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Pharmacological Evidence that Histamine H_3 Receptors Mediate Histamine-Induced Inhibition of the Vagal Bradycardic Out-flow in Pithed Rats

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Abstract: In vivo stimulation of cardiac vagal neurons induces bradycardia by acetylcholine (ACh) release. As vagal release of ACh may be modulated by autoreceptors (muscarinic M_2) and heteroreceptors (including serotonin 5-HT_1), this study has analysed the pharmacological profile of the receptors involved in histamine-induced inhibition of the vagal bradycardic out-flow in pithed rats. For this purpose, 180 male Wistar rats were pithed, artificially ventilated and pre-treated (i.v.) with 1 mg/kg atenolol, followed by i.v. administration of physiological saline (1 ml/kg), histamine (10, 50, 100 and 200 μg/kg) or the selective histamine H_1 (2-pyridylethylamine), H_2 (dimaprit), H_3 (methimepip) and H_4 (VUF 8430) receptor agonists (1, 10, 50 and 100 μg/kg each). Under these conditions, electrical stimulation (3, 6 and 9 Hz; 15 ± 3 V and 1 ms) of the vagus nerve resulted in frequency-dependent bradycardic responses, which were (i) unchanged during the infusions of saline, 2-pyridylethylamine, dimaprit or VUF 8430; and (ii) dose-dependently inhibited by histamine or methimepip. Moreover, the inhibition of the bradycardia caused by 50 μg/kg of either histamine or methimepip (which failed to inhibit the bradycardic responses to i.v. bolus injections of acetylcholine; 1–10 μg/kg) was abolished by the H_3 receptor antagonist JNJ 10181457 (1 mg/kg, i.v.). In conclusion, our results suggest that histamine-induced inhibition of the vagal bradycardic out-flow in pithed rats is mainly mediated by pre-junctional activation of histamine H_3 receptors, as previously demonstrated for the vasopressor sympathetic out-flow and the vasodepressor sensory CGRPergic (calcitonin gene-related peptide) out-flow.

Heart rate is a cardiovascular parameter that mostly depends on the sympathovagal balance [1]. Hence, activation of the sympathetic nervous system causes cardio-excitation (by increasing heart rate, blood pressure and myocardial contractility), whereas activation of the parasympathetic nervous system (whose major component is the vagus nerve) results in cardio-inhibition (by decreasing these parameters) [1]. Indeed, electrical stimulation of cardiac vagal neurons, which involves acetylcholine release, induces frequency-dependent bradycardic responses in pithed rats [2]. As continuous vagal stimulation increases the size of cardiac infarct by sympathetic co-activation [3], the activation of modulatory mechanisms on vagal acetylcholine release may be a therapeutic target in cardiac problems. In this respect, our group has already shown that activation serotonin 5-HT_1 receptors inhibit the vagal bradycardic out-flow in normoglycaemic and diabetic pithed rats [2,4,5].

Histamine is another relevant biogenic monoamine that plays a key role in the control of a variety of central and peripheral functions as well as a pathophysiological role in various allergic disorders by interacting with histamine H_1, H_2, H_3 and H_4 receptors [6]. At the cardiovascular level, direct activation of H_1 and H_2 receptors may induce (depending, amongst other factors, on the experimental conditions, administration route, dose and species used) vasodilatation, vasoconstriction and positive chronotropic effects in mammals [7–10]. The complex pharmacological effects of histamine may also be explained by its interactions with histamine H_3 and H_4 receptors [11,12], which are coupled to $G_{i/o}$ proteins [13,14].

Histamine H_4 receptors have been detected at peripheral and central neurons, as well as in peripheral tissues; yet, cells that clearly express functional H_4 receptors are mainly haematopoietic, playing a significant role in regulating dendritic and T-cell function [15]. In contrast, histamine H_3 receptors (i) are mainly found in the central and peripheral nervous systems, airways, the cardiovascular system and the gastrointestinal tract; (ii) play a role as presynaptic autoreceptors (modulating the release of histamine [16]) and as heteroreceptors on non-histaminergic neurons (modulating the release of acetylcholine, noradrenaline, dopamine or serotonin [6,17–21]); (iii) modulate the release of acetylcholine in rat entorhinal cortex [22,23]; and (iv) seem to protect against an excess of parasympathetic bronchoconstriction (due to their location on post-ganglionic cholinergic nerves) and inhibit the muscarinic receptor-activated calcium signalling in rat carotid body Type I cells [24]. Moreover, it has been suggested that histamine H_3 receptor antagonists with central actions may have clinical efficacy in cognitive-related disorders, especially those in which ACh neurotransmission is compromised [25].

Accordingly, histamine's interactions with the peripheral nervous system may play an important role in cardiovascular

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regulation. Indeed, activation of pre-junctional H_3 receptors in pithed rats inhibits the vasopressor sympathetic out-flow [17] and the vasodepressor sensory CGRPergic (calcitonin gene-related peptide) out-flow [21]. This study has now investigated in pithed rats whether histamine can inhibit the vagal bradycardic out-flow and, if so, the pharmacological profile of the receptors involved. For this purpose, in addition to the potential inhibition induced by the endogenous ligand and by selective agonists at histamine H_1, H_2, H_3 or H_4 receptors, the effect of the selective H_3 receptor antagonist, JNJ 10181457 [25], was analysed on the above inhibition of the vagal bradycardic out-flow.

