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Pharmacological characterization of histamine H_3 receptors in isolated guinea pig pulmonary artery and ileum

Charles A. Rizzo *, Salvatore Tozzi, Mary Ellen Monahan, John A. Hey

Schering-Plough Research Institute, Kenilworth, NJ 07033, USA

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Abstract

We characterized the histamine H_3 receptors involved in the modulation of electrical field stimulated neurogenic contraction of guinea pig pulmonary artery sympathetic, and guinea pig ileum parasympathetic preparations. Simultaneous measures of electrical field stimulation-evoked ^3H overflow and tension in [^{3}H]norepinephrine-loaded pulmonary artery were sensitive to tetrodotoxin (300 nM) and insensitive to hexamethonium (100 μM). Only the contractile response was inhibited by prazosin (100 nM). (*R*)-α-Methylhistamine's inhibition of the pulmonary artery contraction and ^3H overflow were dose-dependently antagonized by thioperamide (30–100 nM). (*R*)-α-Methylhistamine also inhibited the neurogenic contractions of the isolated ileum ($pD_2 = 8.2$). In the pulmonary artery, the relative potency of the histamine H_3 receptor antagonists vs. (*R*)-α methylhistamine innibition of neurogenic contractions ($pD_2 = 7.1$) was thioperamide ($pA_2 = 8.6 \pm 0.1$) > burimamide ($pA_2 = 7.6 \pm 0.2$) > impromidine ($pA_2 = 6.9 \pm 0.02$). Similarly, the relative potency of histamine H_3 receptor antagonists in the isolated ileum was thioperamide > burimamide ≥ impromidine, with pA_2 estimates of 8.7 ± 0.1, 7.3 ± 0.1 and 7.1 ± 0.1, respectively. Antagonist potencies suggest a predominant histamine H_{3A}-like receptor population on postganglionic sympathetic neurons innervating the pulmonary artery and parasympathetic neurons innervating the ileum longitudinal muscle.

Keywords: Histamine H_3 receptor subtype; Parasympathetic neuron; (*R*)-α-Methylhistamine; Sympathetic neuron

1. Introduction

Neuronal histamine H_3 receptors modulate the release of a variety of central and peripheral neurotransmitters, including histamine, acetylcholine, serotonin and norepinephrine (Arrang et al., 1983; Ishikawa and Sperelakis, 1987; Poli et al., 1991; Schlicker et al., 1988, 1992). Recently, West et al. (1990) used competition versus [^{3}H]N^{α}-methylhistamine to identify two binding sites for the histamine H_3 receptor antagonists thioperamide and burimamide in rat brain and proposed histamine H_3 receptor subtypes associated with these high (H_{3A}) and low (H_{3B}) affinity sites. Study of isolated mouse cortex norepinephrine release (Schlicker et al., 1992) has demonstrated a prejunctional inhibition by histamine H_{3A}-like receptors. Although the existence of functional histamine H_{3B}-like receptors is suggested by an in vivo study of guinea pig hypertensive responses (Hey et al., 1992), functional histamine H_{3B}-like receptors have yet to be unequivocally demonstrated. Characterization of histamine H_3 receptors in various isolated tissue preparations will strengthen the evidence for functional histamine H_{3A}-like receptors and allow further exploration of whether the histamine H_{3B} binding site is associated with a functional receptor.

Aims of the present studies were 2-fold: (1) to develop an isolated vascular sympathetic preparation containing a neuronal inhibitory histamine H_3 receptor; and (2) to characterize the histamine H_3 receptor subtype(s) modulating neuronal function in this vascular sympathetic neuroeffector system and in an isolated parasympathetic neuroeffector system as a test of the histamine H_3 receptor heterogeneity hypothesis. Demonstration of functional histamine H_3 receptor heterogeneity would be important since it could open an avenue for development of new selective therapeutic agents.

* Corresponding author. Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA. Tel.: (908) 298-7234; fax: (908) 298-7175.

