci et al. (1995) demonstrated that both rat peritoneal mast cells and rat

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basophilic leukaemia (RBL)-2H3 cells expressed binding sites for cannabinoids and the gene encoding the CB_2 receptor. Together with the observation that mediator release from these cells was inhibited by palmitoylethanolamide but not by anandamide, these authors concluded that activation of mast cell cannabinoid CB_2 receptors might contribute to the endogenous control of inflammation. This postulation was further supported by the in vivo study of Mazzari et al. (1996) which reported that orally administered palmitoylethanolamide could reduce oedema formation and inflammatory hyperalgesia by suppressing mast cell activation. However, contradicting results to this early belief were reported recently. In their study comparing the affinity of a series of palmitoylethanolamide homologues and analogues to cannabinoid receptors in various systems, Lambert et al. (1999) concluded that it was unlikely for palmitoylethanolamide to be an endogenous agonist of the CB_2 receptor and the previously reported suppression of mediator release from RBL-2H3 cells was independent of cannabinoid receptor activation. They also demonstrated in a more recent study that certain cannabinoids modulated mast cell histamine release through receptor-independent effects (Bueb et al., 2001).

With the recent advance in the pharmacology of cannabinoid receptors and the availability of various cannabinoid receptor-directed ligands from commercial sources, the current study investigated the effects of cannabinoid analogues on histamine release from immunologically activated mast cells in order to determine if functional cannabinoid receptors actually exist in mast cells.

2. Materials and methods

2.1. Sensitization of rats with ovalbumin

All the experiments and procedures described in this paper complied with the European Community guidelines for the use of experimental animals and were approved by the Animal Research Ethics Committee of the Chinese University of Hong Kong. Male Sprague–Dawley rats (200–250 g) obtained from the Laboratory Animal Services Centre, Faculty of Medicine, Chinese University of Hong Kong were sensitized by a single intraperitoneal injection of a 0.5-ml emulsion containing ovalbumin (60 mg/ml), aluminium hydroxide (240 mg/ml) and pertussis vaccine (1.33 i.u./ml) in 0.01 M phosphate-buffered saline. The animals remained sensitized for 3 to 6 weeks after sensitization (Pauwels et al., 1979) and were used within this period.

2.2. Isolation and purification of rat peritoneal mast cells

Sensitized rats were killed by decapitation and exsanguination. Rat peritoneal mast cells were isolated and purified over Percoll density gradient as previously described (Chan et al., 2000). Briefly, mixed peritoneal cells (containing 4–5% mast cells) were obtained by peritoneal lavage following the intraperitoneal injection of HEPES buffer (137 mM NaCl, 5.6 mM glucose, 10 mM *N*-2-hydroxyethylpiperazine-*N*′ -2-ethanesulphonic acid (HEPES), 2.7 mM KCl, 0.4 mM NaH_2PO_4 and 1 mM $CaCl_2$, pH 7.4) supplemented with 50 i.u./ml of heparin. The mixed peritoneal cells were then washed twice by centrifugation (180 × *g*, 5 min, 4 °C) and were resuspended in 1 ml of HEPES buffer supplemented with 1 mg/ml of bovine serum albumin. The cell suspension was then mixed with 4 ml of 90% Percoll and 1 ml of bovine serum albumin-supplemented HEPES buffer was then carefully layered over the Percoll–cell mixture. Purification was performed by centrifugation (150 × *g*, 25 min, 4 °C) which allowed cell separation and gradient formation simultaneously. Mast cells gathered in a layer at the bottom of the tube, whereas other cells formed a rather compact layer on top of the gradient and could easily be removed by aspiration. The mast cell fraction was then washed twice in HEPES buffer by centrifugation and finally resuspended to the desired cell density in HEPES buffer prewarmed at 37 °C.

