

The histamine H₄ receptor as a new target for treatment of canine inflammatory skin diseases

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Conflict of Interest

None declared.

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Abstract

Histamine is a well known mediator of allergic skin diseases and, with the discovery of the histamine H₄ receptor, the role of histamine is re-evaluated. There are only limited published data elucidating the role of the histamine H₄ receptor in dogs. Twelve beagles intradermally injected with histamine (0.25 µmol and 2.5 µmol/site) reacted with a classical wheal and flare reaction. None of the dogs showed signs of pruritus. The dogs reacted with a wheal and flare reaction after intradermal injection of histamine H₄ receptor agonist/H₃ receptor antagonist clobenpropit (0.1 µmol) and selective histamine H₄ receptor agonist VUF 8430 (1.5 µmol). Again, no scratching occurred in any of the dogs. The highly selective histamine H₄ receptor antagonist JNJ 7777120 reduced the histamine-induced wheal reaction in nine out of 12 dogs. To determine whether canine mast cells are susceptible to histamine H₄ receptor-mediated reactions, effects of clobenpropit and VUF 8430 were tested in canine mastocytoma cells (C2). Incubation with histamine H₄ receptor agonists (up to 10 µmol/L) induced a distinct calcium²⁺ influx. C2 cells also responded with enhanced chemotaxis when stimulated with histamine, VUF 8430 and clobenpropit. Neither VUF 8430, nor clobenpropit (up to 10 µmol/L) led to a modulation of histamine concentration in supernatants of canine mastocytoma cells, whereas mastoparan, used as a positive control, enhanced histamine concentration in supernatants. For treat-

ment of allergic skin diseases in dogs, a combination of H₁R and H₄R antagonists might be advantageous.

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Introduction

Allergic skin diseases in dogs are common, and the most prevalent nonparasitic allergic skin disease is atopic dermatitis. According to Hillier and Griffin, approximately 10% of the canine population is affected by this pruritic skin disease.¹ Treatments currently used for canine atopic dermatitis include glucocorticoids, H₁ antagonist-type antihistamines, and the recently introduced ciclosporin A. Corticosteroids are effective in reducing inflammation and pruritus, but they can cause serious adverse effects. Ciclosporin A, in contrast, is as effective as corticosteroids and seems to have a lower risk of severe adverse reactions. The most frequent adverse effects seen during ciclosporin A therapy are gastrointestinal disorders (vomiting and diarrhoea).² However, the safety of long-term administration of ciclosporin A in dogs is not yet well documented. A trend for increased incidence of adverse effects was noted in dogs after long-time-treatment (>6 months) with ciclosporin A in a retrospective study.³ H₁ antihistamines have only minimal efficacy in allergic diseases and treatment with H₁ antihistamines alone will not adequately control moderate to severe atopic dermatitis.^{4–7} Histamine is a potent biogenic amine with multiple activities in various physiological and pathological conditions. It is a well-known mediator in allergic diseases and has long been recognised as a classical inducer of pruritus in man. The histamine H₄ receptor (H₄R) is the most recently identified histamine receptor. It was characterized by several groups in 2000 and 2001.^{8–12} The H₄R is primarily located in the immune system, in particular on several haematopoietic cells, including mast cells, eosinophils, dendritic cells and T lymphocytes. Recent findings have shown that the H₄R plays a role in activation, migration, cytokine and chemokine production of these cells.^{13–15} Furthermore, this receptor seems to be involved in inflammatory responses and mediation of pruritus *in vivo*.^{16–20} H₄R antagonists have been effective in reducing inflammatory reactions in different disease models^{18,19} and in our studies we have shown that the selective H₄R antagonist JNJ 7777120 clearly inhibits hapten-induced scratching in a murine model of allergic contact dermatitis.²¹ Thus, H₄R antagonists may offer potential to treat a range of allergic and

immunological disorders in dogs. Limited knowledge on the canine H4R exists to date.

In the present study, the H4R ligands clobenpropit (H4R agonist/H3 receptor antagonist), VUF 8430 (selective H4R agonist) and JNJ 7777120 (selective H4R antagonist) were studied to evaluate their ability to modulate canine mast cell activity. Mast cells have long been recognized as key cells of hypersensitivity reactions. For this study, the canine mastocytoma cell line C2 was used. Furthermore, effects of histamine and H4R ligands after intradermal injection were studied for pruritogenic activity in dogs, and the reactions were compared to mice.