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2. Materials and methods

2.1. Tissue preparation and general procedures

All experiments were approved by the Animal Care and Use Committee of the Schering-Plough Research Institute, an AAALAC (American Association for Accreditation of Laboratory Animal Care) accredited facility. Isolated tissues were obtained from male Charles River Hartley strain guinea pigs. The guinea pigs were stunned by a blow to the head and/or cervically dislocated before death was assured by exsanguination. The heart and lungs were removed to room temperature low (0.75 mM) Ca^{2+} Krebs buffer supplemented with 30 nM EDTA and 2 μM indomethacin and aerated with 95% O_2-5% CO_2. A 5 mm long main pulmonary artery ring was removed from just above the right ventricle and either used intact or cut open for use as a tissue strip. One pulmonary artery preparation was obtained from each animal. A 10 cm segment of ileum, starting 20 cm from the ileocecal junction, was removed to room temperature Tyrode's buffer aerated with 95% O_2-5% CO_2. 2 cm long whole ileum segments were then prepared for use in the assay. Pulmonary artery preparations were obtained from 500–900 g guinea pigs. Ileum preparations were obtained from 350–500 g animals.

Electrical stimuli generated by a Grass S48 or S88 Stimulator were amplified and distributed to field stimulating electrodes in the baths by means of a Buxco Electronics Stimulus Distributor. Grass FT03 force displacement transducers and Harvard physiographs were used to record isometric tension.

2.2. Pulmonary artery studies: simultaneous evoked ^3H overflow and tension in [^3H]norepinephrine-loaded tissues

Isolated guinea pig pulmonary artery strips were incubated for 60 min under a 95% O_2-5% CO_2 atmosphere in 37°C low (0.75 mM) Ca^{2+} Krebs buffer containing *l*-[7,8-^{3}H]norepinephrine (0.25 μM, 10 μCi/ml), 30 nM EDTA and 2 μM indomethacin. The [^{3}H]norepinephrine-loaded tissues were mounted in 1.5 ml Radnoti organ baths with built-in platinum wire electrodes (Radnoti Glass Technology, Monrovia, CA, USA). The baths were filled with 37°C low Ca^{2+} Krebs buffer containing 30 nM EDTA, 2 μM indomethacin, 1 μM desipramine and 1 μM rauwolscine and continuously aerated with 95% O_2-5% CO_2. Indomethacin, desipramine and rauwolscine were added to the baths to block prostaglandin synthesis, neuronal uptake of norepinephrine and α_2-adrenoceptor mediated inhibition of norepinephrine release respectively. Tissues were equilibrated 40 min under an initial 4.0 g resting tension with bath fluids changed every 10 min, allowing for ^{3}H washout and stabilization of resting tissue tension. After the equilibration period, bath fluids were changed every 5 min. Isometric tension was monitored throughout the experiment. ^{3}H overflow and tension were evoked by a 1 min train of electrical field stimulation (12 V, 100–200 mA, 0.2 ms pulse duration, 2 Hz). Two trains of electrical field stimulation (S_0, S_1, 20 min wash and recovery interval) were followed by a 20 min incubation with hexamethonium, tetrodotoxin or a histamine H_3 receptor antagonist, or a 60 min incubation with prazosin and where indicated, a 5 min incubation with (*R*)-α-methylhistamine before the final stimulus (S_2). At each change of bath fluid, the entire 1.5 ml bath contents were collected, mixed with 10.0 ml of Scintiverse BD and counted on an LKB 1219 Rackbeta Liquid Scintillation Counter for assessment of tritium overflow. While baseline ^{3}H release decreased throughout the experiment, electrical field stimulation-evoked ^{3}H release remained constant. In a representative series of experiments, baseline cpm at S_0, S_1 and S_2 respectively, was 9604 ± 854, 7168 ± 784 and 4015 ± 439, while the electrical field stimulation-evoked increase over baseline cpm was 5384 ± 1178, 5202 ± 1100 and 5129 ± 1030 cpm (values = average ± S.E.M. of 8 tissues).

2.3. Evoked tension responses in isolated pulmonary artery: assessment of histamine H_3 receptor antagonist activity

Isolated guinea pig pulmonary artery rings were mounted between two platinum wire ring electrodes in a 15.0 ml organ bath containing low (0.75 mM) Ca^{2+} Krebs buffer with 30 nM EDTA, 2 μM indomethacin, 1 μM rauwolscine, 1 μM chlorpheniramine maleate and 10 μM cimetidine. Baths fluids were continuously aerated with 95% O_2-5% CO_2. Indomethacin and rauwolscine were included to block prostaglandin synthesis and α_2-adrenoceptor mediated feedback inhibition of norepinephrine release while chlorpheniramine and cimetidine were added to minimize histamine H_1 and H_2 receptor mediated effects. Tissues were equilibrated 90 min under 4.0 g initial resting tension and isometric tension was recorded throughout the experiment. Tension was stimulated by a 20 s train of electrical field stimulation (4 Hz, 0.3 ms pulse duration, half-maximal voltage) during six stimulus periods (S_0–S_5) allowing 30 min intervals between stimulus periods. Tissues were washed immediately after peak tension was achieved. Stimulus periods S_1–S_5 were preceded by 20 min incubations with histamine H_3 receptor antagonists. 5 min incubations with rising noncumulative (*R*)-α-methylhistamine concentrations preceded stimulus periods S_2–S_5.

