*Inflammation, Vol. 38, No. 3, June 2015 (© 2014)*
DOI: 10.1007/s10753-014-0057-1

# Histamine $H_3$ and $H_4$ Receptor Ligands Modify Vascular Histamine Levels in Normal and Arthritic Large Blood Vessels *In Vivo*

**Konstantinos Kyriakidis,[1] Evangelia Zampeli,[1] Marina Palaiologou,[2] Dina Tiniakos,[2,3] and Ekaterini Tiligada[1,4]**

*Abstract*—Growing evidence associates histamine with arthritis, but its implication in shaping vascular function in chronic inflammation remains largely elusive. This study explored the involvement of vascular histamine in the extra-articular responses in peripheral large blood vessels using a rat model of adjuvant-induced arthritis. Histamine levels were increased in the abdominal aorta and the inferior vena cava of arthritic animals. Contrary to the $H_1$ receptor antagonist dimetindene, histamine induction was observed following administration of the $H_3$ and $H_4$ receptor ligands GSK334429 and JNJ7777120, respectively. In arthritis, prophylactic treatment with GSK334429 partially attenuated the clinical signs and restored basal histamine levels only in the abdominal aorta. This study is the first to implicate the $H_3$ and $H_4$ receptors in a concerted constitutive regulation of basal vascular histamine in the rat large blood vessels and to identify the $H_3$ receptor as a component that may influence arterial histamine during the onset of arthritis.

**KEY WORDS:** arthritis; histamine; inflammation; peripheral blood vessels; rat.

## INTRODUCTION

Histamine is synthesized in several cell types and mediates a wide spectrum of effects through four known types of receptors, designated as $H_1$, $H_2$, $H_3$, and $H_4$ [1]. Although histamine has been one of the most studied and therapeutically exploited substances in medicine for more than a century, its strong association with the pluripotent mast cell and allergy has deterred the investigation of its functional significance in homeostatic mechanisms [2]. The identification of the $H_3$ and $H_4$ receptors some years ago revived the interest in histamine research and exposed attractive perspectives for the potential therapeutic exploitation of these new drug targets in neurological and immunological disorders, respectively [3–5].

In cardiovascular (patho)physiology, considerable evidence has accumulated over the years to implicate histamine in functions such as blood pressure and nitric oxide regulation and in the pathogenesis of atherosclerosis [6, 7]. Besides the recognized $H_1$ and $H_2$ receptor-mediated effects in blood vessels, presynaptic and post-synaptic $H_3$ receptors appear to affect basal cardiovascular parameters in various species [8]. Despite the reports of the histaminergic influence on blood vessels, the role of vascular histamine in chronic multisystem inflammatory diseases, such as rheumatoid arthritis (RA), and the likely contribution to the increased prevalence of cardiovascular disease remain largely elusive. The mechanisms leading to an elevated cardiovascular mortality rate in RA patients are complex and cannot be fully explained by the classic risk factors [9]. In this regard, growing evidence suggests that additional mechanisms underlie vascular dysfunction and assigns a role to the uncontrolled systemic inflammation as a non-traditional cardiovascular risk factor [9]. Along this line of research, the $H_4$ receptor has been linked to inflammation. Although its exact function in autoimmune disease has yet to be established, supporting evidence identifies the $H_4$ receptor as a key player in both innate and adaptive immune responses that drive arthritic disorders [10–12].

[1] Department of Pharmacology, Medical School, National and Kapodistrian University of Athens, 11527 Athens, Greece
[2] Laboratory of Histology and Embryology, Medical School, National and Kapodistrian University of Athens, Athens, Greece
[3] Institute of Cellular Medicine, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, UK
[4] To whom correspondence should be addressed at Department of Pharmacology, Medical School, National and Kapodistrian University of Athens, 11527 Athens, Greece. E-mail: aityliga@med.uoa.gr

Moreover, $H_3$ receptor antagonists have been reported to reverse neuropathic hypersensitivity [13] and to relieve osteoarthritic pain [14] in disease-related rodent models. Consequently, studies designed to identify the implication of vascular histamine in the extra-articular disease characteristics are pertinent to explore the systemic mechanisms associated with arthritis onset and progression. Most related investigations have focused on articular changes and on a range of circulating immune components [12, 15].

Even though the anti-inflammatory properties of histamine in human RA have been described [16], $H_4$ receptor activation was recently shown to induce pro-inflammatory responses. For instance, JNJ7777120, which is reported to be equipotent antagonist at the human, mouse, and rat $H_4$ receptor orthologs [17], exhibited prophylactic and therapeutic anti-inflammatory and anti-arthritic activity in a Balb/c mouse model of collagen antibody-induced arthritis [15]. Interestingly, however, JNJ7777120 was shown to dose-dependently increase histamine levels in the normal rat conjunctiva, but no related effect was detected in compound 48/80-induced acute ocular inflammation *in vivo* [18]. These findings were indicative of a peripheral constitutive automodulatory action of histamine that is elicited, at least in part, via the $H_4$ receptor. Until now, these potential properties of histamine have received very limited attention in the literature, despite the functional identification of $H_4$ receptors on the histamine-producing mast cells that may promote chronic inflammation [19, 20] and the well-documented autoinhibitory action of neuronal $H_3$ receptors controlling amine release [4].

Taken together, the data imply a putative role of $H_3$ and $H_4$ receptors in modulating constitutive histamine levels, which are likely modified in response to inflammatory stimuli to shape systemic inflammation. Therefore, we used an experimental model of adjuvant-induced arthritis in a high responder rat strain, which is widely used in studies of chronic inflammation and pain responses and in the preclinical evaluation of anti-inflammatory agents [21, 22], in order to explore for the first time the hypothesis that the onset of arthritis may influence vascular histamine levels *in vivo*. Considering the species and tissue variations [23], as well as the complex pharmacological profile of histamine receptor ligands, mostly related to off-target effects and biased signaling [24, 25], we used GSK334429 and JNJ7777120 in this translational animal model to dissect the role of $H_3$ and $H_4$ receptors, respectively, in modifying the response in the rat large arteries and veins *in vivo*.