2.3. Experimental procedures

Mast cells were resuspended to a cell density of 10^5 cells/ml and were equilibrated at 37 °C for 10 min in HEPES buffer. In experiments studying the effects of the cannabinoids on mast cells, 200 μl of the equilibrated cells was added to 200 μl of buffer with or without a cannabinoid. Anti-rat immunoglobulin E antibody (anti-IgE) was either added to the buffer immediately before the addition of cells or 10 min after the addition of cells for studying the effect of preincubation with cannabinoids on anti-IgE activation of mast cells. Histamine release from activated cells was then stopped 10 min later with the addition of 1 ml of ice cold HEPES buffer. Cells and supernatants were separated by centrifugation (180 × *g*, 5 min, 4 °C). The cell pellets were resuspended in 1.4 ml of HEPES buffer and were boiled for 10 min to liberate the residual histamine. Histamine content in both supernatants and cell pellets were determined spectrofluorometrically using a Bran + Luebbe auto-analyser which automatically extracted histamine from samples using the method of Shore et al. (1959). In experiments studying the effect of cannabinoid receptor antagonists, cells were first incubated with an antagonist for 10 min and subsequently with a cannabinoid for a further 10 min before being activated with anti-IgE. In all the experiments, control tubes containing 0.01% DMSO alone were included to ensure that both the spontaneous and anti-IgE-induced histamine release were not affected by the solvent.

2.4. Materials

All the cannabinoid receptor-directed ligands were purchased from Tocris and these included the endocannabi-

noids anandamide and palmitoylethanolamide, the synthetic cannabimimetics CP 55,940, WIN 55,212-2 and HU-210 as well as the cannabinoid receptor antagonists AM 281 and AM 630. All compounds were dissolved in dimethyl sulphoxide (DMSO) as 100 mM stock and were diluted to the desired concentrations in HEPES buffer. The final concentration of DMSO at the highest concentrations of cannabinoids tested was not more than 0.01% (v/v). Sheep anti-rat IgE for the activation of mast cells and pertussis vaccine for the active sensitization of animals were purchased from ICN Biomedicals, Costa Meesa, USA and Pasteur Merieux SV, France, respectively. All other chemicals were of analytical grade and were purchased from Sigma, St. Louis, USA.

2.5. Data presentation

The results are expressed as a percentage of the total histamine content of the cells which had been released into the supernatant:

$$\text{Histamine release } (\%) = \{S/(S+C)\} \times 100\%$$

where S = amount of histamine released into the supernatant, C = amount of histamine remaining in the cell pellet. All histamine releases were adjusted for the spontaneous value in buffer only, which is generally less than 10% of total cellular histamine content. Data were presented as mean ± standard error of the mean (S.E.M.) for n experiments. Where appropriate, data were compared using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test to determine statistical differences after multiple comparisons (GraphPad Prism; GraphPad Software, San Diego, USA). A probability value of less than 0.05 was considered significant.

3. Results

3.1. Effects of anandamide, palmitoylethanolamide and synthetic cannabinoid receptor agonists on mast cell histamine release

When incubated in buffer alone, rat peritoneal mast cells released spontaneously less than 10% of total cellular content of histamine; cells in control tubes containing 0.01% of DMSO released similar level of histamine. With the exception of anandamide, all the tested compounds did not induce significant histamine release when they were incubated with rat peritoneal mast cells for up to 20 min at the highest concentration of 10^{-5} M tested. Anandamide induced significant histamine release from mast cells at concentrations higher than 3×10^{-6} M after 20-min incubation. At the highest concentration of 10^{-5} M tested, anandamide induced the release of 12.1 ± 5.6% of total cellular histamine after incubating with mast cells for 20 min (Fig. 1).

3.2. Effects of anandamide, palmitoylethanolamide and synthetic cannabinoid agonists on anti-IgE-induced histamine release from rat peritoneal mast cells

In general, anti-IgE at 1:300 dilution induced around 20% of histamine release from actively sensitized rat peritoneal mast cells; similar level of histamine was released by