Materials and Methods

Animals. A total of 180 male Wistar rats (300 ± 25 g) were maintained at a 12/12-hr light/dark cycle (with light beginning at 07:00 hr) and housed in a special room at constant temperature (22 ± 2°C) and humidity (50%), with food and water freely available in their home cages. Housing conditions and experimental procedures were in accordance with regulations provided by the European Union on the use of animals for scientific purposes (2010/63/UE). This was enacted by Spanish legislation on 1 February 2013 (R.D. 53/2013). All protocols were approved by the University of Salamanca Institutional Bioethics committee (006N°201400037737).

General methods. After anaesthesia with sodium pentobarbital (60 mg/kg, i.p.) and cannulation of the trachea, the rats were pithed as reported earlier [2,5,21,26]. This procedure basically consists in inserting a stainless-steel rod through the orbit, the foramen magnum and into the vertebral foramen to avoid the influence of the central nervous system. Immediately afterwards, the animals were artificially ventilated with room air using a Harvard respiratory pump (1 ml/100 g, 50 strokes/min; Harvard Apparatus, South Natick, MA, USA). The right and left jugular veins were cannulated for the infusion of agonists and antagonists, respectively. Diastolic blood pressure and heart rate were monitored from the left carotid artery which was coupled to a PRS 206 amplifier (Cibertec, Madrid, Spain) and connected to a Power Laboratory System (AD Instruments, Oxford, UK) to display the recordings of blood pressure and heart rate in the software Labchart® Scope™. Both vagus nerves were isolated, ligated at the cervical level, and cut rostrally to the ligature to prevent afferent and efferent vagal reflexes. At this point, the 180 rats were initially divided into two main sets (fig. 1), so that the effects produced by the different histaminergic agents could be investigated on the bradycardic responses induced by (i) electrical stimulation of the vagus nerve (set 1; n = 160) or (ii) i.v. bolus injections of exogenous acetylcholine (set 2; n = 20).

The bradycardic stimulus–response curves (S-R curves) and dose–response curves (D-R curves) elicited by electrical stimulation and exogenous ACh, respectively (fig. 1), were completed in about 15 min., with no changes in diastolic blood pressure. Before electrical stimulation, rats were systematically pre-treated i.v. with heparin (1000 UI/kg; to avoid clotting) and atenolol (1 mg/kg; to prevent possible cardiac sympatho-excitatory effects). Rats were kept warm (37.5 ± 0.5°C) with a heating lamp. The electrical stimuli (3, 6 and 9 Hz; at 15 ± 3 V; 1 ms duration), as well as the i.v. bolus injections of exogenous ACh (1, 5 and 10 μg/kg), were given using a sequential schedule at 3- to 5-min. intervals, as previously reported [2,4].

Experimental protocols. After the hemodynamic status of the rats had been stable for ≥10 min., baseline values for diastolic blood pressure (a more accurate indicator of peripheral vascular resistance) and heart rate were determined. Subsequently, the next experimental protocols were followed, with five additional animals (not considered in fig. 1) being used to evaluate each dose of agonist or antagonist, and each animal preparation to evaluate only one agonist or antagonist.

Protocol I: Electrical stimulation of the vagus nerve. The first set of rats (n = 160) was used to study the influence of histaminergic agonists and antagonists on the bradycardic responses induced by electrical vagal stimulation, that is square-wave pulses from a Cibertec Stimulator CS-9 (supramaximal intensity: 15 ± 3V; 1 ms; 3, 6, and 9 Hz for 15 sec. at 5-min. intervals) with a platinum bipolar electrode connected to the caudal stump of the right cervical vagus nerve. Thus, the control S-R curve (E0) was completed in about 15 min. Then, this set of rats was divided into three subsets (n = 110, 40 and 10).

The first subset (n = 110, divided into seven groups) received i.v. bolus injections of (i) nothing (control, n = 5); (ii) saline (1 ml/kg, n = 5); (iii) histamine (10, 50, 100 and 200 μg/kg; n = 5 each); (iv) the H_1 selective agonist, 2-pyridylethylamine (1, 10, 50 and 100 μg/kg; n = 5 each); (v) the H_2 selective agonist, dimaprit (1, 10, 50 and 100 μg/kg; n = 5 each); (vi) the H_3 selective agonist, methimepip (1, 10, 50 and 100 μg/kg; n = 5 each); and (vii) the H_4 selective agonist, VUF 8430 (1, 10, 50 and 100 μg/kg; n = 5 each). After 5 min., a new S-R curve (E1) was obtained as previously described for the S-R curve E0 (fig. 1).

The second subset (n = 40) was conducted to confirm the histaminergic receptors involved in the modulation of bradycardic responses induced by vagal stimulation. This subset, divided into two groups (n = 20 each), received an i.v. bolus injection of vehicle (saline, 1 ml/kg) and JNJ 10181457 (H_3 receptor antagonist; 1 mg/kg), respectively. The corresponding curve ($E0_{saline}$, $E0_{JNJ\ 10181457}$) was completed after 10 min. Then, each group was subsequently subdivided into four treatment subgroups (n = 5 each) that received an i.v. bolus injection of, respectively, (i) nothing (control), (ii) saline (1 ml/kg), (iii) histamine (50 μg/kg) and (iv) methimepip (50 μg/kg). After 5 min., a new S-R curve (E1) was obtained.

The third subset (n = 10) was used to validate the cholinergic muscarinic nature of the bradycardic responses induced by electrical vagal stimulation. Accordingly, this subset (divided into two groups) received an i.v. bolus injection of atropine (0.3 and 0.5 mg/kg; n = 5 for each dose) before electrical stimulation.

