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Central endogenous histamine modulates sympathetic outflow through H_3 receptors in the conscious rabbit

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1 This study examined the role of histamine H_3 receptors in vagal and sympathetic autonomic reflexes in the conscious rabbit, and in rabbit and guinea-pig isolated right atria.

2 The baroreceptor-heart rate reflex (baroreflex), Bezold-Jarisch-like and nasopharyngeal reflexes were assessed after these treatments (i.v.; with H_1 and H_2 receptor block): (i) vehicle (saline; $n=11$); (ii) H_3 receptor agonist, (*R*)-α-methylhistamine (*R*-α-MH) $100\,\mu g\,kg^{-1}+100\,\mu g\,kg^{-1}\,h^{-1}$ ($n=9$); (iii) H_3 receptor antagonist, thioperamide $1\,mg\,kg^{-1}+1\,mg\,kg^{-1}\,h^{-1}$ ($n=11$); (iv) *R*-α-MH and thioperamide ($n=6$); and (v) H_2 and H_3 antagonist, burimamide $6.3\,mg\,kg^{-1}+6.3\,mg\,kg^{-1}\,h^{-1}$ ($n=4$).

3 *R*-α-MH caused a thioperamide-sensitive fall in mean arterial pressure (MAP) of 8 ± 1 mmHg and tachycardia of 18 ± 2 bpm ($P<0.0005$). Burimamide was without effect, however thioperamide elicited an increase in MAP of 4 ± 1 mmHg ($P<0.01$), but no change in heart rate (HR).

4 *R*-α-MH caused a 44% decrease in the average gain of the baroreflex ($P=0.0001$); this effect was antagonised by thioperamide. Thioperamide caused a parallel rightward shift in the barocurve with an increase in MAP of 5 mmHg ($P<0.05$). Burimamide had no effect on the baroreflex. The vagally mediated bradycardia elicited by the Bezold-Jarisch and nasopharyngeal reflexes was unaffected by H_3 receptor ligand administration.

5 *R*-α-MH ($\leqslant10\,\mu M$) caused a thioperamide-sensitive depression of both sympathetic and vagal responses in guinea-pig atria, but had no effect in rabbit atria.

6 As H_3 receptor activation caused a significant decrease in baroreflex gain without affecting HR range, the former is unlikely to be simply due to peripheral sympatholysis (supported by the lack of effect in isolated atria). Central H_3 receptors may have a tonic role in the baroreflex as thioperamide caused a rightward resetting of the barocurve. In contrast, the peripherally acting H_3 antagonist burimamide was without effect. These findings suggest a role for central histamine H_3 receptors in cardiovascular homeostasis in the rabbit.

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Abbreviations: baroreflex, Baroreceptor-heart rate reflex; HR, heart rate; MAP, mean arterial pressure; *R*-α-MH, (*R*)-α-methylhistamine

Introduction

Interest in the physiological role of histamine has been rekindled by the discovery of the H_3 autoreceptor, which mediates inhibition of histamine release and synthesis in the CNS (Arrang *et al*., 1983). Presynaptic H_3 inhibitory receptors have been shown to exist on histaminergic neurones in the CNS (autoreceptors) and nonhistaminergic neurones of the CNS and peripheral nervous system (heteroreceptors) (for a review, see Hill *et al*., 1997). The first evidence for the existence of H_3 receptors in the cardiovascular system was found in the guinea-pig mesenteric artery where histamine attenuated the amplitude of electrically evoked junction potentials (Ishikawa & Sperelakis, 1987). Inhibition of sympathetic neurotransmission *in vitro* has also been shown in human saphenous vein (Molderings *et al*., 1992), and guinea-pig atria where inotropic and chronotropic responses to transmural stimulation were depressed (Luo *et al*., 1991; Endou *et al*., 1994). H_3 receptor activation attenuates acetylcholine release from vagus nerves in the rat isolated stomach (Yokotani *et al*., 2000), and modulates proliferation and migration of cells in rat fundic mucosa (Morini *et al*., 2002).

In conscious guinea-pigs, McLeod *et al*. (1991) found that i.v. administration of the selective H_3 receptor agonist *R*-α-methylhistamine (*R*-α-MH) elicited a rapid fall in mean arterial pressure (MAP) without affecting heart rate (HR). A role for central H_3 receptors in the control of cardiovascular function was also demonstrated in this study, where i.c.v. injections of *R*-α-MH decreased HR, suggested to be *via* a vagal enhancement, with a subsequent decrease in MAP. In other studies, H_3 receptor activation attenuated electrically stimulated increases in MAP and HR in pithed rats (Malinowska & Schlicker, 1991; Godlewski *et al*., 1997a) and anaesthetised guinea-pigs (Hey *et al*., 1992a), without influencing basal MAP. In anaesthetised dogs, *R*-α-MH decreased noradrenaline release following electrical stimulation of cardiac sympathetic nerves (Mazenot *et al*., 1999).

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Whether H_3 receptors are quiescent under physiological conditions or tonically activated by an endogenous agonist is under debate. The autonomic nervous system is endowed with H_3 receptors that may be important in cardiovascular control. In addition, the H_3 receptor has been autoradiographically localised within areas of the brainstem (Schwartz *et al*., 1990), the major cardiovascular control centre. The competitive H_3 receptor antagonist thioperamide (Arrang *et al*., 1987) enhanced electrically induced increases in diastolic blood pressure in the pithed rat (Godlewski *et al*., 1997a). Inhibition of histamine biosynthesis led to arterial hypertension in rats (Campos *et al*., 1996), while Acuña *et al*. (1998) found thioperamide facilitated vasopressor responses to footshock in conscious rats. Conversely, other studies have failed to find any evidence for a putative involvement of H_3 receptors on resting cardiovascular function (Hedge *et al*., 1994; Coruzzi *et al*., 1995).

The aim of this study was to explore the importance of histamine H_3 receptors in cardiovascular autonomic reflexes in the conscious rabbit. To do this the cardiovascular effects of selective H_3 receptor agonists and antagonists, as well as effects on the baroreceptor-heart rate reflex (baroreflex), and vagal components of the Bezold-Jarisch-like and nasopharyngeal reflexes, were assessed under conditions of H_1 and H_2 receptor antagonism. Further, the role of H_3 receptors in sympathetic and vagal responses in rabbit and guinea-pig isolated right atria were investigated.