Materials and methods

Animals

Two different groups of beagles were used. Six beagle dogs (three intact males and three intact females) were obtained from the Animal Science Group, Lelystad, Netherlands. Dogs were housed in a group of six. The other six beagles (six intact females) were housed in pairs in the Department of Parasitology, University of Veterinary Medicine Hannover and were obtained from Harlan-Winkelmann, Borcheln, Germany. Dogs ranged in age from 5 months to 1 year when they were included, and were fed a standard diet and water *ad libitum*.

Female BALB/c mice were obtained from Charles River (Sulzfeld, Germany) at the age of 8 weeks (body weight = 20 g). All animals were healthy and housed in groups of six mice per cage at 22°C with a 12-h light/dark cycle. Water and a standard diet (Altromin, Lage/Lippe, Germany) were available *ad libitum*. The animal experiments were approved by the LAVES Institute, Oldenburg, Germany.

Cell culture

The canine mastocytoma cell line C2 was obtained from H. Fuhrmann, Faculty of Veterinary Medicine, University of Leipzig. C2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM/Ham's F12) supplemented with 5% fetal bovine serum, histidine, HEPES, glutamine and antibiotics (penicillin and streptomycin) at 5% CO₂ and 37°C. Cells were passaged every seven days.

Chemotaxis assay

The Boyden chamber assay was performed with histamine (Sigma, Steinheim, Germany), the partial H4R agonist clobenpropit (Calbiochem, La Jolla, CA, USA) and the selective H4R agonist VUF 8430 (provided by R. Leurs, Vrije Universiteit, Amsterdam, The Netherlands) as chemotactic agents. This system consists of a lower chamber containing the putative chemoattractive factor and an upper chamber containing a polycarbonate filter with 8 µm pores (Greiner, Frickenhausen, Germany). C2 cells were counted and suspended in DMEM. C2 cell suspension containing about 5×10^5 cells was placed in the upper chamber. Chemotaxis was allowed for 2 h, and the number of cells was counted after staining with trypan blue (Sigma, Steinheim, Germany) in a Neubauer counting chamber (Neubauer; VWR, Mannheim, Germany). The selective H4R antagonist JNJ 7777120 (synthesised by K. Sander and H. Stark, Johann Wolfgang Goethe University, Frankfurt/Main, Germany) was added to the cells 30 min before stimulating with the chemotactic agents.

Calcium²⁺ (Ca²⁺) mobilization assay

Effects of H4R ligands on intracellular calcium concentration (Ca²⁺) in canine mast cells (C2 cells) were tested. C2 cells (5×10^4) were seeded on black 96-well plates and loaded with the Ca²⁺-sensitive fluorescent dye FLUO-4 according to the manufacturer's instructions

(Invitrogen GmbH, Karlsruhe, Germany). This indicator has high affinity binding for Ca²⁺ and very large fluorescence intensity increase in response to Ca²⁺ binding (>100-fold). Ca²⁺ mobilization was measured in a FluoStar plate reader (BMG Lab Technologies GmbH, Jena, Germany). The Ca²⁺ signal per second was measured for 50 s at excitation and emission wavelengths of 485 and 525 nm. Five seconds after starting the measurement, the H4R ligands histamine, clobenpropit or VUF 8430 (10 µmol/L) were added to the cells. A Ca²⁺ ionophore (A23187; 20 nmol/L, Sigma) was used as positive control and phosphate buffered saline (PBS) as negative control.

Histamine ELISA

To demonstrate a possible direct effect of H4R agonists on mast cell secretion, histamine from canine mast cells was measured using an ELISA. Histamine was determined in supernatants of stimulated C2 cells. Cells were washed twice with calcium- and magnesium-free Tyrode's buffer and then aliquots with 2×10^6 cells in Tyrode's buffer were prepared. To stimulate histamine release, cells were incubated with clobenpropit (1 µmol/L/10 µmol/L), VUF 8430 (1 µmol/L/10 µmol/L) and, as a positive control, with the wasp venom peptide mastoparan (25 µmol/L) for 45 min at 37°C. PBS was used as negative control. After incubation, cells were placed on ice for 10 min to stop the reaction. Histamine was measured in the supernatants by ELISA (IBL, Hamburg, Germany) according to manufacturer's protocol.