2.4. Neurogenic contractile responses in ileum: assessment of histamine H_3 receptor antagonist activity

Guinea pig whole ileum segments were mounted between coaxial stainless steel electrodes in a 25.0 ml organ bath containing 37°C Tyrode's buffer with 1 μM chlorpheniramine maleate to block histamine H_1 receptor mediated effects and continuously aerated with 95% O_2-5% CO_2. Buffer also included 10 μM cimetidine during the impromidine experiments to block impromidine-induced changes in baseline tension. Tissues were equilibrated under 0.3 g resting tension and isometric tension was recorded throughout the experiment. Transient evoked tension responses were stimulated by repetitive 1 s trains of electrical field stimulation (3 V, 40 Hz, 80% maximal pulse duration) at 1 min intervals. Histamine H_3 receptor antagonists were added to the bath 5 min prior to initiation of repetitive electrical field stimulation trains. After the first electrical field stimulation train, rising cumulative additions of (*R*)-α-methylhistamine were performed 1 min before each of the following stimulus trains.

2.5. Materials

The following chemicals were used: chlorpheniramine maleate, indomethacin, burimamide, cimetidine and (*R*)-α-methylhistamine dihydrochloride were supplied by the Chemical Research Dept., Schering-Plough Research Institute, Kenilworth, NJ, USA. Hexamethonium bromide, tetrodotoxin, and *l*-norepinephrine bitartrate were obtained from the Sigma Chemical Co., St. Louis, MO, USA. Desipramine hydrochloride, rauwolscine hydrochloride, prazosin hydrochloride and thioperamide maleate were obtained from Research Biochemicals International, Natick, MA, USA. Impromidine trihydrochloride was a gift from Smith-Kline-Beecham, King of Prussia, PA, USA. *l*-[7,8-^{3}H]norepinephrine in 0.02 M acetic acid/ethanol (9:1), 1 mCi/ml, 30–50 Ci/mmol, was obtained from Amersham Life Science, Arlington Heights, IL, USA.

All compounds were prepared as concentrated stock solutions before addition to the assay buffer. Stock solutions of EDTA, desipramine hydrochloride, rauwolscine hydrochloride, chlorpheniramine maleate, cimetidine, (*R*)-α-methylhistamine dihydrochloride, and hexamethonium bromide were all prepared in water. Dimethyl sulfoxide (DMSO) was used to prepare stock solutions of prazosin hydrochloride, thioperamide maleate, burimamide and impromidine. Maximal final concentrations of DMSO did not exceed 0.33% by volume in the baths. The indomethacin stock solution was dissolved in a slight molar excess of sodium hydroxide and the tetrodotoxin stock solution was prepared in 10 mM acetate buffer, pH 4.5. *l*-[7,8-^{3}H]Norepinephrine in 0.02 M acetic acid/ethanol (9:1) was dried under a stream of N_2 in a glass vial. The appropriate buffer was added and the vial vortexed to redissolve the *l*-[7,8-^{3}H]norepinephrine at the final concentration.

2.6. Data analysis

Agonist activity was expressed as a pD_2, where $pD_2 = -\log EC_{50}$. Antagonist activity was estimated as an apparent pA_2 using agonist dose responses obtained in the absence and presence of concentrations of each antagonist which produced significant rightward shifts of the agonist dose-response curve. Apparent pA_2 was taken as $-\log$ of K_i, where K_i was estimated from the relations between agonist, competitive antagonist and K_i (Furchgott, 1955).

Stimulus-induced ^{3}H overflow was assessed as the electrical field stimulation-induced increase in counts per minute (cpm) over baseline cpm. Stimulus responses were normalized by comparison to an initial electrical field stimulation response (S_1) in each tissue to obtain an S_2/S_1 ratio. No evidence of quenching or fluorescence was noted with any of the concentrations of the compounds tested.