## MATERIALS AND METHODS

### Animals

Male Wistar rats of 200–250-g body weight were used throughout the study. They were obtained from the Hellenic Pasteur Institute (Athens, Greece), housed in groups of five per cage, and allowed to acclimate for at least 2 days prior to initialization of experiments. They were maintained under controlled conditions of 12:12 h light–dark cycle, 24 °C, and 60±5 % humidity and received a standard commercial rat chow and tap water ad libitum. A total of 62 animals were randomly divided into groups according to the treatment that they received.

### Drugs and Reagents

The $H_3$ receptor antagonist GSK334429 (1-(1-methylethyl)-4-({1-[6-(trifluoromethyl)-3-pyridinyl]-4-piperidinyl}carbonyl)hexahydro-1H-1,4-diazepine) was provided by GlaxoSmithKline (Essex, UK). The $H_4$ receptor ligand JNJ7777120 (1-[(5-chloro-1H-indol-2-yl)carbonyl]-4-methylpiperazine) was obtained from Johnson & Johnson Pharmaceutical Research and Development (San Diego, CA, USA). The $H_1$ receptor antagonist dimetindene, sodium deoxycholate, and complete Freund's adjuvant (CFA), containing 1 mg of heat killed and dried *Mycobacterium tuberculosis* (H37Ra, ATCC 25177) in 0.85 ml paraffin oil and 0.15 ml mannide monooleate, were purchased from Sigma Chem. Co. (St. Louis, MO, USA). All chemical reagents were of analytical grade.

### Induction and Assessment of Experimental Arthritis

Experimental arthritis was induced on day 0 by a single intradermal injection of 0.1 ml of CFA at the base of tail. The adjuvant was administered in the lowest effective volume and by slow injection to reduce the incidence of severe local inflammatory lesions, granulomas, and abscessation. The respective control animals (normal) received 0.1 ml of normal saline [NaCl 0.9 % (*w*/*v*)].

Following treatment at day 0, the animals were evaluated daily for clinical signs of polyarthritis, during a 3-week observation period by a single observer blinded to their treatments. A clinical severity score of arthritis assigned for each paw was based on a semiquantitative 0 to 4 scale according to the degree of swelling, erythema, and ankylosis as follows: grade 0, no macroscopic signs of swelling, erythema, or ankylosis; grades 1–3, swelling, redness, and ankylosis in one to three digits; and grade 4, severe swelling of the entire paw with ankylosis. The total

score for each paw was calculated by summing the scores of the individual digits with arthritis. The maximum possible score for each paw was, therefore, 4, and the maximum total clinical severity score was 16. Disease onset occurred at 9 to 12 days post-immunization, and by day 20, all animals had reached the maximum score of arthritis severity.

All procedures employed in this study were carried out by fully trained and experienced personnel. They were approved by the institutional and regional committees for animal care and use (license nos. K/3428/2007 and 6089/2012 of the Region of Attica Veterinary Authorities, Greece).

### Drug Administration

GSK334429 and JNJ7777120 were readily dissolved and diluted in normal saline. They were administered under light sevoflurane anesthesia, for less than 1 min, to both normal and CFA-treated animals on day 0 (prophylactic model dosing) by a single 0.1-ml intraperitoneal injection at the left lower quadrant part of the abdomen. Based on the dosing data in the available literature [13, 14, 17, 18, 23], the histamine $H_1$, $H_3$, and $H_4$ receptor ligands were applied at the following doses: GSK334429, $H_3$ receptor antagonist, 1 and 3 mg/kg; JNJ7777120, $H_4$ receptor ligand, 10 and 30 mg/kg; and dimetindene, $H_1$ receptor antagonist, 50 and 100 mg/kg body weight.

### Surgical Preparation and Isolation of Blood Vessels

The animals were sacrificed by decapitation after deep sevoflurane anesthesia, on day 21 following treatment with CFA or normal saline, between 09.00 and 10.00 h in order to minimize circadian fluctuations in histamine levels. Immediately after sacrifice, the abdomen was opened through a 3-cm midline incision and the abdominal cavity was exposed and examined thoroughly macroscopically in order to exclude profound pathology of the visceral organs. Following retraction of the viscera, the abdominal aorta, from below the renal arteries to its bifurcation into the iliac branches, and the inferior vena cava, from its bifurcation in hepatic veins to below the diaphragm, were first ligated proximally and distally and measured in order to minimize differences in lengths and in mass content of each tissue sample and then dissected out. Tissue specimens were rinsed free of blood in normal saline, carefully cleaned of adhering tissue within 5 min after sacrifice, blotted dry, weighted, and assayed.

### Endothelium Removal

In pilot experiments, the vascular endothelium was chemically removed from the isolated vessels, immediately after dissection, by perfusion with 1.8 mg/ml sodium deoxycholate in Krebs solution for 30 s. Perfusion was performed using two short back-cut needle (blue, 1′) 22-G intravenous veterinary peripheral catheters (Abbott Animal Health), connected proximally to each end of the dissected vessel and distally to a catheter hub for free needle venous access (7′ MicroCLAVE® Smallbore with a T-connector, injection site, and a slip luer; Abbott Animal Health), having at each ending a 5-ml luer lock syringe connection (Abbott Animal Health) in order to rinse with sodium deoxycholate-free Krebs solution [26]. The 7′ MicroCLAVE® Smallbore system was used because of its neutral displacement feature, minimizing back flow into the catheter upon disconnection and maintaining the flow rate (gravity 165/min). During perfusion, no clamping protocol was used.

### Extraction and Quantification of Histamine

Tissues were homogenized with 0.4 N perchloric acid using a Teflon glass homogenizer (TRI-R Instruments, NY, USA). Histamine was extracted successively, using Quickfit® tubes (Bibby Scientific Ltd, Staffordshire, UK), in *n*-butanol, sulfuric acid, and *n*-heptane, any residual amounts of histidine being removed by washing in NaCl-saturated NaOH [27]. Tissue histamine was quantified fluorophotometrically, at 360-nm excitation and 450-nm emission, after condensation with *o*-phthalaldehyde, and expressed as nanogram per milligram wet tissue [18, 27]. Control experiments were performed to rule out any interference of the histamine receptor ligands with the histamine determination assay.

### Histological Analysis and Immunohistochemistry

Immediately after dissection, isolated specimens of the abdominal aorta were fixed in 10 % neutral formalin for 18 h and then were routinely processed and embedded in paraffin. Samples were cut in 4-μm-thick sections and stained with hematoxylin and eosin stain for histological evaluation. Elastic fibers were highlighted with orcein histochemical stain (orcein in 70 % ethanol).