Intradermal testing (dogs)

Histamine, the H4R agonists clobenpropit and VUF 8430 were dissolved in phosphate buffered saline (PBS) to the final desired concentration. JNJ 7777120 was dissolved in dimethyl sulphoxide (2.5 mg in 50 µL) then diluted with PBS. Injection of PBS was used as a negative control. An area of 8 cm × 10 cm was clipped on the lateral thorax and dogs were rested for 1 day before starting the experiments. All substances were injected intradermally using a 27 gauge needle at a dose of 0.05 mL. In experiments involving the H4R antagonist JNJ 7777120, the substance was administered intradermally 30 min before injection of histamine into the same area. Areas of wheal reactions were evaluated planimetrically after 15 min by drawing their borders on a transparent sheet. They were scanned using Epson Easy Photo fix software and analysed with the public domain Windows Scion Image program. Results were expressed in cm². To determine the pruritogenic effects, dogs received on subsequent days histamine (0.25 and 2.5 µmol), clobenpropit (0.1 and 0.3 µmol) and VUF 8430 (0.15 and 1.5 µmol). After each injection of each concentration of the tested substances, dogs were video-recorded (Canon UC-X45 Hi) or directly observed for 30 min to 1 h and their scratching behaviour was quantified. Itching was defined as scratching with the hind paw, licking, rubbing or rolling.

Intradermal testing (mice)

The rostral part of each mouse's back was depilated with a depilation cream (Veet, Reckitt Benckiser Deutschland GmbH, Mannheim, Germany). Mice were placed in acrylic cages for about 30 min to become acclimatized. Histamine was then injected intradermally at a volume of 50 µL. Mice were directly observed for 30 min immediately after histamine injection. Control mice received a PBS injection in place of histamine. Scratching responses to the injected site with the hind paws were enumerated as an itch response.

Statistical evaluation

Figures are presented as mean ± SEM. Statistically significant differences between the drug treatments and controls were assessed by one-way ANOVA followed by post-hoc test (Dunnett's test). For all analyses $P < 0.05$ was considered significant.

Results

Chemotactic response of canine mastocytoma cells to H₄R agonists

Histamine, clobenpropit and VUF 8430 markedly stimulated C2 cell migration at 10 $\mu\text{mol/L}$ concentration. Pre-treatment with the H₄R antagonist JNJ 777120 (10 $\mu\text{mol/L}$) significantly inhibited the migratory response provoked by clobenpropit (Figure 1a), VUF 8430 (Figure 1a) and histamine (Figure 1b). The migratory response to the selective H₄R agonist VUF 8430 was dose-dependent (Figure 1c).

Calcium²⁺ mobilization assay

Activation by the H₄R agonists histamine (1 and 10 $\mu\text{mol/L}$; Figure 2a), clobenpropit (10 $\mu\text{mol/L}$; data not shown) and VUF 8430 (10 and 100 $\mu\text{mol/L}$; Figure 2b), induced calcium²⁺ influx in canine mastocytoma cells.

Histamine ELISA

Neither clobenpropit nor VUF 8430 (up to 10 $\mu\text{mol/L}$) modulated histamine concentration in supernatants of canine mastocytoma cells (Figure 3). Mastoparan increased histamine level up to 1103.42 ng/mL (mean 334.36 ng/mL). The histamine level measured in the supernatants of H₄R agonists stimulated C2 cells varied between 30–50 ng/mL, which was approximately the same level detected in supernatants of vehicle treated control cells.

Intradermal testing (dogs)

Histamine (0.25 μmol and 2.5 μmol) induced a classical wheal and flare reaction, but none of the dogs showed a scratching behaviour. The H₄R agonist/H₃R antagonist clobenpropit (0.1 μmol and 0.3 μmol) induced extravasation in nine out of 12 beagles and the selective H₄R agonist VUF 8430 (1.5 μmol) in six out of 12 dogs, but no scratching was evoked. The diameter of the wheals induced by clobenpropit (0.3 μmol) ranged from 0.16 to 1.70 cm² and the wheals caused by VUF 8430 (1.5 μmol) had a diameter of 0.91–1.93 cm² (Figure 4). A lower dose of VUF 8430 (0.15 μmol) did not cause extravasation. Intradermal injection of the vehicle (PBS) did not induce any wheal and flare reaction.