Results are given as the mean ± S.E.M. of *n* experiments. For comparison of two means, a Student's *t*-test was performed. Analysis of variance (ANOVA) was used when comparing two or more groups to the same control.

Table 1
Characterization of the electrical field stimulation response in [^{3}H]norepinephrine-loaded pulmonary artery

Treatment	% Control [a] electrical field stimulation-evoked ^{3}H overflow [b]	% Control [a] electrical field stimulation-evoked tension [b]	*n*
Hexamethonium 100 μM	99 ± 8	97 ± 12	4
Tetrodotoxin 300 nM	3 ± 1 [c]	19 ± 2 [c]	4
Prazosin 100 nM	70 ± 4	29 ± 13 [c]	3

[a] Values report % of the vehicle control response. [b] Control and treated electrical field stimulation-evoked ^{3}H overflow and tension assessed as *S2/S1* ratios. [c] Significantly different from the control response ($P < 0.05$).

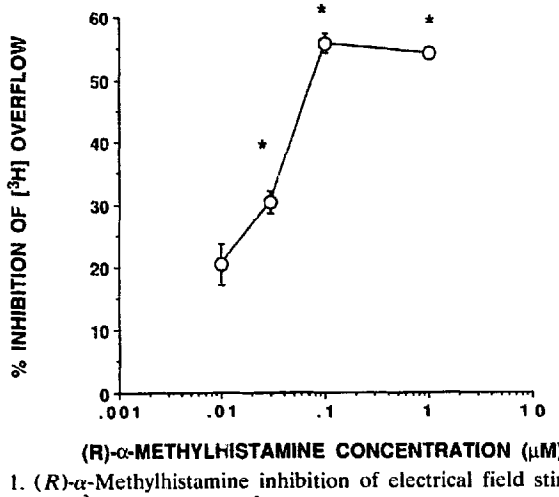

Fig. 1. (*R*)-α-Methylhistamine inhibition of electrical field stimulation-evoked ^3H overflow in *l*-[^3H]norepinephrine-loaded pulmonary artery. * Significantly different from the control response, $P < 0.05$, $n = 4$.

3. Results

3.1. Isolated guinea pig pulmonary artery: histamine H_3 receptor modulation of norepinephrine release

Electrical field stimulation of [^3H]norepinephrine-loaded pulmonary artery strips evoked ^3H overflow and tension (average tension = 0.26 ± 0.03 g). Both electrical field stimulation-evoked ^3H overflow and tension were found to be insensitive to the ganglionic blocker hexamethonium (100 μM) but inhibited by the Na^+ channel blocker tetrodotoxin (300 nM, Table 1). α_1-Adrenoceptor blockade with prazosin (100 nM) significantly inhibited only the evoked tension response (Table 1). (*R*)-α-Methylhistamine inhibited both ^3H overflow and tension ($pD_2 = 7.7$ vs. ^3H overflow, Fig. 1). (*R*)-α-Methylhistamine (300 nM) did not antagonize exogenous norepinephrine (30 nM–1 μM)-induced tension (norepinephrine ED_{50} with and without (*R*)-α-methylhistamine was 253 nM and 247 nM respectively, $n = 4$). The effect of (*R*)-α-methylhistamine (100 nM) on both evoked ^3H overflow and tension was dose dependently antagonized by the selective histamine H_3 receptor antagonist thioperamide (30 and 100 nM, Table 2).