Immunohistochemistry for the endothelial marker CD31 was performed on sections mounted onto poly-L-lysine-coated slides using the Novolink Polymer Detection System Kit (Novocastra Laboratories–Menarini, Greece) [28]. Briefly, sections were heated at 58 °C, deparaffinized in xylene, rehydrated in graded ethanol, and washed in

Tris-buffered saline (pH 7.6). Antigen retrieval was performed in citrate buffer (pH 6.0) using microwaves (600 W) for 12 min. Sections were then incubated overnight at 4 °C with anti-CD31 primary antibody (mouse monoclonal, clone JC70A, DAKO, USA) at 1:50 dilution. 3′,3′-Diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO, USA) was used as chromogen and Harris hematoxylin (Merck, Germany) for counterstaining. In negative control sections, the primary antibody was replaced with non-immune diluent compatible with the kit (RE7133, Novocastra Laboratories–Menarini, Greece).

### Statistical Analyses

The results were expressed as mean±standard error of the mean (SEM). Statistical evaluation of the data was performed by analysis of variance (ANOVA) followed by Scheffé or Dunnett's *post hoc* test for multiple comparisons. Statistical dependence between variables was assessed by the non-parametric Spearman's rank correlation coefficient ($\rho$). All statistical analyses were carried out using IBM® SPSS® Statistics version 20. All statistical tests were two-tailed, and a $P$ value <0.05 was considered significant with a confidence interval of 95 %.

## RESULTS

### Experimental Arthritis Increases Vascular Histamine Levels

To study the extra-articular effects of arthritis in peripheral blood vessels, CFA-induced arthritis in the rat was used as an experimental model [21, 22]. CFA administration resulted in the progressive development of arthritic signs in the joints of the rat paws, including profound ankylosis and edema (Fig. 1a). The development of the clinical arthritic phenotype was reproducible in all CFA-treated rat paws, yielding a severity score of 16 by day 21. Therefore, no test animal was excluded from the study.

Interestingly, the histamine levels in the abdominal aorta were significantly increased from 0.66±0.15 ng/mg in normal rats to 1.10±0.18 ng/mg in arthritic animals (Fig. 1b). Similar increases from 0.73±0.20 ng/mg to 1.01±0.15 ng/mg were observed in the inferior vena cava (Fig. 1c). No significant difference between the histamine contents of the tissues examined was observed in either normal or arthritic animals ($P$>0.05), and there was no significant correlation between tissue weight and histamine levels in the specimens ($\rho$=0.001 and $\rho$=0.042 for abdominal aorta and inferior vena cava, respectively; $P$>0.05).

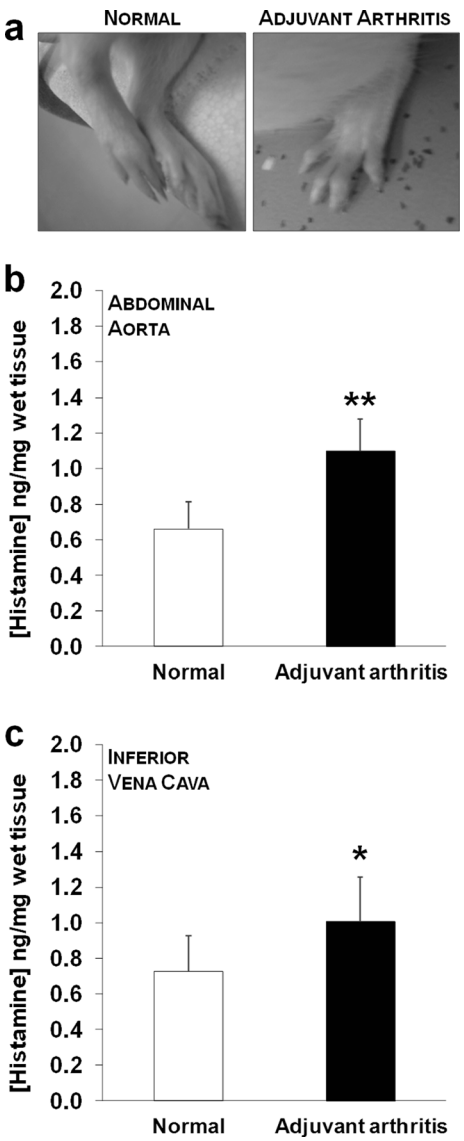


**Fig. 1.** Adjuvant arthritis induces joint destruction and increases histamine levels in the large blood vessels. **a** Representative images of paws. Histamine levels in the **b** abdominal aorta and **c** inferior vena cava of normal rats ($n$=9) and animals with adjuvant arthritis ($n$=12). Data are expressed as mean±SEM. *$P$<0.05, **$P$<0.01 compared to the respective normal tissue.

Considering that histamine is a key mediator of inflammation, these data provide evidence for the induction of systemic response in this experimental model and are reported for the first time. The changes in histamine levels in the abdominal aorta and the inferior vena cava were used as an indicator for the investigation of extra-articular histamine actions in the blood vessels of animals with arthritis.

### GSK334429 Increases Basal Vascular Histamine Levels and Partially Restores the Normal Phenotype in Arthritic Rats

Given the existing data on the cardiovascular effects of the $H_3$ receptor [7], its dual role to regulate neuronal histamine as presynaptic autoreceptor and to act as heteroreceptor [4], as well as the observation that experimental arthritis and histamine content of the examined blood vessels were associated, we then investigated the contribution of $H_3$ receptors in the putative peripheral autoregulatory action of histamine. In normal animals, a single injection of GSK334429 dose-dependently increased tissue histamine levels in both the abdominal aorta ($\rho$=0.953, $P$<0.001) and the inferior vena cava ($\rho$=0.850, $P$<0.05), reaching 1.57±0.08 and 1.84±0.06 ng/mg, respectively, upon administration of 3 mg/kg GSK334429 (Fig. 2a).