Methods

This study was approved by the University of Melbourne Animal Ethics Experimentation Committee in accordance with the guidelines of the National Health and Medical Research Council of Australia.

Autonomic reflexes in the conscious rabbit

Experimental procedure Male and female New Zealand white rabbits (mean 2.5 ± 0.1 kg, 95% confidence interval 2.2–2.8 kg; $n = 11$) were used. Rabbits were kept on a 12 h light/dark cycle with free access to food and water. On the morning of each experiment under local anaesthesia (0.5% lignocaine hydrochloride, Xylocaine, Astra, North Ryde, NSW, Australia), rabbits underwent minor operative procedures to insert catheters into the central ear artery and marginal ear veins. The arterial catheter was connected to a pressure transducer (CDX, Cobe, Lakewood, Co, U.S.A.) for measurement of phasic and MAP. The phasic pressure signal triggered a rate meter (model 173, Baker Medical Research Institute, Melbourne, Australia) for the recording of HR. Cardiovascular variables were continuously recorded on a Grass 7D polygraph (Grass Instruments, Quincy, MA, U.S.A.). Rabbits sat quietly in a polycarbonate box (Nalgene, Nalge, Rochester, NY, U.S.A.) for a minimum recovery period of 20 min before commencement of the experimental protocol.

Experimental design

Treatment protocols were completed in random order on separate experimental days. Drugs were administered as an i.v. bolus followed by a maintenance i.v. infusion at $10\,ml\,h^{-1}$. Treatments were:

(i) *Vehicle*: 0.9% saline $1\,ml + 10\,ml\,h^{-1}$;
(ii) *H_1 and H_2 receptor block*: cimetidine $15\,mg\,kg^{-1} + 15\,mg\,kg^{-1}\,h^{-1}$ and mepyramine $0.8\,mg\,kg^{-1} + 0.8\,mg\,kg^{-1}\,h^{-1}$.
(iii) *H_1 and H_2 block + H_3 receptor agonist*: *R*-α-MH $100\,\mu g\,kg^{-1} + 100\,\mu g\,kg^{-1}\,h^{-1}$.
(iv) *H_1 and H_2 block + H_3 receptor antagonist*: thioperamide $1\,mg\,kg^{-1} + 1\,mg\,kg^{-1}\,h^{-1}$.
(v) *H_1 and H_2 block + H_3 receptor agonist and antagonist*: *R*-α-MH $100\,\mu g\,kg^{-1} + 100\,\mu g\,kg^{-1}\,h^{-1}$ followed 10 min later by thioperamide $1\,mg\,kg^{-1} + 1\,mg\,kg^{-1}\,h^{-1}$.
(vi) *H_1 and H_2 block + alternate H_3 receptor agonist*: imetit, a more potent H_3 receptor agonist used for comparison with *R*-α-MH, $30\,\mu g\,kg^{-1} + 10\,\mu g\,kg^{-1}\,h^{-1}$.
(vii) *H_1 and H_2 block + burimamide*: burimamide, a nonselective H_2 and H_3 receptor antagonist (which does not enter the CNS), $6.3\,mg\,kg^{-1} + 6.3\,mg\,kg^{-1}\,h^{-1}$. This dose of burimamide is 2–3-fold higher than that required to block depressor responses to histamine $30–40\,\mu g\,kg^{-1}$ i.v. in the rabbit (Parsons & Owen, 1973; Angus *et al*., 1977; 1978). A similar dose of burimamide ($6\,mg\,kg^{-1}$ i.v.) was shown to inhibit the effects of *R*-α-MH ($300\,\mu g\,kg^{-1}$ i.v.) in anaesthetised guinea-pigs (Hey *et al*., 1992a).

Each treatment (iii–vii) was given 10 min after administration of the H_1 and H_2 receptor antagonists (regimen as in ii).

On each experimental day, the baroreflex was assessed followed by the Bezold-Jarisch-like and nasopharyngeal reflexes. A stabilisation period of 20–30 min following the start of the maintenance infusion of each drug treatment was allowed before reflex assessment. A baroreflex curve was constructed by inducing alternate increases and decreases in MAP ranging between 5 and 30 mmHg around the resting MAP, and measuring the corresponding reflex changes in HR (Head & McCarty, 1987; Wright *et al*., 2000). Alternate pressor and depressor effects were induced to prevent any change/drift from the resting MAP. To decrease MAP, a bolus dose of sodium nitroprusside (0.025–1.5 ml; $1\,mg\,ml^{-1}$ i.v.) was administered, while phenylephrine (5–150 μl; $250\,\mu g\,ml^{-1}$ i.v.) was used to increase MAP. The Bezold-Jarisch-like reflex was elicited with bolus doses of 5-HT (3, 10 and $30\,\mu g\,kg^{-1}$ i.v.) given over 4–6 s (Wright & Angus, 1989); reflex changes in HR were measured. The nasopharyngeal reflex typified by profound bradycardia was induced by cigarette smoke (12 mg tar; $\sim 20\,cm^3$) gently blown from a syringe across the rabbit's nostrils (Whorlow *et al*., 1998; Wright *et al*., 2000; Devlin *et al*., 2002). The peak decrease in HR was recorded.

Validation of H_1, H_2 and H_3 receptor blockade

To ensure there was adequate block for the duration of the experiment with the various antagonists used, histamine or *R*-α-MH dose–MAP response curves were completed before, 30 and 120 min after the respective antagonist under the following conditions:

(i) *Test of H_1 receptor block*: in the presence of H_2 block with cimetidine ($15\,mg\,kg^{-1} + 15\,mg\,kg^{-1}\,h^{-1}$), histamine (10–

1280 $\mu g\,kg^{-1}$ i.v.) dose–response curves were assessed before and after the H_1 antagonist mepyramine (0.8 $mg\,kg^{-1}$ + 0.8 $mg\,kg^{-1}\,h^{-1}$).