Pre-treatment with the selective H₄R antagonist JNJ 777120 (1.25 μmol) reduced the histamine-induced wheal reaction in nine out of 12 dogs. The diameter of wheals 15 min after histamine injection was approximately 30% reduced after pre-treatment with JNJ 777120 (Figure 5). Some differences in sensitivity to histamine were detected among the 12 beagles [the range of histamine (0.25 μmol) wheal areas was 1.30–2.80 cm²; Figure 5] and also in their response to JNJ 777120 [the range of histamine (0.25 μmol) wheal areas pre-treated with JNJ 777120 was 0.90–1.70 cm²]. Repeating the experiment led to similar results.

Intradermal testing (mice)

Histamine provoked dose-dependent pruritus in BALB/c mice (Figure 6). Approximately 34 scratching bouts were noted during the 30 min observation period after intradermal injection of 0.25 μmol or 2.5 μmol of histamine.

A lower dose of histamine (0.025 μmol) did not provoke enhanced scratching behaviour compared to negative control (PBS).

Discussion

Since the H₄R was cloned in 2000, the evidence for a significant role of this receptor in the regulation of immune responses and modulation of allergic disorders has been growing. Histamine H₁ receptor antagonists (blockers), are minimally effective in many clinical conditions (e.g. atopic dermatitis). The lack of efficacy of H₁ antihistamines in the treatment of allergic skin lesions might partly be due to lack of blockage of the H₄R-mediated histamine effects. The first series of H₄R selective antagonists provided evidence for anti-inflammatory and antipruritic *in vivo* activity in human beings.^{17,19,21} The canine H₄R has not been intensively studied. To date, genes encoding the human, monkey, pig, mouse, rat and guinea pig H₄R have been cloned and just recently the canine H₄R

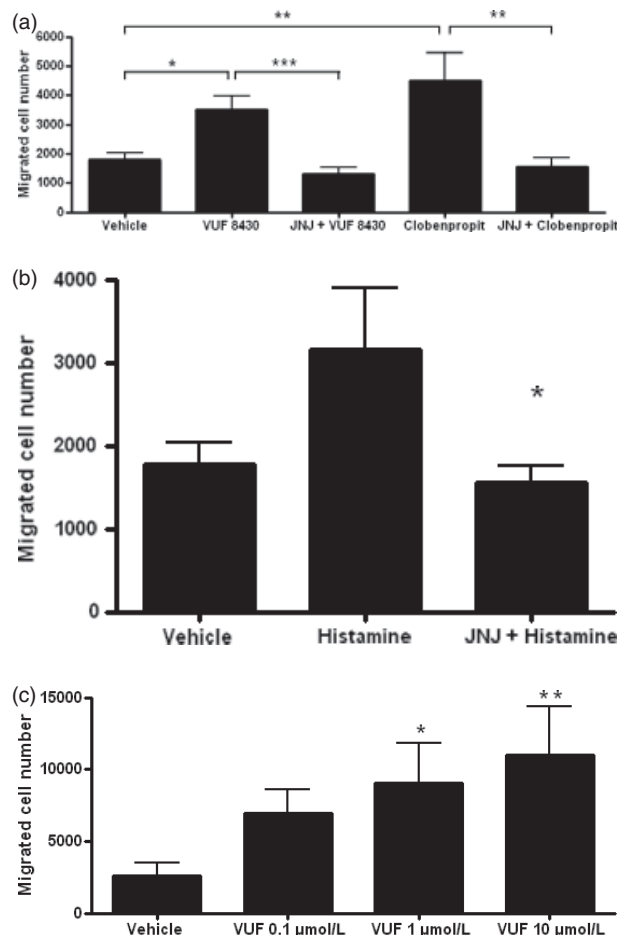


Figure 1. Effects of clobenpropit, VUF 8430 (a) and histamine (b) on canine mast cell migration. To block the migration, cells were pre-treated with the selective H₄R antagonist JNJ 777120. Clobenpropit and VUF 8430 enhanced the cell migration significantly and these effects were inhibited by pre-treatment with JNJ 777120. Pooled data of four independent experiments of $n = 3$ –5 each concentration; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. (c): VUF 8430 induced mast cell migration in a dose-dependent manner. Pooled data of three experiments of $n = 3$ –4 each concentration, mean ($\pm$ SEM). * $P < 0.05$; ** $P < 0.01$.