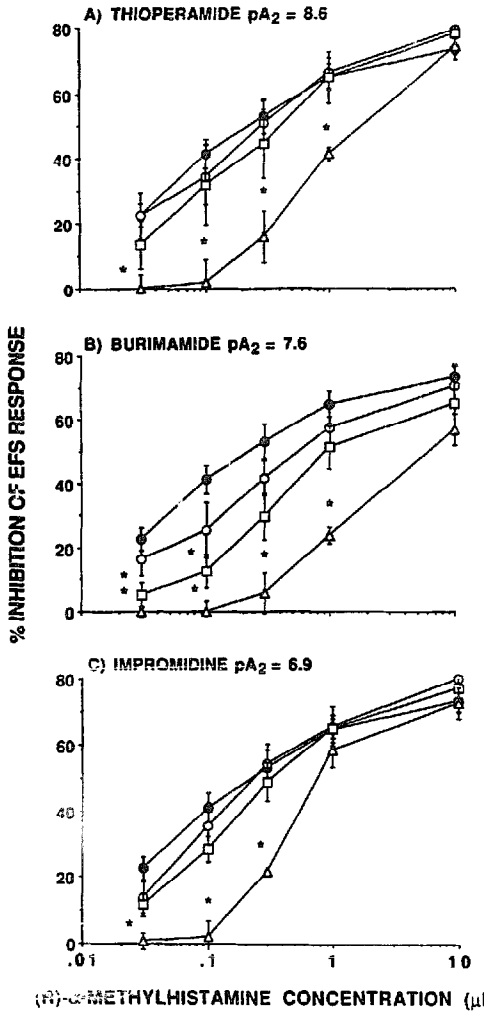

Fig. 2. pA_2 estimates for the histamine H_3 receptor antagonists thioperamide, burimamide, and impromidine vs. (*R*)-α-methylhistamine inhibition of electrical field stimulation-induced contraction in pulmonary artery. pA_2 estimated using agonist dose responses obtained in the absence and presence of concentrations of each antagonist which produced significant rightward shifts of the agonist dose-response curve. Symbols represent: (A) vehicle-treated (●) and 3 nM (○), 10 nM (□) or 30 nM (△) thioperamide-treated responses; (B) vehicle-treated (●) and 0.1 μM (○), 0.3 μM (□) or 0.6 μM (△) burimamide-treated responses; and (C) vehicle-treated (●) and 0.1 μM (○), 0.3 μM (□) or 1.0 μM (△) impromidine-treated responses. $n = 15$ for vehicle-treated responses and $n = 3$–7 for antagonist-treated responses. * Significantly different from the vehicle control response, $P < 0.05$.

Table 2
Thioperamide antagonism of (*R*)-α-methylhistamine inhibition of electrical field stimulation-evoked ^3H overflow and tension in [^3H]norepinephrine-loaded pulmonary artery

Thioperamide concentration (nM) [a]	(*R*)-α-Methylhistamine [b] % inhibition of electrical field stimulation-evoked ^3H overflow	(*R*)-α-Methylhistamine [b] % inhibition of electrical field stimulation-evoked tension	*n*
0	44 ± 2	37 ± 4	8
30	26 ± 2 [c]	19 ± 4 [c]	4
100	9 ± 11 [c]	8 ± 4 [c]	3

[a] Thioperamide addition was noncumulative. [b] (*R*)-α-Methylhistamine concentration was 100 nM. [c] Significantly different from the response to (*R*)-α-methylhistamine in the absence of thioperamide ($P < 0.05$).

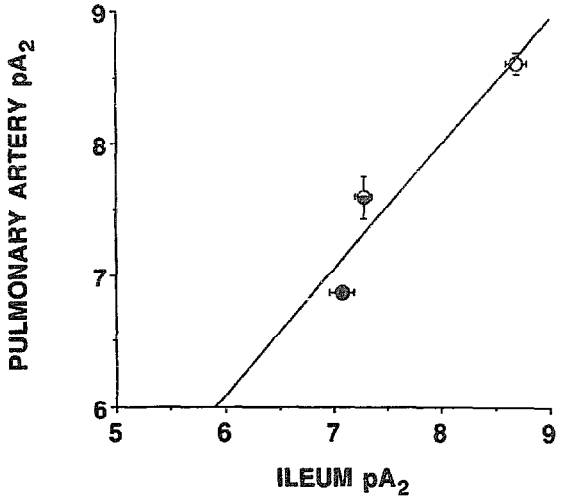

Fig. 3. Regression analysis of histamine H_3 receptor antagonist pA_2 values obtained in guinea pig pulmonary artery vs. pA_2 values obtained in guinea pig ileum shows a strong correlation ($r = 0.95$). The symbols represent pulmonary artery pA_2 vs. ileum pA_2 values for impromidine (●), burimamide (half-open circle) and thioperamide (○).