(ii) *Test of H_2 receptor block*: in the presence of H_1 block with mepyramine (0.8 $mg\,kg^{-1}$ + 0.8 $mg\,kg^{-1}\,h^{-1}$), histamine (10–1280 $\mu g\,kg^{-1}$ i.v.) dose–response curves were assessed before and after the H_2 antagonist cimetidine (15 $mg\,kg^{-1}$ + 15 $mg\,kg^{-1}\,h^{-1}$).

(iii) *Test of H_3 receptor block*: in the presence of H_1 and H_2 block with mepyramine and cimetidine (as above), *R*-α-MH (10–1000 $\mu g\,kg^{-1}$ i.v.) dose–response curves were assessed before and after the H_3 antagonist thioperamide (1 $mg\,kg^{-1}$ + 1 $mg\,kg^{-1}\,h^{-1}$).

Sympathetic responses in rabbit and guinea-pig isolated right atrium

Young New Zealand white rabbits (1.1–1.3 kg) were given an i.v. dose (~70 $mg\,kg^{-1}$) of pentobarbitone sodium (Virbac, NSW, Australia) and killed by exsanguination. Male Hartley guinea-pigs (500–750 g) were anaesthetised by exposure to 80% CO_2 in O_2 and killed by exsanguination. Right atria were isolated and placed vertically on stainless-steel S-shaped hooks on an acrylic leg in a 20 ml glass-jacketed organ bath heated to 37°C. The organ bath was filled with physiological salt solution of composition (in mM): NaCl 119; KCl 4.7; KH_2PO_4 1.18; $MgSO_4$ 1.17; $NaHCO_3$ 25; $CaCl_2$ 2.5; EDTA 0.026; glucose 11; saturated with 5% CO_2 in O_2. The partially stretched atrium rested against two punctate electrodes 3 mm apart protruding from the acrylic leg that recorded the spontaneous surface electrogram (monitored on a dual-beam 10 MHz storage oscilloscope, Model T912, Tektronix, Guernsey, U.K.). This signal was amplified (Baker Medical Research Institute amplifier Model 108) and used to trigger an internal period meter in a Maclab data acquisition system (AD Instruments, Castle Hill, NSW, Australia). Atrial period was continuously recorded on the Maclab. Electrical field stimulation was delivered to the tissue *via* a Grass S88C dual stimulator and a pair of platinum wire field electrodes that were arranged parallel to the atrium. This equipment delivered 1, 2, 4 or 8 field pulses to the tissue (2 ms duration, 50 V (dial setting), 2 Hz) for the sympathetic preparation and 1–4 pulses (2 ms duration, 50 V, 100 Hz, delivered in the refractory period to avoid rhythm disturbances) for the vagal preparation (Angus & Harvey, 1981; Wright & Angus, 1995; 1996; Wright *et al*., 2000).

To examine H_3 receptor-mediated effects on sympathetic responses, atria were equilibrated for 45 min in the presence of 1 μM atropine, 0.1 μM mepyramine and 10 μM cimetidine. To assess vagal responses, atria were equilibrated with 10 μM propranolol, 0.1 μM mepyramine and 10 μM cimetidine. One set of control field pulse stimuli (1–8 pulses for sympathetic and 1–4 pulses for vagal) was applied before adding *R*-α-MH (1 μM guinea-pig atria and 10 μM rabbit atria). There was a 20 min equilibration period after addition of *R*-α-MH before electrical field stimulation. Any effect on atrial responses was then assessed for H_3 receptor involvement by addition of thioperamide (1 μM). Appropriate time control (equilibration with vehicle, H_2O) experiments were also completed in separate rabbit and guinea-pig tissues.

Analysis and statistical methods

Data are presented as mean ± 1 standard error of the mean (s.e.m.). Average MAP and HR values presented in the text and tables have been rounded to the closest whole number. *In vitro*, sympathetic and vagal responses to electrical field stimulation of right atria are expressed as absolute values and as changes in atrial rate. Analysis of the baroreflex involved fitting the MAP and HR changes to a sigmoidal logistic equation characterised by four parameters (P_1 to P_4) where P_1 = lower HR plateau (bpm), P_2 = HR range between upper and lower curve plateaus (bpm), P_3 = a curvature coefficient which is independent of range and P_4 = median blood pressure at half the HR range (MAP_{50}; mmHg) (Head & McCarty, 1987; Wright *et al*., 2000). The average gain is the slope of the linear portion of the curve between the upper and lower plateaus (bpm $mmHg^{-1}$). The upper plateau was calculated by addition of P_1 and P_2.

The average s.e.m. within animals (or atria) was calculated from repeated measures analysis of variance (ANOVA) using the pooled estimate of error from the residual mean square as (error mean square × number of animals (or atria)$^{-1}$)$^{0.5}$ after subtracting the sums of squares 'between animals (or atria)' and 'between times or doses (or pulses)' from the 'total' sum of squares for each treatment group (Wright *et al*., 1987). Average s.e.m.'s are located on the mean lines for each variable in Figures 1, 2, 4 and 5.

The acute effects of H_1 and H_2 receptor blockade on resting MAP and HR were analysed by paired Student's *t*-test. Parameters within and between groups (or isolated tissues) were tested by repeated measures ANOVA, with Greenhouse–Geisser correction for correlation (Ludbrook, 1994), calculated by means of the statistical program SuperANOVA 1.11 for Macintosh. Bradycardic effects elicited by the nasopharyngeal reflex, as well as baroreflex curve parameters, were compared between groups by one-way ANOVA with Dunnett's *post hoc* test where appropriate. Probability values less than 0.05 were accepted as statistically significant.

Drugs

Drugs used and their sources were: atropine sulphate (Sigma, St Louis, MO, U.S.A.), cimetidine (100 $mg\,ml^{-1}$; Tagamet™, SmithKline Beecham, Melbourne, VIC, Australia), histamine acid phosphate (British Drug Houses, London, U.K.), imetit dihydrobromide (Tocris Cookson, Bristol, U.K.), mepyramine maleate (Tocris), L-phenylephrine hydrochloride (Sigma), propranolol hydrochloride (Sigma), (−)*R*-α-methylhistamine (Tocris), serotonin (5-hydroxytryptamine creatinine sulphate; Sigma), sodium nitroprusside (David Bull Laboratories, Mulgrave, VIC, Australia) and thioperamide maleate (Tocris), all dissolved in 0.9% saline. Burimamide (gift from James Black Foundation, Dulwich, U.K.) was first dissolved in 1 M HCl and then neutralised in 0.1 M NaOH. Drugs used *in vitro* were made up in H_2O.