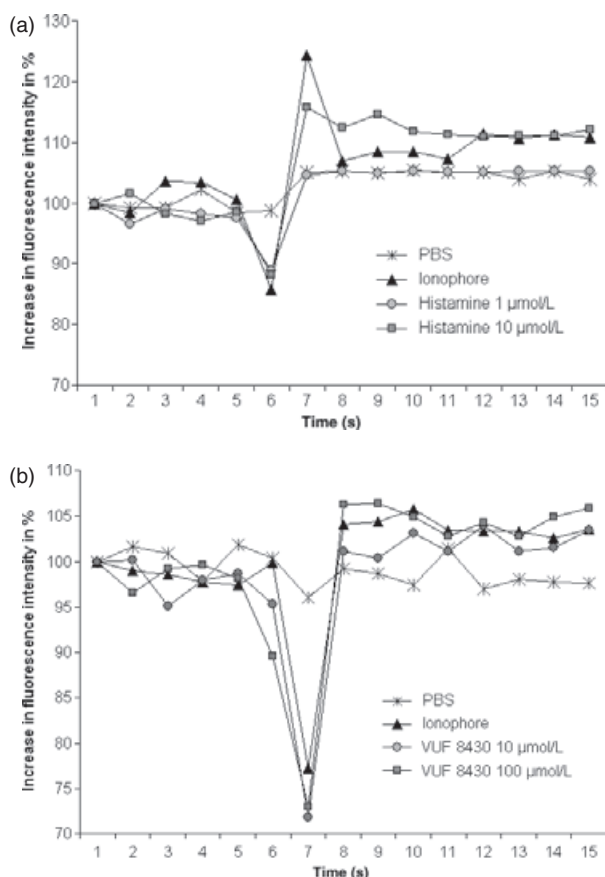


Figure 2. Histamine (a) and VUF 8430 (b) induced Ca²⁺ mobilization in canine mastocytoma cells (C2) in a dose-dependent manner. The dashed line represents the negative control (PBS). The fluorescence intensity is illustrated in per cent in relation to the initial measured value.

has been cloned and characterized.^{8,9,22,23} The H4R shares only low homology among the different species (about 70%).^{9,23} The low homology follows differences in the receptor pharmacology. However, all the H4R ligands studied by Jiang *et al.*²³ have affinity for the canine H4R, even though with different efficacy. The H4R expression profile in the dog is similar to other species, as the canine H4R was detected in bone marrow, spleen, heart, liver, skeletal muscle, small intestine and trachea.^{9,23} However, distribution and function of the H4R receptor in

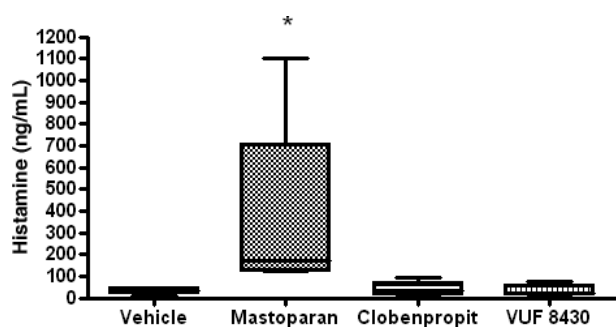


Figure 3. Histamine release from canine mastocytoma cells (C2 cells) activated with mastoparan, clobenpropit and VUF 8430. Pooled data from three independent experiments (each performed in duplicate), **P* < 0.05.

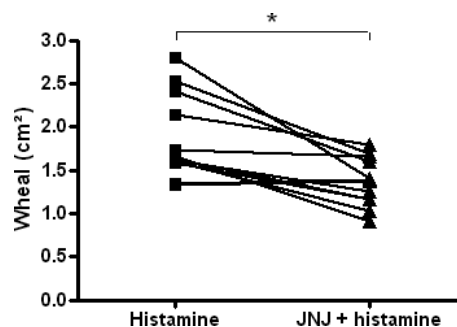


Figure 4. Histamine-induced wheal diameter in cm² (squares) and histamine-induced wheal diameter after pre-treatment with JNJ 777120 (triangles), **P* < 0.05.

the skin has not been evaluated. The expression profile suggests a similar physiological function of the H4R among different vertebrate species.