Protocol II: Intravenous administration of exogenous acetylcholine. The second set of rats (n = 20) was prepared as described above, but the platinum bipolar electrode was not used in view that the vagus nerve was not stimulated electrically. Instead, D-R curves induced by i.v. bolus injections of exogenous ACh (1, 5 and 10 μg/kg) were constructed before (E'0) and 5 min. after (E'1) i.v. administration (n = 5 each) of (i) nothing (control group), saline (1 ml/kg), histamine (50 μg/kg) or methimepip (50 μg/kg).

Data presentation and statistical evaluation. All data in the text, tables and figures, unless otherwise stated, are presented as mean ± S.E.M. The changes in heart rate induced by electrical vagal stimulation or exogenous ACh were expressed as decreases in beats/min. from the corresponding baseline value. Comparison of the results from the experimental groups and their corresponding control group was evaluated with one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls *post hoc* test. Statistical significance was accepted at $p < 0.05$. It is noteworthy that the decreases in heart rate by i.v. saline were similar to those produced in the control group (receiving nothing); therefore, for simplicity, statistical evaluation was only performed *versus* saline.

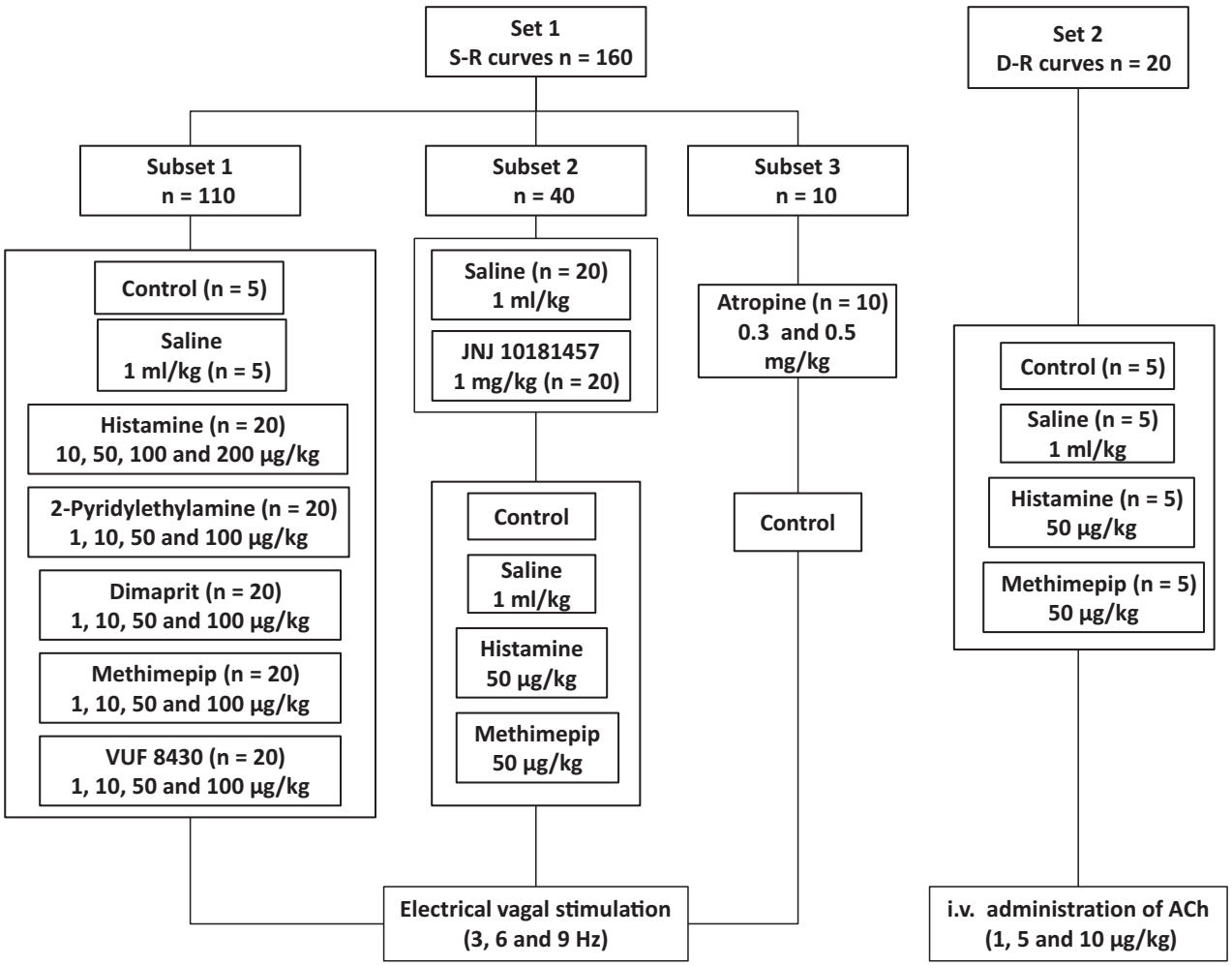

Fig. 1. Experimental protocols showing the number of animals used in the two main sets of rats as well as in the different groups used in the present study. The bradycardic responses were obtained by electrical vagal stimulation (set 1; n = 160) or i.v. bolus injections of exogenous acetylcholine (ACh) (set 2; n = 20). All drugs were administered as i.v. bolus injections. S-R curves: stimulus–response curves; D-R curves: dose–response curves to exogenous ACh; n = 5 for each dose.