On the contrary, in CFA-treated animals (Fig. 2b), prophylactic treatment with the $H_3$ receptor antagonist dose-dependently restored normal histamine levels in the abdominal aorta ($\rho$=−0.953, $P$<0.01) but did not alter the histamine content of the inferior vena cava ($\rho$=−0.371, $P$>0.05). Despite the increases in the histamine content of the normal vascular tissues, GSK334429 induced no detectable arthritic manifestations in the normal rat paws, whereas the signs of ankylosis and edema were attenuated in the CFA-treated animals (Fig. 2c). Collectively, this pharmacological evidence pointed to a direct or indirect $H_3$ receptor-mediated modulation of the basal physiological and/or biochemical properties of endogenous vascular histamine *in vivo*. Furthermore, $H_3$ receptor-mediated changes of vascular histamine seemed to be partially associated with the onset of the arthritic phenotype in the rat.

### JNJ7777120 Increases Basal Vascular Histamine Levels But Fails to Restore the Normal Phenotype in Arthritic Rats

The finding that histamine content of blood vessels and joint destruction in experimental arthritis were differentially modulated by $H_3$ receptor blockade and the recent reports on the implication of the $H_4$ receptor in inflammation [5, 20], particularly in the pathophysiology of arthritic disease [10–12], led the investigation on the involvement of $H_4$ receptors in the putative automodulation of histamine levels in the peripheral blood vessels. In normal rats, JNJ7777120 injection dose-dependently increased the histamine content of the abdominal aorta ($\rho$=0.857, $P$<0.001) up to 1.81±0.14 ng/mg and of the inferior vena cava ($\rho$=0.795, $P$<0.01) up to 1.99±0.22 ng/mg (Fig. 3a).

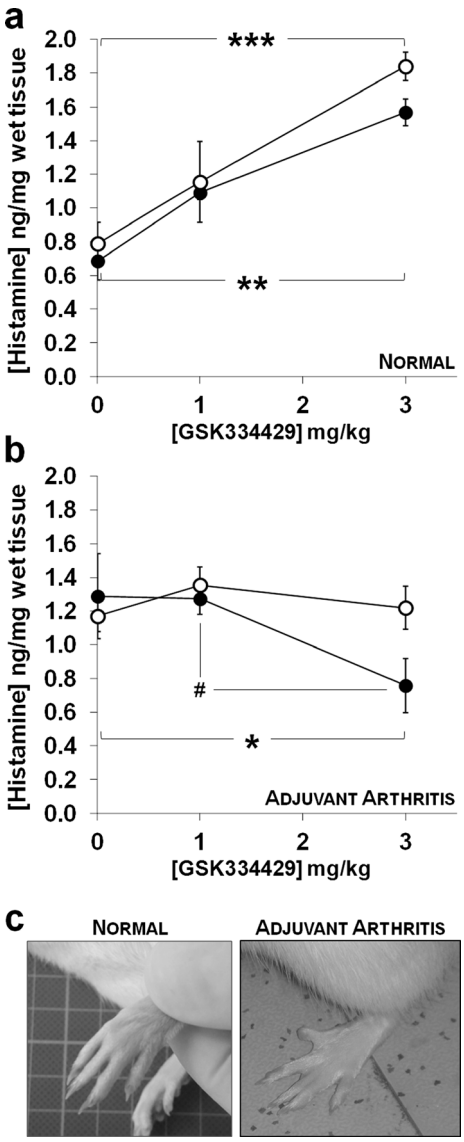


**Fig. 2.** Effects of the $H_3$ receptor antagonist GSK334429 on vascular histamine content and arthritic manifestations. **a** In normal rats, blockade of $H_3$ receptors with 1 mg/kg ($n$=3) or 3 mg/kg ($n$=4) of the antagonist increased histamine levels in the abdominal aorta (*closed circles*) and the inferior vena cava (*open circles*). **b** In animals with adjuvant arthritis, prophylactic treatment with 1 mg/kg ($n$=8) or 3 mg/kg ($n$=8) GSK334429 restored the normal histamine content of the abdominal aorta (*closed circles*) but not of the inferior vena cava (*open circles*). **c** Representative images of paws from normal and arthritic animals treated with 3 mg/kg GSK334429 illustrating that the $H_3$ receptor antagonist did not induce any alterations in the normal paws and reversed the arthritic signs in animals with adjuvant arthritis. Data are presented as mean±SEM. *$P$<0.05, **$P$<0.01, ***$P$<0.001 compared to the respective GSK334429-untreated control groups ($n$=4); $^{\#}P$<0.05 compared to treatment with 1 mg/kg GSK334429.

However, the $H_4$ receptor ligand failed to induce any significant alteration in the histamine content of either the abdominal aorta ($\rho$=−0.155, $P$>0.05) or the inferior vena cava ($\rho$=−0.080, $P$>0.05) in experimental arthritis (Fig. 3b). Furthermore, JNJ7777120 administration did not induce the arthritic phenotype in normal animals, and signs of ankylosis and edema were developed in the paws of CFA-treated animals (Fig. 3c). These observations indicated that, similarly to $H_3$ receptors, $H_4$ receptors are also implicated in the modulation of endogenous vascular histamine levels in normal rats and suggest a differential action of $H_3$ and $H_4$ receptors in the related mechanisms underlying the onset of arthritis.

### Dimetindene Induces No Alteration in Basal Vascular Histamine Levels

Given the higher affinity of $H_3$ and $H_4$ receptors for histamine compared to the low-affinity $H_1$ receptors [3] and the adaptation of vascular $H_1$ receptor density to the actual requirements of the circulation [29], we tested whether the modulation of endogenous vascular histamine in normal blood vessels is confined to $H_3$ and $H_4$ receptors by using dimetindene, an $H_1$ receptor antagonist with known therapeutic efficacy in inflammatory conditions. The intraperitoneal injection of dimetindene induced no significant alteration in the histamine content of either the abdominal aorta ($\rho$=0.316, $P$>0.05) or the inferior vena cava ($\rho$=0.170, $P$>0.05) in normal rats (Fig. 4). Moreover, no signs of ankylosis or edema were developed in the paws of normal animals upon treatment with the $H_1$ receptor antagonist (data not shown). The modulation of normal vascular histamine levels by GSK334429 and JNJ7777120, but not by dimetindene, implies that this histamine action in the rat large blood vessels may be selectivity elicited by targeting the high-affinity $H_3$ and $H_4$ receptors.