In this study, it is demonstrated that canine mastocytoma cells (C2 cells) have a functional histamine H4R. C2 cells share many anatomical and functional similarities with canine mast cells and are a good model to evaluate canine mast cell function.²⁴ Mast cells are major effector cells in immune responses and they are best known for their role in allergic diseases. Histamine and the H4R agonists clobenpropit and VUF 8430 stimulated intracellular Ca²⁺ influx in canine mast cells (C2 cells). Similar Ca²⁺ mobilization induced by H4R stimulation has been previously demonstrated in murine mast cells as well as in human eosinophils.^{13,25} Hofstra *et al.*¹³ proposed that histamine binding to the H4R on mast cells activates an intracellular signalling pathway, which leads to release of intracellular Ca²⁺ from the endoplasmic reticulum. Increased Ca²⁺ level triggers a still unknown signalling pathway, which provokes mast cell migration towards histamine.¹³ In this study, it is demonstrated that canine mast cells migrate towards histamine and that this effect is mediated by the H4R. When the H4R was blocked by a selective H4R antagonist, the chemotactic effects of histamine were abrogated, while H4R agonists enhanced the number of migrating mast cells in comparison to untreated controls. These results are in accordance to previously reported migratory effects of H4R on murine mast cells, murine eosinophils, murine skin dendritic cells and human monocyte-derived dendritic cells.^{13,15,26,27}

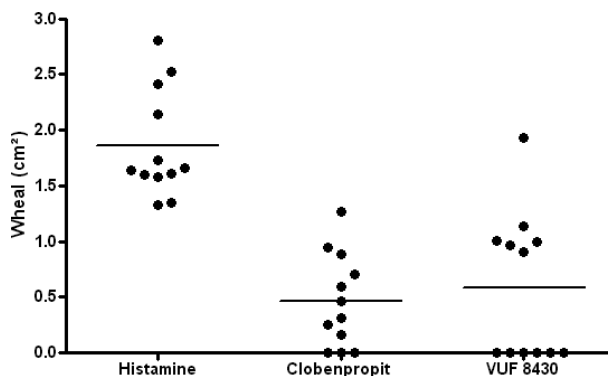


Figure 5. Histamine- (0.25 µmol), clobenpropit- (0.1 µmol) and VUF 8430- (1.5 µmol) induced wheal diameters in cm². Each dog is represented by one plot and the bar denotes medians of each group.

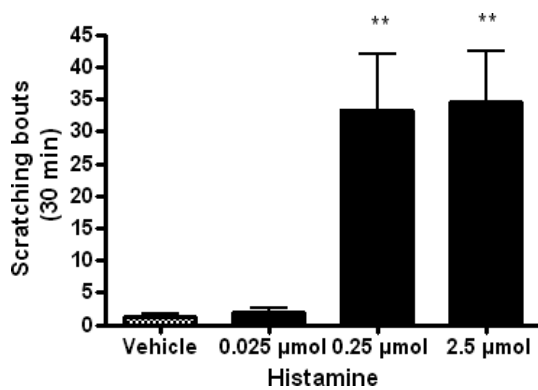


Figure 6. Histamine induced dose-dependent (0.025 µmol, 0.25 µmol and 2.5 µmol) scratching in BALB/c mice during the 30 min observation period after intradermal injection. $n = 6$ each group, ** $P < 0.01$.

Experiments in mice showed that mast cells from wild-type and H3R-deleted mice migrated in response to histamine, whereas mast cells from H4R-deleted mice did not.¹³ Thus, chemotaxis of murine mast cells seems to be mainly triggered by the H4R. There is some controversy as to whether the skin mast cell number is increased in canine atopic dermatitis. Wilkie *et al.* (1990) demonstrated an increased number of mast cells in lesional skin from dogs with chronic atopic dermatitis at specific body sites compared to skin samples from healthy dogs, while other authors described moderate or no increase in the density of skin mast cells.^{28–30} Notably, a significant correlation between the number of mast cells in dog skin in specific body sites and pruritus has been described in canine atopic skin lesions.³¹ Additionally, mast cells from atopic skin lesions are more reactive in terms of mediator release than mast cells from healthy dogs in response to a range of stimuli.³² *In vivo* testing in this study provided evidence that the effect of histamine on extravasation is partially mediated by the H4R (Figures 4 and 5). Therefore, it is questioned whether H4R agonists directly modulate mast cell activity and stimulate release of histamine. Canine mast cells were stimulated with H4R agonists and histamine concentration was determined in supernatants. One of the main biological functions of mast cells is to synthesise and release specific inflammatory mediators after activation. This process is usually triggered by the interaction between allergens and allergen-specific IgE bound to high affinity FcεR1 receptors expressed on mast cell surface. After the cross-linking of IgE-FcεR1 high affinity receptors, degranulation occurs with release of histamine and many other inflammatory mediators. The present work shows that histamine H4R agonists do not stimulate histamine secretion from canine mast cells. According to this, histamine seems not to have any direct effect on canine mast cell degranulation mediated by H4R. An effect on blood vessels by histamine and H4R agonists (influencing the vascular tone and permeability/extravasation) could also be caused by a direct effect on endothelial cells. This is currently under investigation. Histamine has been widely used as a pruritogenic agent in experimental settings and,