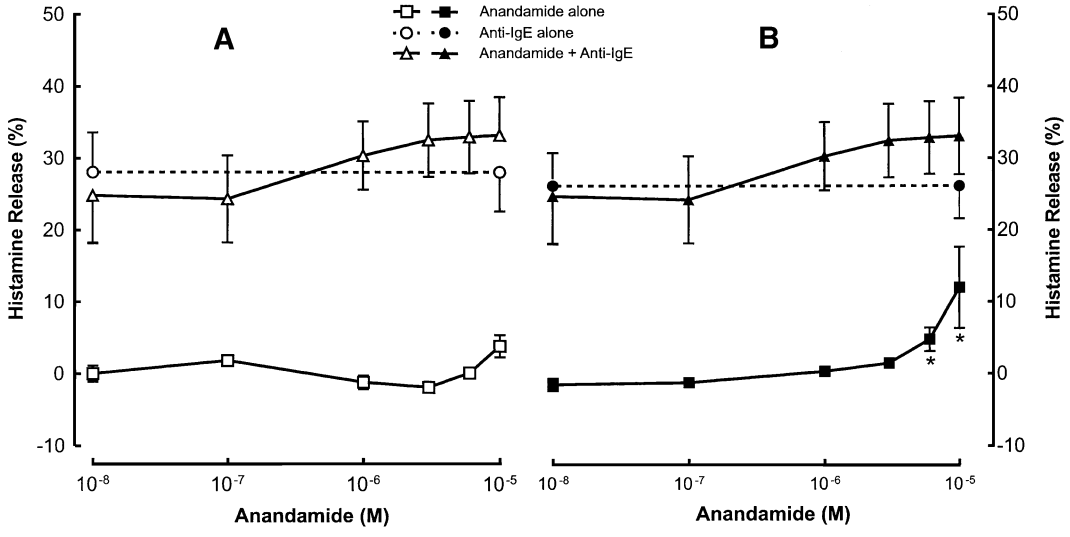

Fig. 1. Effects of anandamide on histamine release from rat peritoneal mast cells. (A) Cells were incubated with anandamide alone, anti-IgE (1:300 dilution) alone or anti-IgE in combination with increasing concentrations of anandamide for 10 min. (B) Cells were incubated with anandamide alone for 20 min or activated with anti-IgE for 10 min following incubation with buffer or anandamide for 10 min. All results were corrected for spontaneous histamine release of (A) 8.9 ± 0.5% and (B) 11.7 ± 0.3% in buffer alone. Data are shown as means ± S.E.M. for $n = 5$. * Indicates $p < 0.05$ when comparing histamine release induced by anandamide alone with spontaneous release in buffer alone.

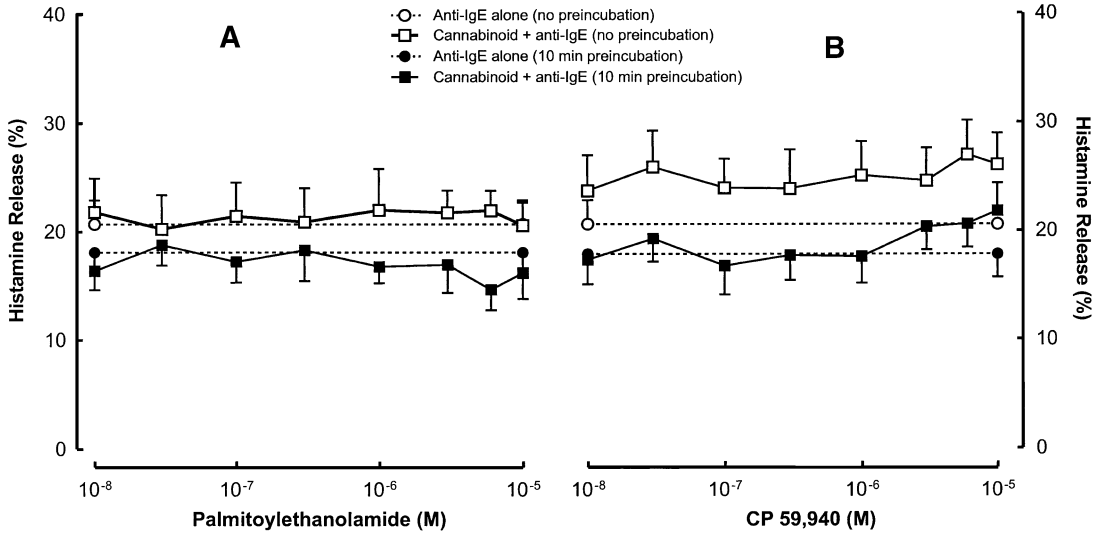

Fig. 2. Effects of (A) palmitoylethanolamide and (B) CP 55,940 on anti-IgE-induced histamine release from rat peritoneal mast cells. Anti-IgE (1:300 dilution) was added to the cells following no preincubation (○, □) or 10-min incubation (●, ■) with the tested cannabinoid. All results were corrected for spontaneous histamine release of 8.2 ± 1.6% (no preincubation) and 8.8 ± 1.8% (10-min preincubation) in buffer. Both palmitoylethanolamide and CP 55,940 alone induced no significant histamine release even at 10^{-5} M. Data are shown as means ± S.E.M. for $n = 4$.