Drugs. The compounds used in this study (obtained from the sources indicated) were heparin sodium (Roche, Madrid, Spain); pentobarbital sodium, atropine and acetylcholine chloride (Sigma-Aldrich, St. Louis, MO, USA); 2-pyridylethilamine dihydrochloride, S-(3-dimethylaminopropyl) isothiourea dihydrochloride (dimaprit), 4-(1H-imidazol-4-ylmethyl)-1-methylpiperidine dihydrobromide (methimepip), 2-[(aminoiminomethyl) amino]ethyl carbamimidothioic acid ester dihydrobromide (VUF 8430) and 4-[3-[4-[piperidinyl]but-1-ynyl] benzyl]morpholine dihydrochloride (JNJ 10181457) and atenolol (Tocris Bioscience (Bristol, U.K.); and histamine dihydrochloride (Merck, Darmstadt, Germany). All drugs were dissolved in physiological saline at the time of experimentation. Saline had no effect on basal diastolic blood pressure or heart rate. The doses of all drugs refer to their free base.

Results

Systemic haemodynamic variables.

The baseline values of diastolic blood pressure and heart rate in the 180 pithed rats were 50 ± 1 mm Hg and 320 ± 4 beats/min, respectively. I.v. administration of physiological saline, 2-pyridylethylamine (H_1 receptor agonist), methimepip (H_3 receptor agonist) and VUF 8430 (H_4 receptor agonist) produced no significant effects on diastolic blood pressure (table 1) or heart rate (not shown). However, i.v. administration of histamine (endogenous ligand) or dimaprit (H_2 receptor agonist) induced a transient (<1 min.) and dose-dependent decrease in diastolic blood pressure (table 1), without altering heart rate (not shown). Moreover, JNJ 10181457 (H_3 receptor antagonist) produced no significant effects on diastolic blood pressure or heart rate (not shown).

Initial effects of electrical vagal stimulation or exogenous acetylcholine on heart rate.

Figure 2 shows an original experimental tracing illustrating that the bradycardic responses to vagal stimulation (3, 6 and 9 Hz) were (i) immediate in onset (appeared about 5 sec. after each stimulus and returned to baseline values immediately after electrical stimulation) and (ii) frequency-dependent. In all

Table 1.

Changes in diastolic blood pressure (ΔDBP) immediately after i.v. bolus injections of saline (1 ml/kg; control group; n = 5), histamine (10, 50, 100 and 200 μg/kg; n = 5 for each dose), 2-pyridylethylamine (H_1 receptor agonist), dimaprit (H_2 receptor agonist), methimepip (H_3 receptor agonist) or VUF 8430 (H_4 receptor agonist) (1, 10, 50 and 100 μg/kg for each agonist; n = 5 for each dose of each agonist).

	ΔDBP (mm Hg)					
Dose *i.v.* (μg/kg)	Saline	Histamine	2-Pyridylethylamine	Dimaprit	Methimepip	VUF 8430
1	1 ± 1	–	−1 ± 0	−3 ± 2	1 ± 1	1 ± 0
10	–	−3 ± 1	1 ± 0	−5 ± 2*	1 ± 1	1 ± 0
50	–	−5 ± 2*	1 ± 1	−9 ± 2*	1 ± 0	1 ± 0
100	–	−9 ± 2*	−1 ± 1	−12 ± 2*	−1 ± 1	1 ± 0
200	–	−10 ± 3*	–	–	–	–

All values (changes in basal diastolic blood pressure) are expressed as mean ± S.E.M.
*$p < 0.05$ *versus* saline.

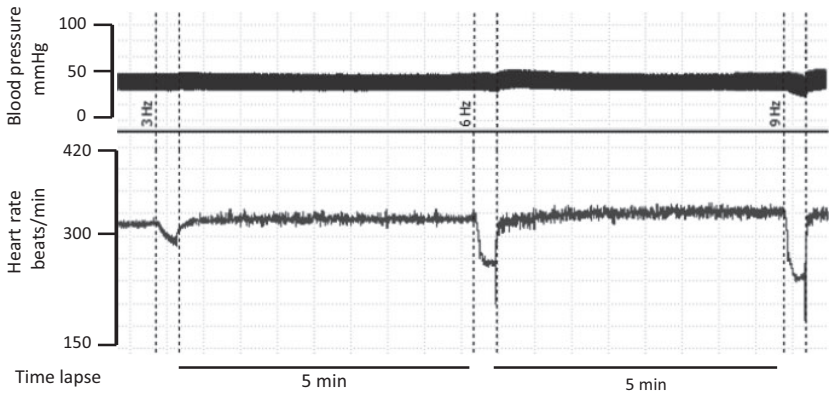

Fig. 2. Original experimental tracings of heart rate (beats/min) and blood pressure (mm Hg) before and after electrical vagal stimulation (3, 6 and 9 Hz). Note that the bradycardic responses resulting from electrical vagal stimulation (i) were not accompanied by changes in systemic blood pressure and (ii) returned to baseline levels immediately after electrical stimulation.

cases, these bradycardic responses were due to cardiac vagal selective stimulation, as only negligible effects in diastolic blood pressure were observed (fig. 2), as previously shown [2,4,5]. Similarly, i.v. administration of increasing doses of ACh (1, 5 and 10 μg/kg) resulted in dose-dependent bradycardic responses (not shown).

At the frequencies and doses of ACh used, the decreases in heart rate were, respectively, −33 ± 3, −59 ± 4 and −81 ± 6 beats/min. (control S-R curve) and −35 ± 4, −53 ± 6 and −99 ± 9 beats/min (control D-R curve). These responses were reproducible as they remained essentially unchanged after i.v. administration of physiological saline (1 ml/kg; see figs 3 and 6, respectively). As expected, the vagally induced bradycardic responses were partially and completely blocked after 0.3 and 0.5 mg/kg of atropine (data not shown), confirming the cholinergic muscarinic nature of these responses.