Results

Validation of H_1, H_2 and H_3 receptor antagonism regimens

Histamine bolus i.v. injections cause a biphasic depressor and pressor response in the conscious rabbit (Angus *et al*., 1977).

Bolus doses of histamine (10–1280 $\mu g\,kg^{-1}$ i.v.) in the presence of the infusion of the H_2 receptor antagonist cimetidine induced a monophasic dose-dependent H_1 receptor-mediated pressor effect in the conscious rabbit (Figure 1; left panel). After administration of mepyramine, there was an ~120-fold right shift in sensitivity to histamine injections for at least 2 h ($n=4$; Figure 1; left panel). Histamine (10–1280 $\mu g\,kg^{-1}$ i.v.), in the presence of the H_1 receptor antagonist mepyramine, caused a dose-dependent putative H_2 and H_3 receptor-mediated hypotension in the conscious rabbit (Figure 1; middle panel). After administration of cimetidine, there was complete abolition of the hypotensive response to histamine, and reversal of the hypotension, for at least 2 h ($P=0.0003$; $n=4$). This H_2 receptor antagonism was also aided by the H_1 receptor pressor effects of higher doses of histamine overcoming the competitive H_1 receptor blockade by mepyramine (Figure 1; middle panel). *R*-α-MH (10–1000 $\mu g\,kg^{-1}$ i.v.) caused a relatively weak H_3 receptor-mediated hypotensive effect in the conscious rabbit (Figure 1; right panel). After administration of thioperamide, there was a ~30–100-fold right shift in sensitivity to *R*-α-MH for at least 2 h ($P=0.0016$; $n=3$). These results demonstrate that the chosen H_1, H_2 or H_3 receptor antagonist dosing regimens were sufficient to maintain a significant blockade of the respective histamine receptor under steady-state conditions for at least 2 h.

Acute agonist and antagonist effects on MAP and HR

Average resting MAP and HR values were measured before and for 20 min after administration of vehicle or drug. Vehicle alone had no effect on either parameter (data not shown). Administration of the H_1 and H_2 receptor antagonists 10 min prior to the test drug(s) caused a small transient rise in MAP and HR (e.g. vehicle group, $P<0.0003$; $n=11$; Figure 2). Both parameters returned to baseline values within 15 min, as seen in the vehicle group. H_1 and H_2 receptor block alone was used as the control for comparison with other treatments. The H_3 receptor agonist *R*-α-MH caused a short-lived, maximum fall in MAP of 8 ± 1 mmHg within 1 min ($P<0.0001$), and increase in HR of 18 ± 2 bpm ($P<0.0005$; $n=9$; Figure 2). The fall in MAP remained significantly lower than the pre-*R*-α-MH value over 20 min ($P<0.002$), while HR had returned to the predrug value by this time ($P=0.12$). These effects of *R*-α-MH were reversed by administration of thioperamide (data not shown). The H_3 agonist imetit caused a more gradual decrease in MAP of 5 ± 2 mmHg by 10 min postadministration, with a concomitant tachycardia of 13 ± 4 bpm ($P<0.01$; $n=7$; data not shown). Administration of the H_3 receptor antagonist thioperamide caused a small increase in MAP of 4 ± 1 mmHg that was maintained over 20 min ($P<0.01$), while HR remained unchanged ($n=11$; Figure 2). After administration of the nonselective H_2 and H_3 receptor antagonist burimamide, there was no change in MAP or HR ($n=4$; data not shown).

Baroreceptor-heart rate reflex

Average baroreflex curves (barocurves) were very similar in the saline group ($n=7$) and in the presence of H_1 and H_2 receptor antagonism ($n=11$; Figure 3, left panel), implying a lack of tonic involvement of these receptors in the baroreflex. All other drug treatments were performed in the presence of H_1 and H_2 receptor blockade. Compared with the H_1 and H_2 receptor antagonist group, *R*-α-MH caused a significant 44% decrease in the average gain (or slope) of the barocurve ($n=9$; $P=0.0001$; Figure 3, right panel), without significantly changing any other parameter (Table 1). Imetit showed a similar effect to *R*-α-MH with a 39% decrease in the average gain ($n=7$; $P=0.01$). In the presence of thioperamide, the decrease in barocurve gain caused by *R*-α-MH (or imetit; data not shown) was prevented ($n=9$; Figure 3, right panel). Thioperamide treatment alone caused a significant parallel rightward shift of the barocurve with an increase in both MAP and MAP_{50} of 5 mmHg ($P<0.05$), without changing the shape of the curve ($n=11$; Figure 3, middle panel; Table 1). In contrast, burimamide administration did not significantly affect any barocurve parameter ($n=4$; data not shown).

Bezold-Jarisch-like and nasopharyngeal reflexes

Bolus i.v. administration of 5-HT (3–30 $\mu g\,kg^{-1}$) induced bradycardia and corresponding decreases in MAP (Wright & Angus, 1989). The bradycardic responses to 5-HT in the H_1 and H_2 receptor antagonism group ($n=11$) were -57 ± 8, -88 ± 8 and -108 ± 10 bpm with 3, 10 and 30 $\mu g\,kg^{-1}$ 5-HT, respectively. These values were not different from the saline only group ($n=7$; data not shown). Treatment with *R*-α-MH,