cells activated with 1:300 dilution of anti-IgE in buffer containing 0.01% DMSO. This level of anti-IgE-induced histamine release was not significantly affected by palmitoylethanolamide or CP 55,940 with or without preincubation (Fig. 2). Despite of the histamine releasing property of anandamide at concentrations above 3×10^{-6} M, histamine release induced by anti-IgE in the presence of anandamide was not significantly different from the sum of individual histamine release induced by the two agents independently (Fig. 1). In contrast, anti-IgE-induced histamine release was enhanced by both HU-210 and WIN 55,212-2 at 10^{-5} M (Fig. 3). Anti-IgE-induced histamine release was increased from 16.1 ± 2.1% to 33.3 ± 5.6% and 40.7 ± 5.7% when the mast cells were activated at the same time as exposure to 10^{-5} M of HU-210 or WIN 55,212, respectively. Preincubation of the cells with the two agents for 10 min, prior to anti-IgE challenge, produced similar level of potentiation; HU-210 and WIN 55,212-2 increased anti-IgE-induced

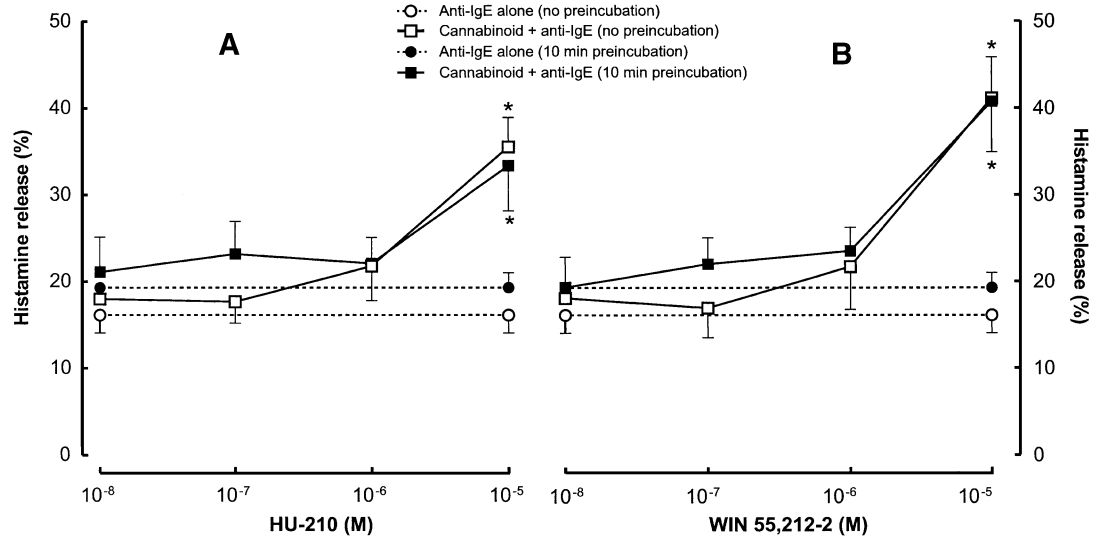

Fig. 3. Effects of (A) HU-210 and (B) WIN 55,212-2 on anti-IgE-induced histamine release from rat peritoneal mast cells. Anti-IgE (1:300 dilution) was added to the cells following no preincubation (○, □) or 10-min incubation (●, ■) with the tested cannabinoid. All results were corrected for spontaneous histamine release of 8.4 ± 0.9% (no preincubation) and 9.8 ± 1.7% (10-min preincubation) in buffer. Both HU-210 and WIN 55,212-2 alone induced no significant histamine release even at 10^{-5} M. Data are shown as means ± S.E.M. for $n = 4$. * Indicates $p < 0.05$ when compared with anti-IgE-induced histamine release in buffer alone.

histamine release from 19.7 ± 1.3% to 35.5 ± 7.4% and 41.1 ± 6.2%, respectively.