Effect of histamine and histaminergic agonists on the bradycardic responses induced by either electrical vagal stimulation or i.v. bolus injections of exogenous ACh.

As shown in fig. 3, i.v. administration of 50, 100 and 200 μg/kg histamine evoked a dose-dependent inhibition of the vagally induced bradycardic responses at all frequencies tested, whereas its lowest dose (10 μg/kg) produced no significant effect. Interestingly, this inhibition by histamine (i) was clearly mimicked by i.v. administration of 10, 50 and 100 μg/kg methimepip (an H_3 receptor agonist), while 1 μg/kg of this agonist had no significant effect (fig. 4); and (ii) was not mimicked by i.v. administration of the same doses of the histamine receptor agonists 2-pyridylethylamine (H_1; fig. 5A), dimaprit (H_2; fig. 5B) and VUF 8430 (H_4; fig. 5C). On the basis of these results, we decided to further investigate whether histamine and methimepip would also inhibit the bradycardic responses induced by i.v. bolus injections of exogenous ACh. Hence, as shown in fig. 6, the bradycardic responses induced by exogenous ACh (1, 5 and 10 μg/kg, i.v.), which remained unaltered after saline (1 ml/kg, i.v.), were not significantly inhibited after i.v. administration of 50 μg/kg of either histamine or methimepip. Accordingly, as this dose of histamine or methimepip selectively inhibited the vagally induced bradycardic responses (figs 3 and 4) without significantly affecting those by exogenous ACh (fig. 6), 50 μg/kg of histamine or methimepip was chosen for further pharmacological analysis with the H_3 receptor antagonist JNJ 10181457.

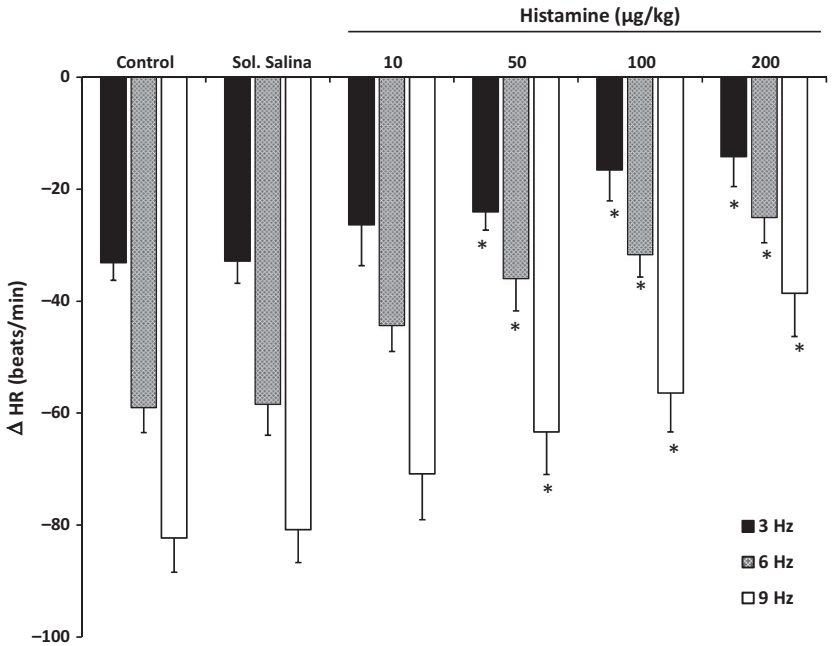

Fig. 3. Effect of i.v. bolus injections of nothing (control group), saline (1 ml/kg) or 10, 50, 100 and 200 μg/kg histamine (n = 5 for each treatment) on the decreases in heart rate (ΔHR) evoked by electrical vagal stimulation (3, 6 and 9 Hz). *$p < 0.05$ *versus* saline.

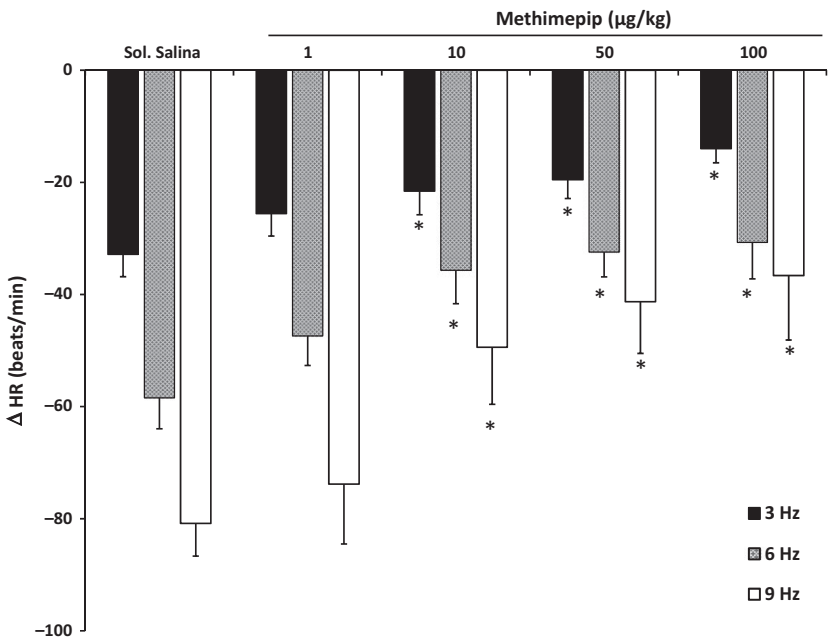

Fig. 4. Effect of i.v. bolus injections of saline (1 ml/kg) or 1, 10, 50 and 100 μg/kg of the H_3 receptor agonist methimepip (n = 5 for each treatment) on the decreases in heart rate (ΔHR) evoked by electrical vagal stimulation (3, 6 and 9 Hz). *$p < 0.05$ *versus* saline. Note that the S-R curve in the animals treated with saline corresponds to the same S-R curve shown in fig. 3.