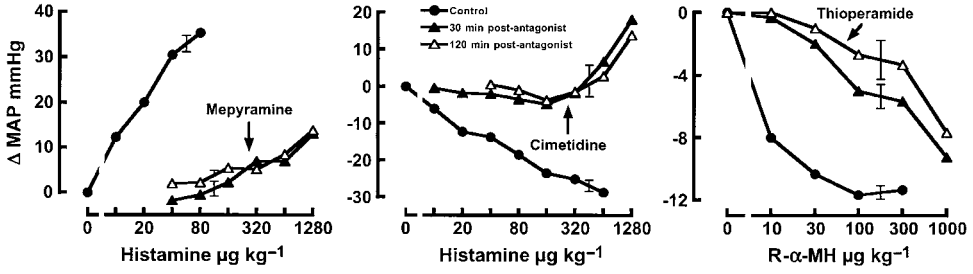

Figure 1 Agonist dose–response curves measured as change in mean arterial pressure (ΔMAP) from baseline before and after H_1, H_2 or H_3 receptor antagonist regimens in conscious rabbits. Left panel: Pressor effects of histamine (10–1280 $\mu g\,kg^{-1}$ i.v., in the presence of H_2 block with cimetidine 15 $mg\,kg^{-1}+15\,mg\,kg^{-1}\,h^{-1}$ i.v.) before (control), 30 or 120 min after administration of the H_1 antagonist mepyramine (0.8 $mg\,kg^{-1}+0.8\,mg\,kg^{-1}$ i.v.; $n=4$). Middle panel: Effects of histamine (10–1280 $\mu g\,kg^{-1}$ i.v., in the presence of H_1 block with mepyramine 0.8 $mg\,kg^{-1}+0.8\,mg\,kg^{-1}$ i.v.) before (control), 30 or 120 min after administration of the H_2 antagonist cimetidine (15 $mg\,kg^{-1}+15\,mg\,kg^{-1}\,h^{-1}$ i.v.; $n=4$). Right panel: Effects of the H_3 agonist *R*-α-MH (10–1000 $\mu g\,kg^{-1}$ i.v.) before (control), 30 or 120 min after administration of the H_3 antagonist thioperamide (1 $mg\,kg^{-1}+1\,mg\,kg^{-1}$ i.v.; $n=3$). Error bars are average s.e.m. from repeated measures ANOVA (see Methods).

thioperamide or their combination (nor imetit or burimamide) had no effect on the 5-HT dose–bradycardia response curve ($P>0.05$; data not shown).

The nasopharyngeal reflex elicited a large bradycardia of 176 ± 8 bpm in rabbits treated with the H_1 and H_2 receptor antagonists ($n=11$). Similar values were obtained in all other drug treatment groups; that is, H_3 receptor ligands were without effect on this vagally mediated reflex (data not shown).

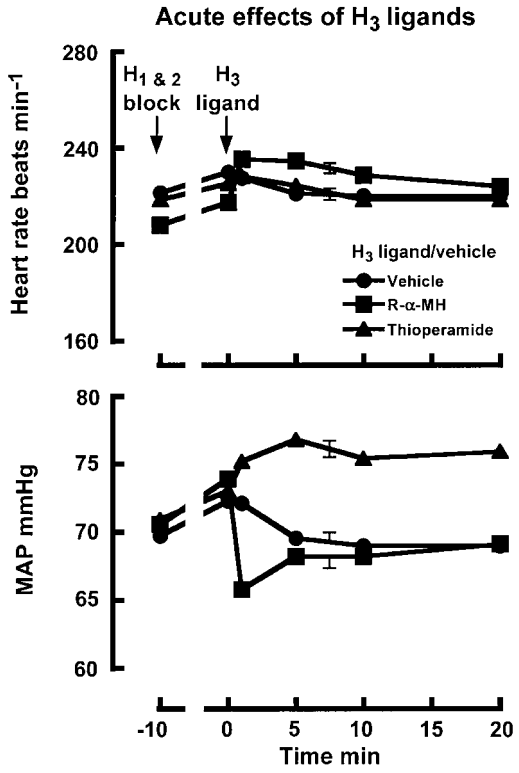

Figure 2 Acute effects of H_3 receptor ligands on HR (upper panel) and MAP (lower panel) in the presence of H_1 and H_2 receptor block (mepyramine and cimetidine; see Methods) given at -10 min. Vehicle (saline, $1\,ml+10\,ml\,h^{-1}$; $n=11$), *R*-α-MH ($100\,\mu g\,kg^{-1}+100\,\mu g\,kg^{-1}\,h^{-1}$; $n=9$) or thioperamide ($1\,mg\,kg^{-1}+1\,mg\,kg^{-1}\,h^{-1}$; $n=11$) were given i.v. at time 0 min. Error bars are average s.e.m. from repeated measures ANOVA (see Methods).

Sympathetic and vagal effects in isolated right atria

Sympathetic tachycardic responses in rabbit isolated right atria to one to eight electrical field pulses were unaffected by H_3 receptor activation. The field pulse–tachycardia response curves before and after 10 μM *R*-α-MH were not different ($P=0.78$), with a maximum 70% increase in rate (~80 bpm) with eight pulses ($n=4$; Figure 4, left panel). In guinea-pig right atria, sympathetic responses were consistent over three vehicle control stimulations ($n=5$; Figure 5, top left panel; $P=0.49$). *R*-α-MH (1 μM) caused a rightward shift of the curve of approximately two-fold ($n=6$; $P=0.0014$, i.e. a doubling of the field pulses required to cause a similar tachycardia; Figure 5, top right panel). This effect was reversed by 1 μM thioperamide.

Vagal responses in rabbit isolated right atria to one to four field pulses were also unaffected by H_3 receptor activation with similar field stimulation–bradycardia response curves before and after 10 μM *R*-α-MH, with a maximum 50% decrease in rate (~100 bpm) with four pulses ($n=4$; Figure 4, right panel). In guinea-pig atria, vagal responses were consistent over three vehicle control stimulations ($n=4$; Figure 5, lower left panel). Similar to the sympathetic preparation in this species, *R*-α-MH (1 μM) caused a shift of the curve to the right of approximately two-fold ($n=5$; $P=0.019$; Figure 5, lower right panel). This effect was reversed by 1 μM thioperamide.

All field stimulation responses in rabbit and guinea-pig tissues were blocked by tetrodotoxin (0.1 μM).