3.3. Effects of cannabinoid receptor antagonists on the potentiation of anti-IgE-induced histamine release mediated by HU-210 and WIN 55,212-2

Since AM 281 (Cosenza et al., 2000) and AM 630 (Ross et al., 1999) are reported to demonstrate antagonist properties against cannabinoid receptors, their effects on the enhancing action of HU-210 and WIN 55,212-2 on anti-IgE-induced histamine release were studied to investigate if cannabinoid receptors are involved. As illustrated in Fig. 4, 10^{-6} M of AM 281 did not affect the spontaneous or anti-IgE-induced histamine release from rat peritoneal mast cells. Furthermore, this CB_1 receptor antagonist also failed to reduce the enhanced anti-IgE-induced histamine release in the presence of 10^{-5} M of HU-210 or WIN 55,212-2. Similarly, the cannabinoid CB_2 receptor antagonist AM 630 at 10^{-6} M also failed to affect histamine release from rat peritoneal mast cells induced by anti-IgE alone or enhanced by either HU-210 or WIN 55,212-2 (Fig. 5). Histamine release induced by 10^{-5} M of anandamide was also not

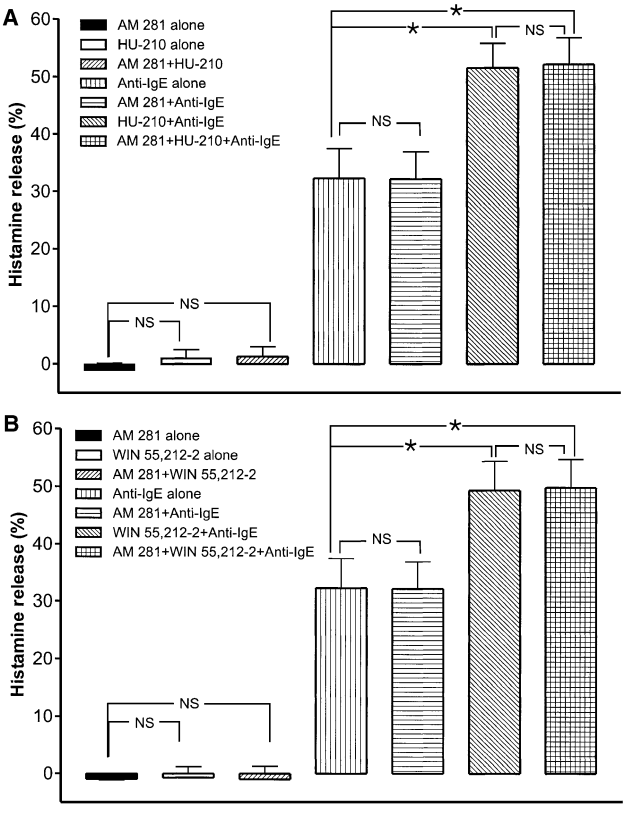

Fig. 4. Effects of 10^{-6} M AM 281 on enhanced anti-IgE (1:300 dilution) induced histamine release mediated by (A) 10^{-5} M HU-210 and (B) 10^{-5} M WIN 55,212-2. All results were corrected for spontaneous histamine release of 9.5 ± 0.6% in buffer. Data are shown as means ± S.E.M. for $n = 6$. * Indicates $p < 0.05$ and NS indicates $p > 0.05$.