Effect of the H_3 receptor antagonist JNJ 10181457 on histamine- and methimepip-induced inhibition of the vagally induced bradycardic responses.

Figure 7 shows that, in contrast to the animals pre-treated with saline (1 ml/kg, i.v.; left panel), i.v. pre-treatment with the selective H_3 receptor antagonist, JNJ 10181457 (1 mg/kg; right panel), (i) completely blocked the inhibition of the vagally induced bradycardic responses produced by histamine (50 μg/kg) or methimepip (50 μg/kg), and (ii) had no effect *per se* on the vagally induced bradycardic responses induced in the animals receiving saline.

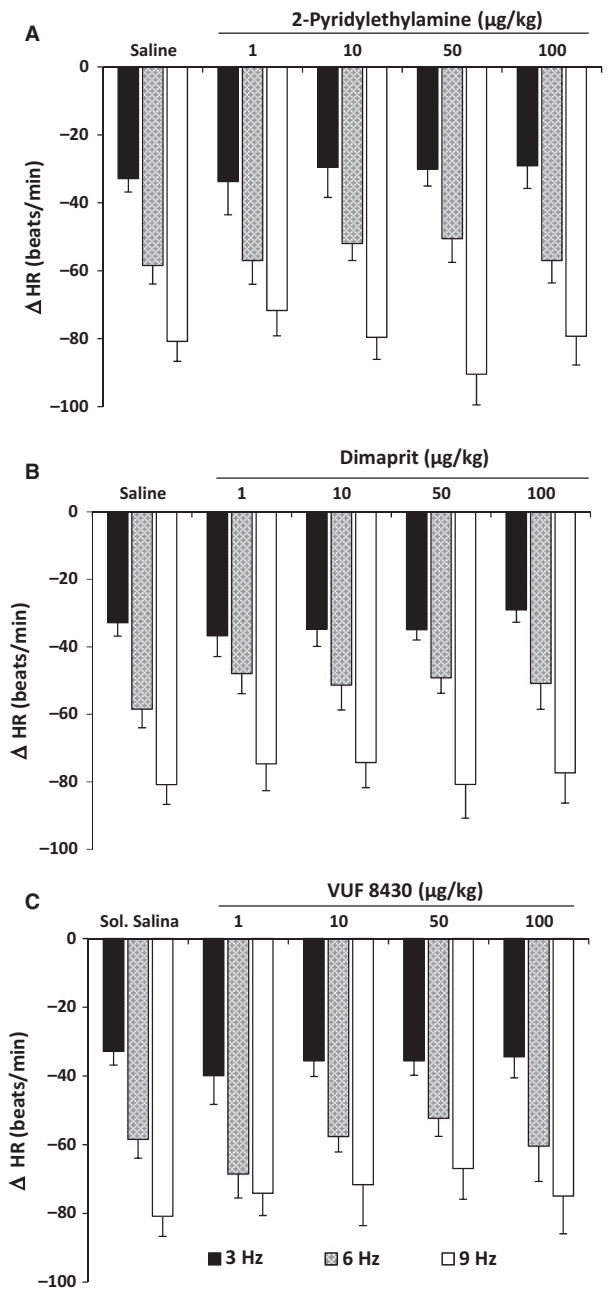

Fig. 5. Effect of i.v. bolus injections of saline (1 ml/kg) or 1, 10, 50 and 100 µg/kg of the histamine receptor agonists 2-pyridylethylamine (H_1, panel A); dimaprit (H_2, panel B) and VUF 8430 (H_4, panel C) (n = 5 for each dose of agonist) on the decreases in heart rate (ΔHR) evoked by electrical vagal stimulation (3, 6 and 9 Hz). The bradycardic responses in the saline group did not significantly differ from those elicited in the animals receiving 2-pyridylethylamine, dimaprit and VUF 8430 ($p > 0.05$). Note that the S-R curve in the animals treated with saline corresponds to the same S-R curve shown in figs 3 and 4.

Discussion

General.

Apart from the implications discussed below, our study shows that inhibition of the vagal bradycardic out-flow by histamine and methimepip (an H_3 receptor agonist [27]) is most probably mediated by H_3 receptors as these responses (i) were not mimicked by 2-pyridylethylamine, dimaprit and VUF 8430 (selective agonists at H_1, H_2 and H_4 receptors, respectively) and (ii) were blocked by JNJ 10181457, a potent H_3 receptor antagonist [25]. Admittedly, our study did not measure vagal nerve activity directly, but the electrically induced ACh release could be estimated indirectly by the measurement of the evoked bradycardic response. Under these conditions, the responses to histamine and methimepip were considered to be vago-inhibitory as both compounds were capable of inhibiting the bradycardic responses to electrical vagal stimulation, but not those to exogenous ACh. These findings highlight the role of histamine H_3 receptors in the modulation of vagal ACh release at cardiac level, as previously shown for serotonin 5-HT_1 receptors [2,4,5]; hence, these receptors may be a therapeutic target in cardiac problems [3,28–30].