### Vascular Histamine Is Undetectable After Chemical Removal of the Endothelium

In an attempt to examine the involvement of the vascular endothelium in the alterations of histamine levels in peripheral blood vessels, tissue histamine was quantified in pilot experiments following removal of the endothelium. The successful *ex vivo* endothelial removal without damage to the tunica media was confirmed in representative tissue samples by routine histological studies and immunostaining with the endothelial cell marker CD31 [28]. The complete removal of the vascular endothelium (Fig. 5a–c)

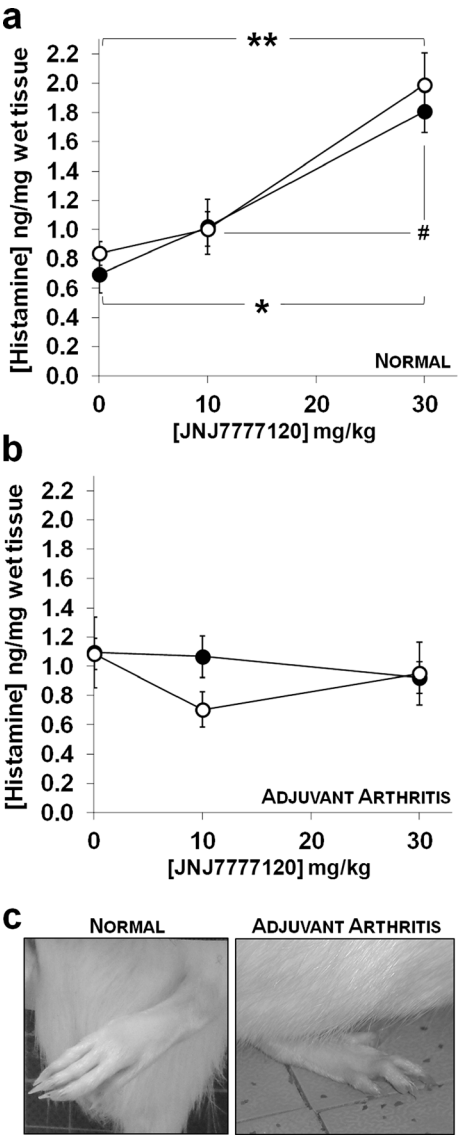


**Fig. 3.** Effects of the $H_4$ receptor antagonist JNJ7777120 on vascular histamine content and arthritic manifestations. **a** In normal rats, 10 mg/kg ($n$=4) or 30 mg/kg ($n$=6) JNJ7777120 increased dose-dependently the histamine content of the abdominal aorta (*closed circles*) and inferior vena cava (*open circles*). **b** In animals with adjuvant arthritis, prophylactic administration of 10 mg/kg ($n$=4) or 30 mg/kg ($n$=8) JNJ7777120 induced no significant alterations in the histamine levels of either the abdominal aorta (*closed circles*) or the inferior vena cava (*open circles*). **c** Representative images of paws from normal rats and from animals with adjuvant arthritis treated with 30 mg/kg JNJ7777120 showing that the $H_4$ receptor antagonist did not induce any alterations in either the normal or the arthritic phenotype. Data are presented as mean±SEM. *$P$<0.05, **$P$<0.01 compared to the respective JNJ7777120-untreated control groups ($n$=4); $^{\#}P$<0.05 compared to treatment with 10 mg/kg JNJ7777120.

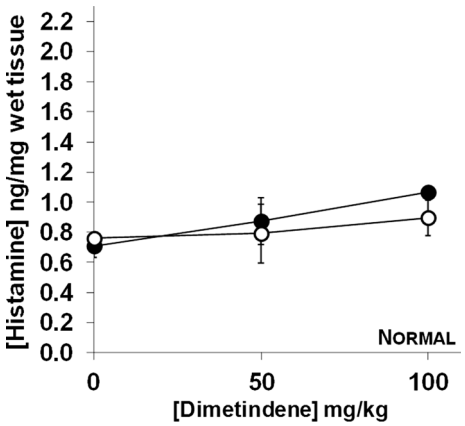


**Fig. 4.** Blockade of $H_1$ receptors with 50 mg/kg ($n$=7) or 100 mg/kg ($n$=4) dimetindene did not significantly affect basal histamine levels in either the abdominal aorta (*closed circles*) or the inferior vena cava (*open circles*) of normal rats compared to the absence of the drug ($n$=7).

was demonstrated, compared to control tissues that were not perfused with sodium deoxycholate (Fig. 5d).

Histamine was not detectable after removal of the endothelium in either the abdominal aorta or the inferior vena cava isolated from normal and arthritic animals following administration of 1 and 3 mg/kg GSK334429 or 10 and 30 mg/kg JNJ7777120 ($n$=4). These findings implied a role of the vascular endothelium in the observed effects of the $H_3$ and $H_4$ receptor ligands and provided the lead for the elucidation of the mechanisms underlying the putative peripheral automodulatory action of histamine in the rat large vessels that is currently under investigation.

## DISCUSSION

This study investigated for the first time the putative peripheral automodulation of histamine in the large blood vessels under physiological and inflammatory conditions *in vivo*. The major findings revealed that tissue histamine levels in the abdominal aorta and the inferior vena cava are increased in experimental arthritis. Moreover, $H_3$ and $H_4$ receptor antagonists induce comparable increases in the basal histamine content of the rat large blood vessels without considerable manifestations of arthritic signs in the joints of the animal paws. It should be pointed out that the fact that these effects were observed 21 days after a single injection of the agents deserves further investigation. The elevation of vascular histamine content in experimental arthritis can be attributed to the induction of a systemic response after CFA administration that may characterize extra-articular inflammation. Contrary to JNJ7777120, prophylactic treatment with GSK334429 partially attenuated the signs of ankylosis and edema in the rat paws and restored basal histamine levels in the abdominal aorta, but not in the inferior vena cava, thus providing pharmacological evidence for the role of the $H_3$ receptor in the