Discussion

These findings demonstrate that activation of histamine H_3 receptors with *R*-α-MH results in acute hypotension and tachycardia, along with considerable attenuation of baroreflex gain in conscious rabbits. Antagonising H_3 receptors with

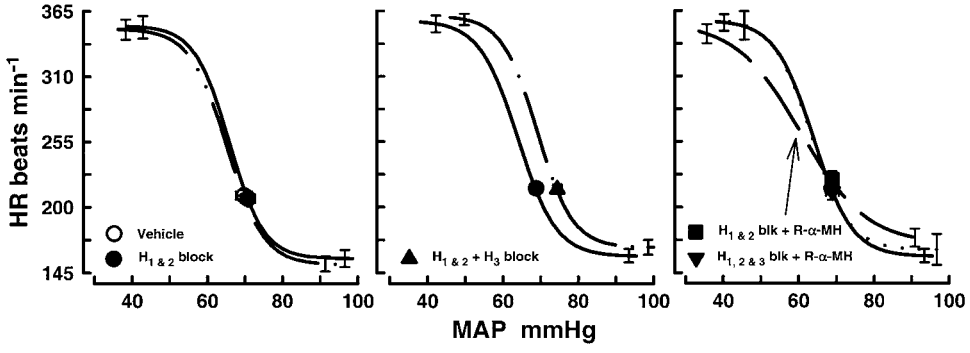

Figure 3 Baroreflex curves relating MAP to HR in conscious rabbits. Left panel: Curves after i.v. administration of vehicle (saline; dash-dot-dot-dash line; $1\,ml+10\,ml\,h^{-1}$; $n=7$) or H_1 and H_2 block with cimetidine and mepyramine (solid line; $15\,mg\,kg^{-1}+15\,mg\,kg^{-1}\,h^{-1}$ and $0.8\,mg\,kg^{-1}+0.8\,mg\,kg^{-1}\,h^{-1}$, respectively; $n=11$). Middle panel: Curves after i.v. administration of H_1 and H_2 block (solid line; $n=11$) or H_1 and H_2 block combined with H_3 block with thioperamide (dash-dot-dash line; $1\,mg\,kg^{-1}+1\,mg\,kg^{-1}\,h^{-1}$; $n=9$). Right panel: Curves after i.v. administration of H_1 and H_2 receptor block (solid line; $n=11$), *R*-α-MH (dashed line; $100\,\mu g\,kg^{-1}+100\,\mu g\,kg^{-1}\,h^{-1}$, in the presence of H_1 and H_2 block; $n=9$), or *R*-α-MH (in the presence of H_1 and H_2 block) combined with thioperamide (dotted line; $1\,mg\,kg^{-1}+1\,mg\,kg^{-1}\,h^{-1}$; $n=9$). The symbols on the curves represent the average resting values for MAP and HR. The error bars on the symbols are ±1 s.e.m. (those not shown are contained within the symbol), and those on the curves represent the s.e.m. of the lower HR plateau (right) and HR range (left).

Table 1 Effect of H_3 receptor ligands on baroreflex curve parameters in conscious rabbits

Treatment (n)	*MAP* (mmHg)	*HR* (bpm)	*HR range* (bpm)	*Lower HR plateau* (bpm)	MAP_{50} (mmHg)	*Average gain* (bpm $mmHg^{-1}$)
Saline (7)	70±2	210±4	199±8	152±6	65±2	8.8±0.8
H_1 and H_2 block (11)	69±2	216±3	198±7	159±6	64±2	8.4±0.8
R-α-MH† (9)	69±2	223±7	187±8	169±7	61±1	4.7±0.3*
Thioperamide† (11)	74±1*	215±4	195±5	166±6	69±1*	9.1±0.7
R-α-MH + thioperamide† (6)	69±2	212±7	194±12	164±13	63±1	8.3±0.4
Imetit† (7)	68±2	231±5	187±7	181±8	61±2	5.1±0.6*

†In the presence of H_1 and H_2 block.
Values are mean±1 s.e.m. Treatments: saline (1 ml + 10 ml h^{-1}); H_1 and H_2 block (mepyramine 0.8 mg kg^{-1} + 0.8 mg kg^{-1} h^{-1} and cimetidine 15 mg kg^{-1} + 15 mg kg^{-1} h^{-1}); *R*-α-MH (*R*-α-methylhistamine; 100 μg kg^{-1} + 100 μg kg^{-1} h^{-1}); thioperamide (1 mg kg^{-1} + 1 mg kg^{-1} h^{-1}); *R*-α-MH + thioperamide; and imetit (30 μg kg^{-1} + 10 μg kg^{-1} h^{-1}).
n, number of rabbits. HR, heart rate; MAP_{50}, MAP at half the HR range. *$P<0.05$, significant difference compared with corresponding value in H_1 and H_2 block group, one-way ANOVA with Dunnett's *post hoc* test.

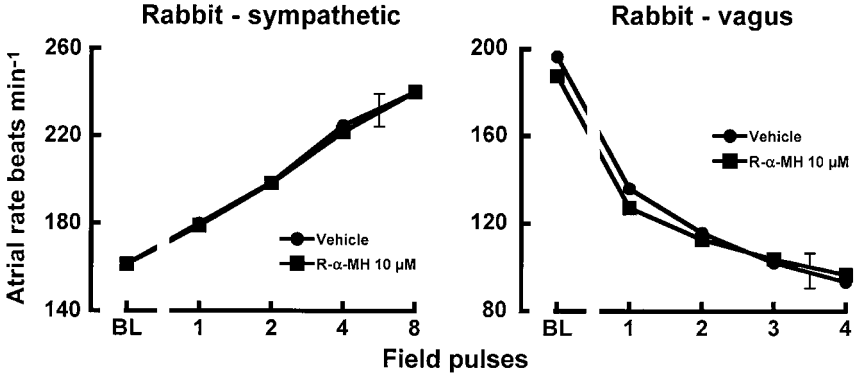

Figure 4 Left panel: sympathetic effects of vehicle (H_2O) or *R*-α-MH (10 μM) on tachycardic responses to one to eight electrical field pulses of rabbit isolated right atria ($n=4$) in the presence of 1 μM atropine, 0.1 μM mepyramine and 10 μM cimetidine. Right panel: Vagal effects of vehicle (H_2O) or *R*-α-MH (10 μM)on bradycardic responses to one to four electrical field pulses (delivered within the refractory period) of rabbit isolated right atria ($n=4$) in the presence of 10 μM propranolol, 0.1 μM mepyramine and 10 μM cimetidine. BL; baseline atrial rate. Error bars are average s.e.m. from repeated measures ANOVA (see Methods).

thioperamide (but not burimamide) led to a small pressor effect and rightward shift of the baroreflex curve, suggesting a tonic level of H_3 receptor activation, which modulates MAP at rest. No evidence was found for a role of H_3 receptors in the vagally mediated Bezold-Jarisch-like or nasopharyngeal reflexes. In rabbit isolated atria, there was no effect of H_3 receptor activation on sympathetically- or vagally mediated responses. Taken together, in the rabbit it appears that central endogenous histamine, acting at H_3 receptors, attenuates sympathetic outflow.