Regarding our experimental protocols, it is noteworthy that each animal was utilized to evaluate only one dose of agonist or antagonist, so that only two (S-R or D-R) curves were carried out in each animal (i.e. control curve and treatment curve). This approach allowed us to avoid phenomena such as cumulative responses by various doses of a drug, and even tachyphylaxis as it occurs when stimulating the vasodepressor sensory out-flow [31,32].

Histamine plays a key role in the control of a variety of central and peripheral functions as well as a pathophysiological role in various allergic disorders by interacting with histamine H1, H2, H3 and H4 receptors [6]. The central histaminergic system influences on cardiac regulation, owing to its neurons innervate many central sites with importance in the cardiovascular homeostasis; this modulation is mainly due to its influence on the autonomic nervous system [33]. It has been demonstrated that histamine H3 receptor activation causes an acute hypotension, tachycardia and marked attenuation of the gain of the baroreflex in conscious rabbits. These effects are most likely due to an inhibition of sympathetic transmission in the periphery and/or interference with the central regulation of sympathetic tone. Moreover, central (not peripheral) endogenous histamine appears to have a role in regulating blood pressure at rest [34]. Obviously, central histaminergic mechanisms involved in cardiovascular regulation [35] are not operative in our experimental model as pithed rats were used.

All drugs utilized were selected on the basis of their corresponding pKi values for each specific histamine receptor, specially methimepip and JNJ 10181457, which were chosen on the basis of their corresponding pKi values for H_3 binding sites, which are higher than (R)-α-methylhistamine and thioperamide, respectively [33,36].

Haemodynamic effects produced by the different treatments.

The frequency-dependent decreases in heart rate produced by electrical vagal stimulation was a selective procedure as diastolic blood pressure was unchanged (fig. 2), as previously reported by our group [2,4,5]. Moreover, i.v. administration of

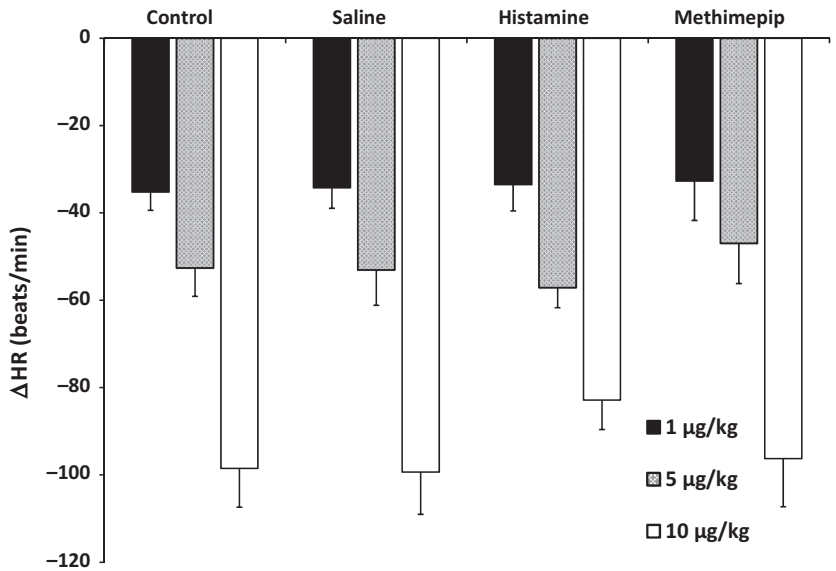

Fig. 6. Effect of i.v. bolus injections of nothing (control group), saline (1 ml/kg), histamine (50 µg/kg) or methimepip (50 µg/kg) (n = 5 for each treatment) on the bradycardic responses (ΔHR) elicited by increasing i.v. doses of exogenous acetylcholine (1, 5 and 10 µg/kg). Note that the bradycardic responses in the control group did not significantly differ from those elicited in the animals receiving saline, histamine or methimepip ($p > 0.05$).