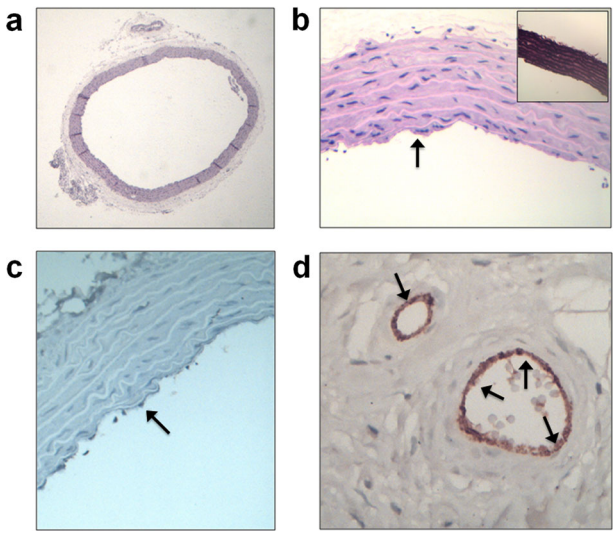


**Fig. 5.** Histological evidence for the removal of the endothelium by perfusion with sodium deoxycholate in transverse sections of the abdominal aorta. **a** Representative hematoxylin and eosin-stained section (magnification, ×25). **b** Absence of endothelium on the luminal surface of the aortic wall (*arrow*) (hematoxylin and eosin, magnification, ×200), *inset*: orcein staining highlights elastic fibers of the aortic wall (magnification, ×100). **c** Staining for the endothelial marker CD31 confirmed the absence of endothelium on the luminal surface of the aortic wall (*arrow*) (magnification, ×200). **d** Positive immunostaining for CD31 in endothelial cells (*arrows*) of aortic vasa vasorum that served as internal positive control (magnification, ×400).

modulation of arterial histamine during the onset of arthritis. Collectively, the data support the differential involvement of $H_3$ and $H_4$ receptors in the regulation of tissue histamine levels in the large blood vessels of normal and arthritic rats (Fig. 6). Furthermore, the findings underscore the need for careful consideration of the local mechanisms that may generate differential responses in different vascular beds following systemic administration of ligands targeting histamine receptors.

How inflammatory diseases target various distant organs and tissues remains elusive. In general, the available data implicate the $H_4$ receptor in the pathogenesis and progression of arthritis [12, 15], but its contribution to the associated extra-articular disease, including cardiovascular dysfunction is far from clear. Most studies performed in various preclinical rodent models of RA have focused on joint pathology and on the association of immune components to the arthritic phenotype [12]. We have shown that CFA-induced arthritis in the rat is characterized by joint destruction accompanied by increases in vascular histamine levels. In the K/BxN serum transfer model of arthritis, histamine has been implicated in the rapid induction of vascular permeability in the joints [30], without corresponding alterations in serum histamine levels [31]. Therefore, tissue histamine levels might be a critical factor that fosters the cross-talk of vascular integrity and immune effector pathways. In a preliminary attempt to identify the source of histamine under the conditions used in our experiments, we failed to detect any amounts of histamine after chemical removal of the endothelium from the blood vessels *ex vivo*, suggesting the likely contribution of the vascular endothelium in these findings. However, the results should be interpreted with caution since cultured rat aortic smooth muscle cells have been suggested to synthesize histamine locally within the vascular wall [32]. Although activation of mast cells and depletion of histamine stores following the chemical removal of the endothelium cannot be excluded, it is worth pointing out that non-mast cell histamine release from rat aortic rings was reported to largely depend on the presence of endothelium [33] and that histamine-mediated endothelium-dependent relaxations were significantly diminished in thoracic aortic rings isolated from a corresponding rat model of adjuvant arthritis [21].

Given the findings that systemic administration of either GSK334429 or JNJ7777120, but not dimetindene, induced tissue histamine levels in the abdominal aorta and the inferior vena cava, it can be suggested that histamine might be under constitutive negative regulation in these tissues. On the other hand, despite the absence of joint manifestations, the histamine receptor ligands may exert pro-inflammatory effects in the rat blood vessels [25]. Similar increases in the conjunctival histamine content have been observed following topical administration of JNJ7777120 in the rat eye [18]. On the basis of pharmacological evidence, it becomes increasingly apparent that the outcome of studies in translational animal models cannot be unequivocally predicted by the *in vitro* ligand pharmacology [26, 25]. Undoubtedly, the functional selectivity of the histamine receptor-targeting compounds that were used in this *in vivo* study cannot be ruled out [24]. Interestingly, however, $H_4$ receptor deficiency coupled to impaired down-regulation of tissue histamine levels has been implicated in Sjogren's syndrome [34]. The recently reported related data showed that constitutive release/transport mechanisms and the metabolizing capacity of non-

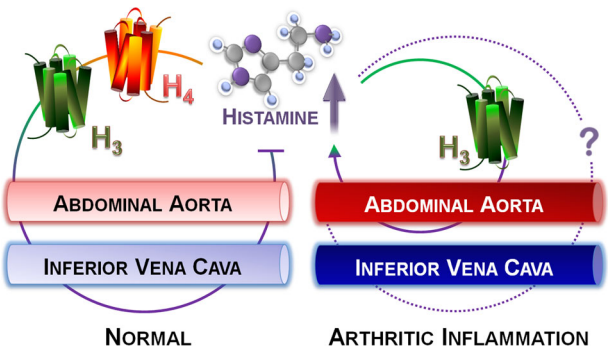


**Fig. 6.** Potential implication of the newest high-affinity histamine $H_3$ and $H_4$ receptors in the constitutive modulation of vascular histamine and their differential action during the onset of arthritis. Schematic representation of the putative concerted role of the receptors in mediating the regulation of basal vascular histamine levels in the normal rat large blood vessels (*left*) and contribution of $H_3$ receptors in the increased arterial histamine levels that partly underlie the systemic extra-articular inflammatory response in a rat translational model of arthritis (*right*). The identification of complementary components that seem to be in operation (*?*) is critical for the elucidation of the role of different vascular beds in systemic inflammatory disorders and for the evaluation of the therapeutic potential of histamine receptor ligands.

professional histamine-producing cells may maintain low tissue histamine concentrations acting on high-affinity $H_4$ receptors, but not on $H_1$ receptors [34]. The latter can be activated following a burst histamine release from professional histamine-producing cells, such as mast cells [34]. This may justify our observations that JNJ7777120 failed to circumvent the elevated vascular histamine content in CFA-induced arthritis. Our findings are also consistent with the inability of JNJ7777120 to reverse the acute inflammatory response in the rat conjunctiva upon mast cell degranulation induced by the histamine releaser compound 48/80 [18]. Furthermore, the reported biphasic action of JNJ7777120 upon mast cell stabilization may reflect the biased signaling of this agent [18, 24, 25].