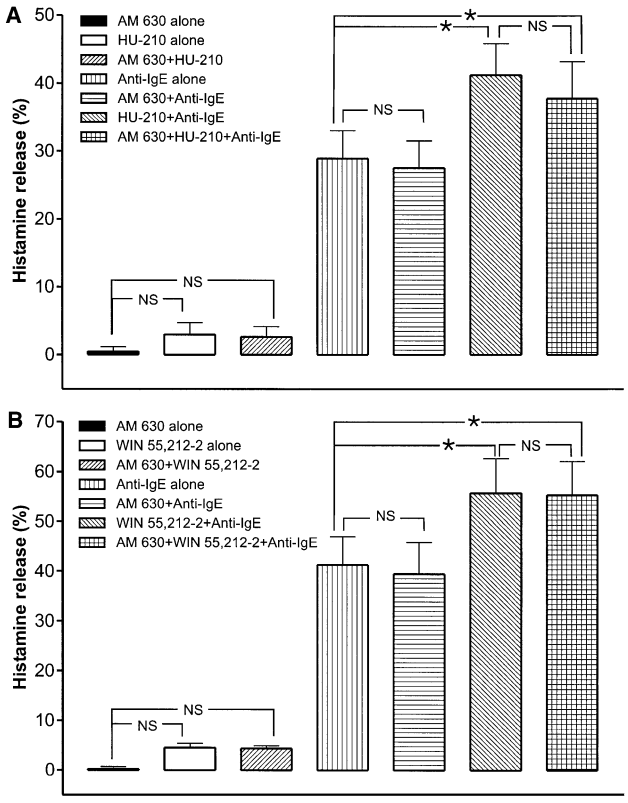

Fig. 5. Effects of 10^{-6} M AM 630 on enhanced anti-IgE (1:300 dilution) induced histamine release mediated by (A) 10^{-5} M HU-210 and (B) 10^{-5} M WIN 55,212-2. All results were corrected for spontaneous histamine release of 10.6 ± 1.2% in buffer. Data are shown as means ± S.E.M. for $n = 6$. * Indicates $p < 0.05$ and NS indicates $p > 0.05$.

significantly altered in the presence of 10^{-6} M of AM 281 or AM 630 (results not shown).

4. Discussion

Aloe et al. (1993) first hypothesized that palmitoylethanolamide behaved as an autacoid, which could locally modulate mast cell activation in response to neurogenic inflammatory stimuli. In addition to demonstrating the anti-inflammatory effects of palmitoylethanolamide using in vivo models of inflammation (Mazzari et al., 1996), these authors also reported that both rat peritoneal mast cells and a mast cell line RBL-2H3 cells expressed both the gene for CB_2 receptor and the functional receptor proteins (Facci et al., 1995). Using radio-ligand binding study, they demonstrated that membrane preparations of RBL-2H3 cells expressed specific binding sites for [^{3}H] WIN 55,212-2. Palmitoylethanolamide, anandamide, nabilone and Δ^8-tetrahydrocannabinol (Δ^8-THC) displaced [^{3}H] WIN 55,212-2 from these specific binding sites with IC_{50} values within the nanomolar range. In the same study, palmitoylethanolamide, nabilone, WIN 55,212-2 and Δ^8-THC were capable of inhibiting serotonin release from immunologically activated RBL-2H3 cells. The EC_{50} values of the inhibitory actions of

these cannabinoids were in the micro-molar range with palmitoylethanolamide being the most potent. Furthermore, anandamide was identified as an antagonist against the inhibitory actions of the active cannabinoids. Hence, it was concluded that the anti-inflammatory effect of palmitoylethanolamide and its derivatives was mediated through suppression of mast cell activation via a CB_2 receptor-dependent mechanism.

In the current study, palmitoylethanolamide demonstrated no effect on spontaneous or anti-IgE-mediated histamine release from rat peritoneal mast cells at concentration as high as 10^{-5} M. Moreover, anandamide at 10^{-5} M was capable of inducing histamine release from non-activated mast cells, whereas WIN 55,212-2 and HU-210 at 10^{-5} M enhanced anti-IgE-induced histamine release. Hence, we have obtained results which are different from those of Facci et al. (1995) and contradicted their original hypothesis (Aloe et al., 1993). A recent study of Bueb et al. (2001) also supported our observations with regards to the effects of anandamide, palmitoylethanolamide and WIN 55,212-2 on non-activated rat peritoneal mast cells. Similarly, in their study, both palmitoylethanolamide and WIN 55,212-2 failed to induce histamine release from peritoneal mast cells even at 10^{-4} M, whereas a significant histamine release was induced by anandamide only at the highest concentration of 10^{-4} M tested. Furthermore, they demonstrated that anandamide induced histamine release from mast cells through a cannabinoid receptor-independent and possibly deleterious mechanism. The discrepancy between observations of Facci et al. (1995) and those reported by Bueb et al. (2001) and ourselves may be due to the different types of mast cells used. The rat peritoneal mast cells are connective tissue mast cells while the RBL-2H3 cells are a model for the mucosal mast cells and they are known to have different morphological as well as functional properties (Zheng et al., 1991; Bottjer et al., 1994).