as histamine specific C-fibres exist, it is postulated that histamine is a mediator of pruritus in human beings.^{33–36} Also, in mice and guinea pigs, histamine is noted to cause scratching^{16,34}, as we could demonstrate in this study. Intradermal injection of histamine (0.025–2.5 µmol) induced pruritus in BALB/c mice, which seems to be mainly mediated by H4R, as intradermal injection of the H4R agonist clobenpropit induced scratching behaviour.^{16,26} Furthermore, histamine-mediated and allergen-mediated pruritus in mice was strongly attenuated in histamine H4R knockout mice or by pre-treatment with JNJ 7777120.^{17,21} As histamine-induced scratching is a well known model for pruritus response in both mice and humans, it was surprising that none of the 12 beagles reacted with scratching in response to intradermal histamine (up to 2.5 µmol) injection. The amount of 2.5 µmol of histamine is the normal dose used for an intradermal test. Conceivably, there could be some differences in sensitivity to histamine among dog breeds. Thus, the beagle dog seems to be insensitive – or only slightly sensitive – to histamine-induced pruritus. On the other hand, histamine injection led to a classic wheal and flare reaction. Neither the H4R agonist/H3R antagonist clobenpropit nor the selective H4R agonist VUF 8430 induced pruritus in dogs, but both induced extravasation. Pre-treatment with JNJ 7777120 reduced the histamine-induced wheal of approximately 30%. This leads to the conclusion that the H4R is involved in the wheal and flare reaction induced by histamine in dogs. On the other hand, histamine seems not to be a major mediator of pruritus in beagle dogs.

In conclusion, our data provide evidence that the H4R is involved in canine allergic skin diseases. The H4R activates chemotaxis of canine mast cells and modulates inflammatory responses to histamine, but has no direct influence on degranulation. These findings might provide new treatment strategies for allergic skin diseases. A combination of H4R antagonists and H1R antagonist may offer potential to treat allergic skin diseases in dogs.

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Résumé L'histamine est un médiateur bien connu des dermatoses allergiques et, depuis la découverte du récepteur H₄, son rôle est réévalué. Les publications restent limitées sur le rôle du récepteur H₄ à l'histamine chez le chien. Douze beagles ont reçu des injections intradermiques d'histamine (0.25 µmol et 2.5 µmol/zone) produisant une réaction classique en plaque ortiée érythémateuse. Aucun des chiens n'a montré de prurit. Les chiens ont réagi avec une plaque ortiée érythémateuse après injection intradermique de clobenpropit (0.1 µmol), un agoniste du récepteur H₄/antagoniste du récepteur H₃ et de VUF 8430 (1.5 µmol) un agoniste sélectif du récepteur H₄. Là encore, aucun signe de prurit n'a été observé. L'antagoniste JNJ 7777120 hautement sélectif du récepteur H₄ diminue la plaque ortiée induite par l'histamine pour 9 des douze chiens. Afin de déterminer si les mastocytes canins sont sensibles aux réactions méditées par les récepteurs H₄, le clobenpropit et le VUF 8430 ont été testés sur des cellules de mastocytomes canins (C2). L'incubation avec l'agoniste des récepteurs H₄ (jusqu'à 10 µmol/L) induisait un influx de Ca²⁺.

Les cellules C2 répondaient également par une augmentation du chimiotactisme à une stimulation par l'histamine, le VUF 8430 et le clobenpropit. Ni le VUF 8430, ni le clobenpropit (jusqu'à 10 µmol/L) ne modifièrent des concentrations d'histamine dans les surnageants des mastocytes canins, tandis que le mastoparan, utilisé comme contrôle positif, augmenta la concentration en histamine dans le surnageant. Une association d'antagonistes aux récepteurs H₁R et H₄R pourraient être avantageuse dans le traitement des dermatoses allergique du chien.