That the $H_3$ receptor likely elicits a distinct action in the arthritic large blood vessels from the $H_4$ receptor is revealed by the restoration of basal histamine levels in the abdominal aorta of arthritic animals (Fig. 6) along with the improvement of clinical signs in the rat joints by GSK334429, contrary to the outcome obtained after JNJ7777120 administration. Regarding the role of $H_3$ receptors in the cardiovascular system, the majority of data concern the sympathetic activity and the modulation of the vascular tone [7, 8]. In addition to the putative non-receptor-mediated histamine signaling that may drive inflammation [35, 36], the $H_3$ receptors have been identified as negative regulators of peripheral immune responses and implicated in experimental allergic encephalomyelitis, the principal autoimmune model of multiple sclerosis [37]. CFA treatment resulted in rapid differential expression of $H_3$ receptor (*Hrh3*) isoforms, and modest early changes in *Hrh3* expression were suggested to significantly alter the subsequent immune response and the course of inflammation [37]. In fact, CFA treatment led to the differential expression of many genes associated with inflammatory and endothelial responses between wild-type and $H_3$ receptor knock-out mice, including various proteins that contribute to vascular tone and inflammation [37]. Consequently, the $H_3$ receptor is likely to be a yet elusive key intermediate integrating the constitutive negative regulation of vascular histamine and the tissue-specific histaminergic response associated with the onset of arthritis following CFA treatment. However, additional work is required to fully understand the mechanisms underlying these putative processes and their potential biological significance and exploitation in arthritic inflammation.

In summary, the evidence gathered during this study implicates the high-affinity $H_3$ and $H_4$ receptors in the modulation of vascular histamine in the large blood vessels *in vivo*. Without disregarding species variations and the recent dramatically increased literature on the complex pharmacological properties of histamine receptor ligands that direct the type and magnitude of functional activity [23–25, 38], our studies provide first evidence for a concerted constitutive inhibitory role of $H_3$ and $H_4$ receptors in the regulation of basal vascular histamine levels (Fig. 6). Although, on the basis of current results, we cannot assess all potential systemic effects of the endogenous histaminergic system that may associate vascular (patho)physiology to inflammatory disorders, our data identify the $H_3$ receptor as a key component influencing arterial histamine during the onset of arthritis and therefore uncover a new mechanism that may affect cardiovascular parameters in inflammation. No data on the potential autoreceptor role of histamine receptors in peripheral blood vessels are currently available, and as yet, no clear picture has emerged unifying all the reported findings that have emerged from studies using biased and unbiased ligands. Hence, in future studies, it will be important to identify the cellular source of histamine and the biochemical processes that regulate vascular histamine levels and to uncover how histamine receptors might contribute to the development of vascular dysfunction that may affect target cells in autoimmune diseases.

## ACKNOWLEDGMENTS

This work was supported by grants from the Greek Ministry of Health (8309) and the University of Athens Research Account (5900), and it was part of the EU European Cooperation in Science and Technology COST Action BM0806, "Recent advances in histamine receptor $H_4$R research."

**Conflict of Interest.** The authors confirm that this article content has no conflicts of interest.

## REFERENCES

1. Parsons, M.E., and C.R. Ganellin. 2006. Histamine and its receptors. *British Journal of Pharmacology* 147(S1): 127–125.
2. Kakavas, S., E. Zampeli, K. Papamichael, B. Delitheos, and E. Tiligada. 2006. The mast cell pathway to inflammation and homeostasis: Pharmacological insights. *Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry* 5: 323–324.
3. Tiligada, E., E. Zampeli, K. Sander, and H. Stark. 2009. Histamine $H_3$ and $H_4$ receptors as novel drug targets. *Expert Opinion on Investigational Drugs* 18: 1519–1531.