3.2. *Isolated guinea pig pulmonary artery: histamine H_3 receptor antagonist activity*

Electrical field stimulation-evoked tension in pulmonary artery rings was used to obtain pA_2 estimates for three histamine H_3 receptor antagonists at the histamine H_3 receptor modulating sympathetic function in this tissue (Fig. 2). (*R*)-α-Methylhistamine inhibition ($pD_2 = 7.1$) of electrical field stimulation-evoked tension (average tension = 0.58 ± 0.04 g) was inhibited by all three histamine H_3 receptor antagonists in the following rank order: thioperamide ($pA_2 = 8.6 \pm 0.1$) > burimamide ($pA_2 = 7.6 \pm 0.2$) > impromidine ($pA_2 = 6.9 \pm 0.02$).

3.3. *Isolated guinea pig ileum: histamine H_3 receptor antagonist activity*

Electrical field stimulation-evoked tension in guinea pig ileum was used to obtain pA_2 estimates for the histamine H_3 receptor antagonists at the histamine H_3 receptor modulating parasympathetic function. A rank order potency of thioperamide ($pA_2 = 8.7 \pm 0.1$) > burimamide ($pA_2 = 7.3 \pm 0.1$) ≥ impromidine ($pA_2 = 7.1 \pm 0.1$) vs. (*R*)-α-methylhistamine ($pD_2 = 8.2 \pm 0.1$) was obtained (data not shown).

3.4. *Correlations of histamine H_3 receptor antagonist pA_2 values and affinities*

Linear regression analysis of thioperamide, burimamide and impromidine histamine H_3 receptor pA_2 values obtained in guinea pig pulmonary artery vs. pA_2 values obtained in guinea pig ileum demonstrates a high correlation ($r = 0.95$, Fig. 3).

A graphic assessment of the fit of our histamine H_3 receptor antagonist data with reported histamine H_3 receptor antagonist affinities for the rat brain binding sites is found in Fig. 4. Regression analyses of pA_2 estimates from both guinea pig ileum and pulmonary artery vs. pK_i values (West et al., 1990) for the rat brain high (H_{3A}, Fig. 4A) and low (H_{3B}, Fig. 4B) affinity binding sites indicate a better fit with the proposed histamine H_{3A} receptor subtype.

4. Discussion

We have demonstrated electrical field stimulation-induced sympathetic contractions of isolated guinea pig

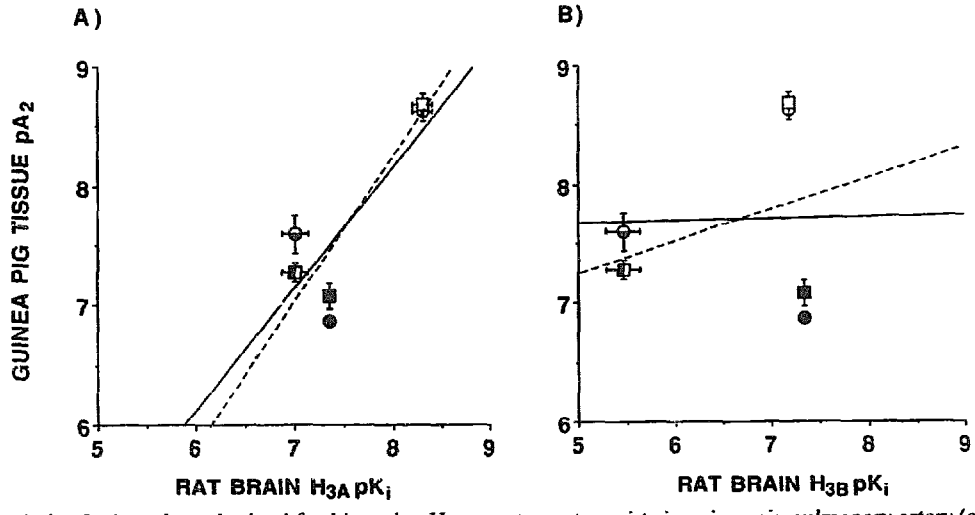

Fig. 4. Regression analysis of pA_2 values obtained for histamine H_3 receptor antagonists in guinea pig pulmonary artery (circles, solid regression lines) and ileum (squares, dashed regression lines) vs. their affinity for the (A) high (H_{3A}) and (B) low (H_{3B}) affinity rat brain binding sites. pA_2 values from guinea pig pulmonary artery and ileum show much higher correlations with rat brain H_{3A} pK_i values ($r = 0.78$ and 0.93 respectively) than with rat brain H_{3B} pK_i values ($r = 0$ and 0.33 respectively). Symbols represent the pA_2 vs. pK_i for impromidine (●, ■), burimamide (half-open circle, half-open square) and thioperamide (○, □). $pK_i = -\log K_i$ reported by West et al. (1990).