Resumen La histamina es un mediador bien conocido de las enfermedades alérgicas de la piel y, con el descubrimiento del receptor de histamina H₄, el papel de la histamina es reevaluado. Sólo hay unos pocos trabajos publicados que estudian el papel de los receptores de histamina H₄ en perros. Doce perros de raza Beagle que se inyectaron con histamina por vía intradérmica (0,25 µmol y 2,5 µmol/lugar) reaccionaron con el clásico habón y enrojecimiento. Ninguno de los perros mostró signos de prurito. Los perros reaccionaron con habón y enrojecimiento tras la inyección intradérmica del agonista del receptor de histamina H₄ / antagonista del receptor H₃ clobenpropit (0,1 µmol) y con el agonista selectivo del receptor VUF 8430 (1,5 µmol). De nuevo, no se observó picor en ninguno de los perros. El antagonista altamente selectivo del receptor JNJ 7777120 redujo el habón producido por histamina en nueve de los 12 perros. Para determinar si los mastocitos caninos son susceptibles a las reacciones mediadas por los receptores de histamina H₄, se probaron los efectos de clobenpropit y VUF 8430 en células de mastocitoma canino (C2). La incubación con agonistas del receptor de histamina H₄ (hasta 10 µmol/l) indujo un influjo claro de calcio²⁺. Las células C2 también respondieron con una quimiotaxis acentuada cuando se estimularon con histamina, VUF 8430 y clobenpropit. Ni VUF 8430, ni el clobenpropit (hasta 10 µmol/L) produjeron una modificación de la concentración de histamina en los sobrenadantes de las células de mastocitoma, mientras que el mastoparan, utilizado como control positivo, aumentó la concentración de histamina en los sobrenadantes. Para el tratamiento de las enfermedades alérgicas en perros puede ser beneficioso utilizar una combinación de antagonistas H₁R y H₄R.

Zusammenfassung Histamin ist ein bekannter Mediator allergischer Hauterkrankungen und mit der Entdeckung des Histamin H₄ Rezeptors wurde die Rolle von Histamin re-evaluiert. Es gibt nur wenig publiziertes Material, welches die Rolle des Histamin H₄ Rezeptors bei Hunden beleuchtet. Zwölf Beagles, denen Histamin (0,25µmol und 2,5 µmol/Injektionsstelle) intradermal injiziert worden war, reagierten mit einer klassischen "Wheal und flare" Reaktion. Keiner der Hunde zeigte Anzeichen von Juckreiz. Die Hunde reagierten nach einer intradermalen Injektion des Histamin H₄ Rezeptor Agonisten/H3 Rezeptor Antagonisten Clobenpropit (0,1µmol) und dem selektiven Histamin H₄ Rezeptor Agonisten VUF 8430 (1,5 µmol) mit einer "Wheal und flare" Reaktion. Wiederum kratzte keiner der Hunde. Der hochselektive Histamin H₄ Rezeptor Antagonist JNJ 7777120 reduzierte die durch Histamin induzierte "Wheal Reaktion" in neun von 12 Hunden. Um festzustellen, ob canine Mastzellen für Histamin H₄ Rezeptor-medierte Reaktionen empfänglich sind, wurden die Auswirkungen von Clobenpropit und VUF 8430 auf Mastozytomzellen (C2) getestet. Die Inkubation mit Histamin H₄ Rezeptor Agonisten (bis zu 10 µmol/l) bewirkte ein deutliches Einströmen von Ca²⁺. Die C2 Zellen reagierten mit zunehmender Chemotaxis, wenn sie durch Histamin, VUF 8430 und Clobenpropit stimuliert wurden. Weder VUF 8430 noch Clobenpropit (bis zu 10 µmol/l) führten zu einer Modulation der Histaminkonzentrationen im Überstand der caninen Mastozytomzellen, während Mastoparan, welches als Positivkontrolle verwendet wurde, die Histaminkonzentration im Überstand erhöhte. Für die Behandlung von allergischen Erkrankungen bei Hunden, könnte eine Kombination von H₁R und H₄R Antagonisten von Vorteil sein.