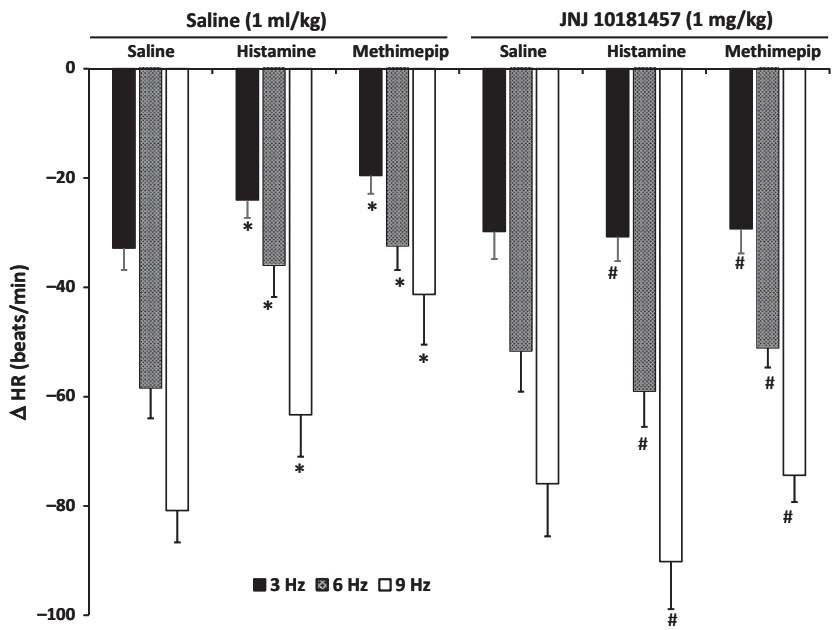

Fig. 7. Effect of i.v. bolus injections of saline (1 ml/kg; n = 5), histamine (50 µg/kg; n = 5) or methimepip (50 µg/kg; n = 5) in the absence (saline, 1 ml/kg) or the presence of i.v. pre-treatment with the H_3 receptor antagonist JNJ 10181457 (1 mg/kg) on the vagally induced bradycardic responses (ΔHR). $^{*}p < 0.05$ *versus* saline. $^{\#}p < 0.05$ *versus* the corresponding responses after histamine or methimepip (not significantly different *versus* saline) in the absence of JNJ 10181457. Note that the S-R curve in control animals (receiving no i.v. saline) did not significantly differ from the S-R curve in saline-treated animals, but it is not shown for the sake of clarity.

physiological saline, 2-pyridylethylamine [37], methimepip [27] and VUF 8430 [38] (histamine H_1, H_3 and H_4 receptor agonists, respectively) (table 1) as well as JNJ 10181457 (a histamine H_3 receptor antagonist [25]) did not significantly affect diastolic blood pressure or heart rate (not shown). Although H_1 receptor activation usually induces a vasodepressor response [39], we may speculate that the doses of 2-pyridylethylamine used in our study (1 to 100 µg/kg, i.v.) are not high enough to induce any vascular effect (table 1). Moreover, regarding H_3 receptor activation, there are controversial data about its effect on blood pressure depending on the agonist used and the vascular bed under study. For example,

R-(α)-methylhistamine induces endothelium-dependent vasodilatation in rat mesenteric arteries [40], but immepip (H_3/H_4 receptor agonist) does not significantly change basal diastolic blood pressure and heart rate in pithed rats [21]. In our experiments, only i.v. administration of histamine or the agonist dimaprit (H_2) induced a transient (<1 min.) decrease in diastolic blood pressure, without altering the heart rate (table 1). Our results are partially consistent with other studies in pithed rats [41] showing that activation of H_1 and H_2 receptors, but not of H_3 receptors, resulted in a decrease in diastolic blood pressure, with longer duration for dimaprit than for 2-(2-thiazolyl) ethylamine (H_1 receptor agonist). Also, H_2 receptor activation mediates a dose-dependent vasodilation in the human forearm and uterine artery [42,43].

The role of H_3 receptors in the inhibition of vagally induced bradycardic responses.

As 50 μg/kg of histamine or methimepip inhibited the bradycardic responses to electrical vagal stimulation (figs 3 and 4) without affecting those to exogenous ACh (fig. 6), a selective vago-inhibitory action is implied, as reported for other compounds [2,4,5]. Moreover, the pharmacological profile of the receptors involved closely resembles that of histamine H_3 receptors as (i) methimepip is a selective agonist at these receptors, which shows a 2000-time preference for the human H_3 receptor over the human H_4 receptor and a more than 10,000-time preference over the human H_1 and H_2 receptors [27]; (ii) agonists at H_1, H_2 and H_4 receptors failed to mimic the above vago-inhibitory action by histamine and methimepip (fig. 5); and (iii) the vago-inhibitory action by histamine and methimepip was abolished by 1 mg/kg of JNJ 10181457 (fig. 7), a potent and selective H_3 receptor antagonist ($pK_i = 8.15$) [36] . In agreement with this view, histamine H_3 receptors are coupled to $G_{i/o}$ proteins [13,14], a signal transduction system usually associated with the inhibition of neurotransmitter release [44].

Our suggestion that peripheral H_3 receptors mediate the vago-inhibitory action by histamine and methimepip is consistent with other findings implying that (i) central H_3 receptors modulate acetylcholine release in rat entorhinal cortex [22, 23] and (ii) peripheral H_3 receptors inhibit the muscarinic receptor-activated calcium signalling in rat carotid body Type I cells [24]. Similarly, other studies have shown that peripheral H_3 receptors inhibit (as heteroreceptors) (i) the vasodepressor sensory CGRPergic out-flow [21] and the vasopressor sympathetic out-flow [45] in rats, and (ii) the noradrenergic nerve-mediated vasoconstriction and the CGRPergic nerve-mediated vasodilatation in rat mesenteric arteries [46].

Possible locus of the cardiac vago-inhibitory H_3 receptors.

Obviously, central mechanisms are not operative in pithed rats, but an action of histamine and methimepip at H_3 receptors located on vagal intramural (cardiac) ganglia and postganglionic parasympathetic neurons cannot be excluded. Thus, our study provides no evidence for the specific location of the H_3 receptors inhibiting the vagal bradycardic out-flow, but other studies imply that H_3 receptors located on (i) perivascular sympathetic and sensory nerves in rat mesenteric arteries modulate the noradrenergic and CGRPergic neurotransmission [46], and (ii) sensory nerves innervating resistance blood vessels inhibit the vasodepressor CGRPergic out-flow in pithed rats [21]. Admittedly, further studies that fall beyond the scope of the present investigation will be required to ascertain the functional role of histamine H_3 receptors on parasympathetic ganglia and post-ganglionic parasympathetic neurons in the heart.

In conclusion, our findings in pithed rats suggest that histamine-induced inhibition of the vagal bradycardic out-flow is mainly mediated by pre-junctional histamine H_3 receptors, with pharmacological evidence excluding the involvement of histamine H_1, H_2 and H_4 receptors.

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Statement of conflicts of interest

The authors state no conflict of interest.

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