The discrepancy may however be due to differences in experimental conditions. In our current studies, we have noticed that when the final concentration of the solvent, DMSO was higher than 0.01% in the incubation medium, significant inhibition of anti-IgE-induced histamine release was produced by the solvent alone. In the study of Facci et al. (1995), a significant reduction of immunogenic activation of RBL-2H3 cells was only observed in medium containing palmitoylethanolamide with 1% of DMSO but not when the DMSO concentration was at 0.2%. Since significant inhibition was only observed in the presence of a very high concentration of DMSO, it may be inappropriate to conclude that the inhibition reported was solely due to palmitoylethanolamide. Furthermore, the radio-ligand binding studies reported by Facci et al. were not confirmed by a similar study by Lambert et al. (1999). In the latter study, palmitoylethanolamide was reported to be a poor binding ligand for CB_2 receptors expressed in various biological models. Moreover, no specific cannabinoid binding sites were detected on the surface of intact RBL-2H3 cells or membrane fractions from these cells. The authors concluded that palmitoylethanolamide was unlikely to be an endogenous agonist of the CB_2 receptors and might produce anti-inflammatory actions via CB_1/CB_2 receptor-independent mechanisms. Indeed, the hypothesis that palmitoylethanolamide was the endogenous ligand for CB_2 receptors was also not supported by a number of studies which investigated the activities of palmitoylethanolamide in various CB_2 receptor-specific systems (Lambert et al., 1999; Ross et al., 2000; Sugiura et al., 2000). The failure of palmitoylethanolamide to modulate anti-IgE-induced histamine release may be due to the lack of binding to CB_2 receptors on mast cells or the absence of CB_2 receptors in mast cells. Since the potent cannabinoid receptor agonist CP 55,940 also demonstrated similar lack of activities against mast cells, it is more likely that these cells do not express functional cannabinoid receptors at all.

For the first time, we have also observed that WIN 55,212 and the more potent cannabinoid receptor agonist HU-210 could enhance anti-IgE-induced histamine release at concentrations higher than 10^{-6} M. In order to elucidate if the enhancing effect of the two compounds were mediated through cannabinoid receptors, we investigated if such effect could be reversed by cannabinoid receptor antagonists. AM 281 is a structural analogue of the highly potent and selective CB_1 receptor antagonist SR141716A. Although AM 281 is less potent than SR141716A, it maintains high selectively for CB_1 receptors and readily reverses the actions of WIN 55,212-2 or CP 55,940 in various cannabinoid receptor containing systems (Gatley et al., 1998; Izzo et al., 2000; Gifford et al., 1997; Cosenza et al., 2000). AM 630 is a potent CB_2 selective antagonist which has been reported to reverse CP 55,940-induced inhibition of forskolin-stimulated cyclic AMP production by human CB_2-transfected CHO cell preparations (Ross et al., 1999). In the current study, neither AM 281 nor AM 630 at 10^{-6} M demonstrated any significant effect on the histamine release enhancing actions of HU-210 and WIN 55,512-2. Therefore, it can be concluded that neither CB_1 nor CB_2 receptors are involved in the enhancing actions of the two cannabinoids. Further studies will be required to delineate the actual mechanisms involved.

The pathophysiological activation of mast cells is via the cross-linking of cell surface high affinity IgE receptors as mediated by anti-IgE in the current study. The failure of any of the tested cannabinoid receptor agonists to reduce anti-IgE-induced histamine release does not support the hypothesis that cannabinoids produced anti-inflammatory effects by inhibiting the pathophysiological activation of mast cells. Instead, some of the synthetic cannabinoids tested enhanced anti-IgE-induced histamine release through cannabinoid receptor-independent mechanisms. In total, the presence of functional CB_1 or CB_2 receptors in rat peritoneal mast cells is not supported by the current study. The mast cell stabilizing action of palmitoylethanolamide originally reported may be due to nonspecific effects produced by

the high concentration of DMSO used in dissolving the amide.

Acknowledgements

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