In preliminary experiments, the H_1, H_2 and H_3 antagonist regimens were shown to provide significant block for the duration of the experimental protocol (~2 h). The H_1 and H_2 regimens were validated over a range of histamine doses (well beyond physiological levels) as it was important to remove any possible activation of these receptors by *R*-α-MH, which has very low affinity for both H_1 and H_2 subtypes (Arrang *et al*., 1987; Hey *et al*., 1992b). In the conscious rabbit, the acute fall in MAP and tachycardia elicited by *R*-α-MH closely confirms findings of McLeod *et al*. (1994). The fall in MAP is consistent with a presynaptic location of the H_3 receptor on sympathetic nerves innervating peripheral resistance vessels, where activation leads to an inhibition of endogenous noradrenaline release and decrease in total peripheral resistance (McLeod *et al*., 1996). This has been suggested to occur *via* an inhibitory $G_{i/o}$ protein decreasing Ca^{2+} current through N-type Ca^{2+} channels (Endou *et al*., 1994). In rabbits, the maximum effect of *R*-α-MH on MAP was shown in this study to be reached with 100 μg kg^{-1} i.v. The immediacy of the depressor effect confirms a peripheral site of action as *R*-α-MH does not easily cross the blood–brain barrier (Taylor *et al*., 1992; Yamasaki *et al*., 1994), comparable with the endogenous ligand histamine. The acute tachycardia is likely due to reflex activation in response to the fall in MAP (McLeod *et al*., 1994). The immediate reversal of *R*-α-MH's depressor effect by the competitive antagonist thioperamide confirms that it was mediated by the H_3 receptor. The H_3 agonist imetit, the most potent and selective to date (Garbarg *et al*., 1992; Hough, 2001), caused a similar, but more gradual, fall in MAP compared with *R*-α-MH.

Thioperamide administration caused an increase in MAP, suggesting a basal activation of H_3 receptors on sympathetic varicosities innervating resistance arteries, where H_3 blockade would prevent tonic inhibition of noradrenaline release. Godlewski *et al*. (1997a) also suggested tonic sympathetic inhibition as they found that H_3 receptor antagonists enhanced electrically induced pressor responses in the pithed rat. Favouring the presence of tonic control of neurotransmitter release *via* H_3 receptors, histamine activates these receptors at a concentration >100 times less than required to activate H_1, or H_2 receptors (Arrang *et al*., 1983). Whether the pressor effect of thioperamide is due to a central and/or peripheral site

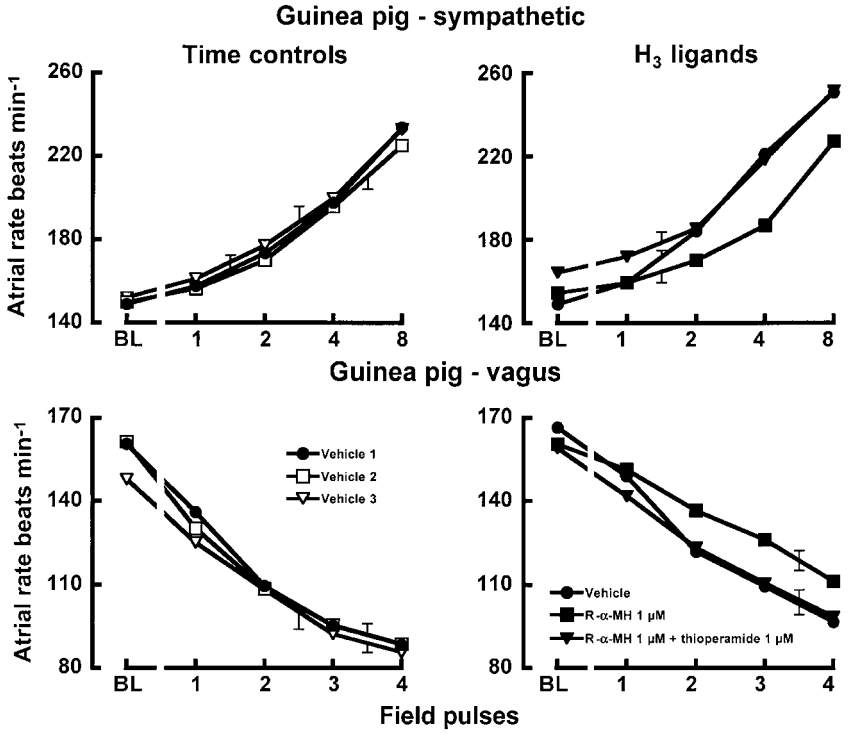

Figure 5 Upper panels: Sympathetic effects of (left) vehicle time controls (H_2O; $n=5$) or (right) vehicle, *R*-α-MH (1 μM) or *R*-α-MH (1 μM)with thioperamide (1 μM) ($n=6$) on tachycardic responses to one to eight electrical field pulses of guinea-pig isolated right atria in the presence of 1 μM atropine, 0.1 μM mepyramine and 10 μM cimetidine. Lower panels: Vagal bradycardic responses with (left) vehicle time controls (H_2O; $n=4$) or (right) vehicle, *R*-α-MH (1 μM) or *R*-α-MH (1 μM) with thioperamide (1 μM) ($n=5$) in response to one to four electrical field pulses (delivered within the refractory period) of guinea-pig isolated right atria. All measurements were in the presence of 10 μM propranolol, 0.1 μM mepyramine and 10 μM cimetidine. BL, baseline atrial rate. Error bars are average s.e.m. from repeated measures ANOVA (see Methods).

of action is not clear as this highly lipophilic drug easily crosses the blood–brain barrier (Taylor *et al*., 1992; Mochizuki *et al*., 1996). In contrast, the nonselective H_2 and H_3 antagonist burimamide, which does not enter the CNS (Ganellin *et al*., 1976), did not cause a change in MAP in the conscious rabbit. This suggests that thioperamide's actions may be centrally mediated.