pulmonary artery (Wiklund et al., 1989) to be a useful preparation in which to characterize the inhibitory histamine H_3 receptor on postganglionic neurons. In [^{3}H]norepinephrine-loaded pulmonary artery, the histamine H_3 receptor selective agonist (*R*)-α-methylhistamine inhibits both electrical field stimulation-evoked tension and ^{3}H overflow in a thioperamide-sensitive manner, confirming the presence of prejunctional histamine H_3 receptors. Additionally, we have obtained affinity estimates (apparent pA_2) for the histamine H_3 receptor antagonists thioperamide, burimamide and impromidine in the sympathetic pulmonary artery preparation and in an established histamine H_3 receptor assay, electrical field stimulation-evoked parasympathetic cholinergic contractions of isolated guinea pig ileum (Poli et al., 1991; Trzeciakowski, 1987). The histamine H_3 receptor antagonists thioperamide and burimamide discriminate between high (H_{3A}, K_{iA} = 4.9 and 97 nM respectively) and low (H_{3B}, K_{iB} = 64 and 3500 nM respectively) affinity rat brain binding sites labeled with [^{3}H]N^{α}-methylhistamine, providing the basis for a hypothesis of histamine H_3 receptor heterogeneity (West et al., 1990). Our affinity estimates of 2.5 and 2.0 nM for thioperamide and 25 and 50 nM for burimamide, in guinea pig pulmonary artery and ileum respectively, define the predominant functional histamine H_3 receptors in these preparations as H_{3A}-like according to the classification proposed by West et al. (1990). Our impromidine affinity estimates are lower than expected (78–100 nM); however, this discrepancy has little effect on our conclusions, because impromidine does not discriminate between the rat brain histamine H_{3A} and H_{3B} binding sites (K_i = 45 nM, West et al., 1990).

Since the proposal by West et al. (1990) of histamine H_3 receptor heterogeneity, only two investigations of neuronal function in isolated tissues have specifically addressed identification of functional histamine H_3 receptor subtypes. Schlicker et al. characterized an inhibitory histamine H_{3A}-like receptor on isolated mouse brain cortex noradrenergic neurons (Schlicker et al., 1992) and suggested that this subtype modulates isolated guinea pig intestine longitudinal muscle parasympathetics, largely on the basis of a thioperamide pA_2 of 8.8 (Schlicker et al., 1994). Our isolated tissue studies confirm a histamine H_{3A}-like modulation of guinea pig ileum parasympathetics and this finding is supported by published affinities for thioperamide (pA_2 = 8.9–9.0) and burimamide (pA_2 = 7.1–7.5) in similar preparations (Hew et al., 1990; Vollinga et al., 1992). The present pulmonary artery findings add the histamine H_3 receptor modulating sympathetic neurons in guinea pig pulmonary artery to the growing list of functional histamine H_{3A}-like receptors. In retrospect, similar pA_2 estimates for thioperamide and/or burimamide reported in a variety of isolated tissue preparations, including rat cerebral cortex histamine release (Arrang et al., 1983, 1987), guinea pig ileum NANC neurons (Menkveld and Timmerman, 1990; Taylor and Kilpatrick, 1992), and dog saphenous vein sympathetics (Gupta and Roberts, 1994), suggest that functional histamine H_{3A}-like receptors are likely widespread. Although thioperamide and burimamide affinities intermediate to the K_{iA} and K_{iB} of West et al. (1990) have been reported in such isolated tissue preparations as rat cerebral cortex histamine synthesis (Arrang et al., 1987), guinea pig mesenteric artery sympathetics (Ishikawa and Sperelakis, 1987), and augmentation of voltage-dependent Ca^{2+} currents in rabbit saphenous artery (Oike et al., 1992), no definitive evidence of functional histamine H_{3B}-like receptors has been obtained in isolated tissue.

In conclusion, our work demonstrates the modulation of sympathetic neurons in isolated guinea pig pulmonary artery by histamine H_3 receptors similar to the histamine H_3 receptors modulating cholinergic parasympathetic neurons in guinea pig ileum. In addition, the characterization of these receptors expands the evidence from isolated tissue studies for functional histamine H_{3A}-like receptors. Confirmation of functional H_{3B}-like receptors is still lacking.

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