4. Tiligada, E., K. Kyriakidis, P.L. Chazot, and M.B. Passani. 2011. Histamine pharmacology and new CNS drug targets. *CNS Neuroscience & Therapeutics* 17: 620–628.
5. Zampeli, E., and E. Tiligada. 2009. The role of histamine $H_4$ receptor in immune and inflammatory disorders. *British Journal of Pharmacology* 157: 24–33.
6. Sasaguri, Y., K.Y. Wang, A. Tanimoto, et al. 2005. Role of histamine produced by bone marrow-derived vascular cells in pathogenesis of atherosclerosis. *Circulation Research* 96: 974–981.
7. Sun, P., X. Jin, T. Koyama, S. Li, Y. Kitamura, and H. Kawasaki. 2010. R-(−)-alpha-methylhistamine, a histamine $H_3$ receptor agonist, induces endothelium-dependent vasodilation in rat mesenteric resistance arteries. *Biological and Pharmaceutical Bulletin* 33: 58–63.
8. Malinowska, B., G. Godlewski, and E. Schlicker. 1998. Histamine $H_3$ receptors—general characterization and their function in the cardiovascular system. *Journal of Physiology and Pharmacology* 49: 191–211.
9. Martín-Martínez, M.A., C. González-Juanatey, S. Castañeda, et al. 2014. Recommendations for the management of cardiovascular risk in patients with rheumatoid arthritis: Scientific evidence and expert opinion. *Seminars in Arthritis and Rheumatism* 44: 1–8.
10. Ohki, E., M. Suzuki, T. Aoe, Y. Ikawa, E. Negishi, and K. Ueno. 2007. Expression of histamine $H_4$ receptor in synovial cells from rheumatoid arthritic patients. *Biological and Pharmaceutical Bulletin* 30: 2217–2220.
11. Grzybowska-Kowalczyk, A., D. Maslinska, M. Wojciechowska, et al. 2008. Expression of histamine $H_4$ receptor in human osteoarthritic synovial tissue. *Inflammation Research* 57(S1): 63–64.
12. Cowden, J.M., F. Yu, H. Banie, et al. 2014. The histamine $H_4$ receptor mediates inflammation and Th17 responses in preclinical models of arthritis. *Annals of the Rheumatic Diseases* 73: 600–608.
13. Medhurst, A.D., M.A. Briggs, G. Bruton, et al. 2007. Structurally novel histamine $H_3$ receptor antagonists GSK207040 and GSK334429 improve scopolamine-induced memory impairment and capsaicin-induced secondary allodynia in rats. *Biochemical Pharmacology* 73: 1182–1194.
14. Cowart, M., G. Hsieh, L.A. Black, et al. 2012. Pharmacological characterization of A-960656, a histamine $H_3$ receptor antagonist with efficacy in animal models of osteoarthritis and neuropathic pain. *European Journal of Pharmacology* 684: 87–94.
15. Abd-Allah, A.R., S.F. Ahmad, I. Alrashidi, et al. 2014. Involvement of histamine 4 receptor in the pathogenesis and progression of rheumatoid arthritis. *International Immunology* 26: 325–340.
16. Adlesic, M., M. Verdrengh, M. Bokarewa, L. Dahlberg, S.J. Foster, and A. Tarkowski. 2007. Histamine in rheumatoid arthritis. *Scandinavian Journal of Immunology* 65: 530–537.
17. Thurmond, R.L., P.J. Desai, P.J. Dunford, et al. 2004. A potent and selective histamine $H_4$ receptor antagonist with anti-inflammatory properties. *Journal of Pharmacology and Experimental Therapeutics* 309: 404–413.
18. Zampeli, E., R.L. Thurmond, and E. Tiligada. 2009. The histamine $H_4$ receptor antagonist JNJ7777120 induces increases in the histamine content of the rat conjunctiva. *Inflammation Research* 58: 285–291.
19. Hofstra, C.L., P.J. Desai, R.L. Thurmond, and W.P. Fung-Leung. 2003. Histamine $H_4$ receptor mediates chemotaxis and calcium mobilization of mast cells. *Journal of Pharmacology and Experimental Therapeutics* 305: 1212–1221.
20. Tiligada, E. 2012. Editorial: Is histamine the missing link in chronic inflammation? *Journal of Leukocyte Biology* 92: 4–6.
21. Fang, Z.Y., J. Fontaine, P. Unger, and G. Berkenboom. 1991. Alterations of the endothelial function of isolated aortae in rats with adjuvant arthritis. *Archives Internationales de Pharmacodynamie et de Thérapie* 311: 122–130.
22. Inglis, J.J., A. Nissim, D.M. Lees, S.P. Hunt, Y. Chernajovsky, and B.L. Kidd. 2005. The differential contribution of tumour necrosis factor to thermal and mechanical hyperalgesia during chronic inflammation. *Arthritis Research and Therapy* 7: R807–816.
23. Coruzzi, G., C. Pozzoli, M. Adami, et al. 2012. Strain-dependent effects of the histamine $H_4$ receptor antagonist JNJ7777120 in a murine model of acute skin inflammation. *Experimental Dermatology* 21: 32–37.
24. Nijmeijer, S., H.F. Vischer, F. Sirci, et al. 2013. Detailed analysis of biased histamine $H_4$ receptor signalling by JNJ 7777120 analogues. *British Journal of Pharmacology* 170: 78–88.
25. Nordemann, U., D. Wifling, D. Schnell, et al. 2013. Luciferase reporter gene assay on human, murine and rat histamine $H_4$ receptor orthologs: Correlations and discrepancies between distal and proximal readouts. *PloS One* 8: e73961.
26. Takenaga, M., H. Kawasaki, A. Wada, and T. Eto. 1995. Calcitonin gene-related peptide mediates acetylcholine-induced endothelium-independent vasodilation in mesenteric resistance blood vessels of the rat. *Circulation Research* 76: 935–941.
27. Tiligada, E., D. Aslanis, A. Delitheos, and D. Varonos. 2000. Changes in histamine content following pharmacologically-induced mast cell degranulation in the rat conjunctiva. *Pharmacological Research* 41: 667–670.
28. Pusztaszeri, M.P., W. Seelentag, and F.T. Bosman. 2006. Immunohistochemical expression of endothelial markers CD31, CD34, von Willebrand factor, and Fli-1 in normal human tissues. *The Journal of Histochemistry and Cytochemistry* 54: 385–395.
29. Galajda, Z., J. Balla, A.J. Szentmiklosi, et al. 2011. Histamine and $H_1$-histamine receptors faster venous circulation. *Journal of Cellular and Molecular Medicine* 15: 2614–2623.
30. Binstadt, B.A., P.R. Patel, H. Alencar, et al. 2006. Particularities of the vasculature can promote the organ specificity of autoimmune attack. *Nature Immunology* 7: 284–292.
31. Blanchet, M.R., M. Gold, S. Maltby, et al. 2010. Loss of CD34 leads to exacerbated autoimmune arthritis through increased vascular permeability. *Journal of Immunology* 184: 1292–1299.
32. Tippens, A.S., S.V. Davis, J.R. Hayes, E.C. Bryda, T.L. Green, and C.A. Gruetter. 2004. Detection of histidine decarboxylase in rat aorta and cultured rat aortic smooth muscle cells. *Inflammation Research* 53: 390–395.
33. Gruetter, C.A., B. Bailey, L. Easterling, and J.L. Szarek. 2002. Release of non-mast cell histamine from rat aorta. *Life Sciences* 70: 1709–1717.
34. Stegaev, V., A.T. Nies, P. Porola, et al. 2013. Histamine transport and metabolism are deranged in salivary glands in Sjogren's syndrome. *Rheumatology* 52: 1599–1608.
35. Saligrama, N., L.K. Case, R. del Rio, R. Noubade, and C. Teuscher. 2013. Systemic lack of canonical histamine receptor signaling results in increased resistance to autoimmune encephalomyelitis. *Journal of Immunology* 191: 614–622.
36. Kyriakidis, D.A., and E. Tiligada. 2013. Non receptor-mediated histamine actions. In *Histamine $H_4$ receptor: A novel drug target for immunoregulation and inflammation*, ed. H. Stark, 309–32. London: Versita.
37. Krementsov, D.N., E.H. Wall, R.A. Martin, et al. 2013. Histamine h3 receptor integrates peripheral inflammatory signals in the neurogenic control of immune responses and autoimmune disease susceptibility. *PloS One* 8: e62743.
38. Tiligada, E. 2013. Perspectives in $H_4$R research and therapeutic exploitation. In *Histamine $H_4$ receptor: A novel drug target for immunoregulation and inflammation*, ed. H. Stark, 333–52. London: Versita.