The existence of H_3 receptors on postganglionic sympathetic, and possibly vagal, nerves in the heart led to an anticipation of an attenuation of the upper and/or lower HR plateaus of the baroreflex curve through inhibition of noradrenaline and/or acetylcholine release, respectively. However, the considerable decrease in baroreflex gain seen with both H_3 receptor agonists, without a change in either of the curve plateaus (or heart rate range), indicates a more complex interaction of the H_3 receptor in this reflex. This attenuation of gain is physiologically significant as it is within the narrow range of changes in MAP around the resting baseline that the baroreflex has most influence. This sympatholytic-like effect (apart from the lack of decrease in HR range) agrees with the notion that H_3 receptors are inhibiting noradrenaline release from cardiac sympathetic nerves. In cardiovascular tissues such as human saphenous vein strips (Molderings *et al*., 1992), guinea-pig pulmonary artery strips (Rizzo *et al*., 1995) and atria (Endou *et al*., 1994), H_3-mediated inhibition of exocytotic noradrenaline release is 30–60%.

It is important to consider whether the site of action of this H_3 receptor-mediated effect on the baroreflex is central and/or peripheral. Although *R*-α-MH can cross the blood–brain barrier, it is thought to do so with difficulty (Taylor *et al*., 1992; Yamasaki *et al*., 1994). To our knowledge, no studies examining CNS penetration of *R*-α-MH (or thioperamide) have been carried out in the rabbit. It may be that over the duration of an experiment (~2 h), *R*-α-MH does enter the CNS and modulate sympathetic output in the rabbit. Also, baroreflex afferent nerves pass through the area postrema, a region with fenestrated capillaries that lack tight endothelial junctions (Eckberg & Sleight, 1992), thereby potentially allowing *R*-α-MH CNS access.

The *in vitro* studies were conducted to further clarify the site of the H_3 receptor-mediated effect. There was no evidence for the functional existence of H_3 receptors on sympathetic nerves in rabbit atria, with a high concentration of *R*-α-MH (10 μM) having no effect on sympathetic responses. This supports the idea that the H_3 agonist-mediated decrease in baroreflex gain may be centrally mediated, and not at the level of postganglionic sympathetic nerves supplying the sinoatrial node in the rabbit heart. In guinea-pig atria, in contrast, *R*-α-MH caused a thioperamide-sensitive attenuation of sympathetic responses, without affecting those to exogenous noradrenaline (data not shown), suggesting the existence of presynaptic H_3 receptors. This agrees with Endou *et al*. (1994) where *R*-α-MH (0.3 μM) significantly depressed the positive chronotropic response to sympathetic nerve stimulation in guinea-pig atria. Some studies have suggested that a functional relation exists between α_2-adrenoceptors and H_3 receptors on noradrenergic neurones, where blocking the α_2-adrenoceptor enhances/unmasks H_3 receptor-mediated effects (Schlicker *et al*., 1990;

1992; Godlewski *et al*., 1997b). In separate guinea-pig isolated atria experiments, tissues were pretreated with yohimbine to block α_2-adrenoceptors; however, this did not affect responses to *R*-α-MH (data not shown). Other studies have also failed to find this interaction (Luo *et al*., 1991; Molderings *et al*., 1992).

The resetting of the baroreflex curve to a higher resting MAP in the presence of the antagonist thioperamide is an exciting finding, suggesting H_3 receptors were endogenously activated and may have a tonic role in the baroreflex. As discussed above, some studies have suggested a potential role for H_3 receptor activation modulating vascular tone at rest; however, these used electrically driven systems or alteration of basal histamine levels (Campos *et al*., 1996; Godlewski *et al*., 1997a, b; Mazenot *et al*., 1999). There is frequency-dependent release of cardiac histamine in response to sympathetic stimulation in guinea-pig heart (Gross *et al*., 1984), while normal sympathetic activity appears to regulate a nonmast cell rapid turnover pool of cardiac histamine in the rat (Yoshitomi *et al*., 1989). The source of histamine responsible for the basal vascular tone in the conscious rabbit is not clear but may be the CNS, as inferred by the results with burimamide which does not cross the blood–brain barrier. Burimamide, in contrast to thioperamide, did not cause a rightward shift of the baroreflex curve.

Endogenous histamine is important in regulating sympathetic overactivity in pathophysiological states such as myocardial ischaemia (Imamura *et al*., 1994; Hatta *et al*., 1997). Enhanced histamine release activates presynaptic H_3 receptors to decrease exocytotic and carrier-mediated noradrenaline release in acute and protracted myocardial ischaemia, respectively. In hearts isolated from transgenic mice lacking H_3 receptors, there was a two-fold greater release of noradrenaline following an ischaemic episode compared to hearts from wild-type mice (Koyama *et al*., 2003). This highlights the likely therapeutic value of H_3 receptor agonists in the negative modulation of noradrenaline release in myocardial ischaemia (Levi & Smith, 2000; Silver *et al*., 2001; 2002; Koyama *et al*., 2003).

The Bezold-Jarisch-like and nasopharyngeal reflexes, both characterised by profound bradycardia mediated by the efferent vagus (Krayer, 1961; White *et al*., 1974; 1975), were unaffected by H_3 receptor agonists or antagonists. This suggests that, in the conscious rabbit, H_3 receptors are not involved in vagally mediated reflexes, supporting the idea that the decrease in baroreflex gain in response to H_3 agonists is predominantly sympathetically mediated. Furthermore, the lack of effect of H_3 ligands on the Bezold-Jarisch-like reflex implies there is no interaction with the 5-HT_3 receptor, at least in the rabbit.

In conclusion, histamine H_3 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 as thioperamide (but not burimamide) caused an increase in MAP and resetting of the baroreflex. Further investigation is needed to confirm whether these effects are due to a central site of action, and to examine the role of H_3 receptors in cardiovascular function in pathological states such as hypertension and ischaemic heart disease.

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