H3 receptor-mediated inhibition of intestinal acetylcholine release: pharmacological characterization of signal transduction pathways

Naunyn-Schmiedeberg's Arch Pharmacol (2001) 363 : 193–202
DOI 10.1007/s002100000351

ORIGINAL ARTICLE

**Corrado Blandizzi · Rocchina Colucci**
**Martina Tognetti · Barbara De Paolis · Mario Del Tacca**

# $H_3$ receptor-mediated inhibition of intestinal acetylcholine release: pharmacological characterization of signal transduction pathways

Received: 12 July 2000 / Accepted: 8 September 2000 / Published online: 18 November 2000


**Abstract** The present study investigates the mechanisms through which prejunctional histamine $H_3$ receptors modulate intestinal cholinergic neurotransmission. The experiments were performed on longitudinal muscle-myenteric plexus preparations of guinea pig ileum, preincubated with [$^3$H]choline, superfused with physiological salt solution containing hemicholinium-3, and subjected to electrical field stimulation. The stimulation-induced outflow of radioactivity was taken as an index of endogenous acetylcholine release.

The electrically induced [$^3$H]acetylcholine release was inhibited by histamine ($EC_{50}$=33.5 nM) or the $H_3$ receptor agonist *R*-α-methylhistamine ($EC_{50}$=41.6 nM), whereas it was not affected by pyridylethylamine ($H_1$ agonist), impromidine ($H_2$ agonist), pyrilamine ($H_1$ antagonist), cimetidine ($H_2$ antagonist), thioperamide or clobenpropit ($H_3$ antagonists). The inhibitory effects of histamine or *R*-α-methylhistamine were antagonized by thioperamide (p$K_d$= 8.31 and 8.53, respectively) or clobenpropit (p$K_d$=9.44 and 9.32, respectively), but not by pyrilamine or cimetidine. The modulatory action of histamine on the evoked tritium outflow was attenuated by pertussis toxin and abolished by *N*-ethylmaleimide, two selective blockers of $G_i/G_o$ proteins. Tetraethylammonium or 4-aminopyridine, acting as inhibitors of voltage-dependent $K^+$ channels, enhanced the evoked tritium outflow when tested alone, and apparently counteracted the inhibitory effect of histamine. However, the blocking actions of tetraethylammonium and 4-aminopyridine were no longer evident when their enhancing actions were compensated by appropriate reductions of $Ca^{2+}$ concentration in the superfusion medium. Histamine-induced inhibition of evoked tritium output was enhanced by ω-conotoxin, a selective blocker of N-type $Ca^{2+}$ channels, or low $Ca^{2+}$ concentration, whereas it was not modified by nifedipine, an antagonist of L-type $Ca^{2+}$ channels. In addition, the inhibitory effect of histamine was not significantly affected by forskolin (activator of adenylyl cyclase), 8-bromo-cyclic AMP (a stable analog of cyclic AMP), rolipram (a selective blocker of type IV phosphodiesterase), phorbol myristate acetate (activator of protein kinase C), H-89 (*N*-(2-[*p*-bromocinnamylamino]ethyl)-5-isoquinolinesulfonamide, inhibitor of protein kinase A), Ro-31-8220 (2-{1-[3-(amidinothio)propyl]-1*H*-indol-3-yl}-3-(1-methylindol-3-yl)-maleimide, inhibitor of protein kinase C), KT5823 (*N*-methyl-(8*R**,9*S**,11*S**)-(-)-9-methoxy-9-methoxycarbonyl-8-methyl-2,3,9,10-tetrahydro-8,11-epoxy-1*H*,8*H*,11*H*-2,7b,11a-triazadibenzo[a,g]cycloocta[c,d,e]-trinden-1-one, inhibitor of protein kinase G), or lavendustin A (inhibitor of tyrosine kinase). The present results indicate that histamine inhibits intestinal cholinergic neurotransmission through presynaptic $H_3$ receptors coupled to $G_i/G_o$ proteins. It is suggested that adenylyl cyclase, serine-threonine protein kinase and tyrosine kinase pathways are not implicated in this regulatory action, and that $G_i/G_o$ proteins modulate the activity of N-type $Ca^{2+}$ channels through a direct link, thus causing a reduced availability of extracellular $Ca^{2+}$ at the level of ileal cholinergic nerve terminals.

**Keywords** Histamine $H_3$ receptors · Acetylcholine release · Enteric nervous system · Ileum · G proteins · $Ca^{2+}$ channels · $K^+$ channels

C. Blandizzi · R. Colucci · M. Tognetti · B. De Paolis
M. Del Tacca (✉)
Division of Pharmacology and Chemotherapy,
Department of Oncology,
Transplants and Advanced Technologies in Medicine,
University of Pisa, Via Roma 55, 56126 Pisa, Italy
e-mail: m.deltacca@do.med.unipi.it,
Fax: +39-050-562020

## Introduction

Histamine is an important endogenous mediator which regulates a variety of digestive pathophysiological events, including intestinal peptic ulcer disease (Pattichis and Louca 1995), motility (Leurs et al. 1991), intestinal inflammatory responses and allergic reactions (Raithel et al. 1995). All these functions are ascribed to specific interactions of histamine with three distinct receptor subtypes, named $H_1$, $H_2$ and $H_3$ (Hill et al. 1997).

$H_3$ receptors can be distinguished from $H_1$ and $H_2$ receptors by virtue of their high affinity for selective agonists and antagonists, such as *R*-α-methylhistamine, imetit, thioperamide and clobenpropit (Hill et al. 1997). The recent molecular cloning and structural analysis of cDNA, encoding the human $H_3$ receptor, have lent considerable support to its pharmacological classification (Lovenberg et al. 1999). Indeed, upon transfection with $H_3$ receptor cDNA, C6 glioma cells could competitively bind several $H_3$ ligands, whereas they showed no affinity for known $H_1$ and $H_2$ antagonists (Lovenberg et al. 1999).

$H_3$ receptors may act as presynaptic heteroreceptors, both in the central and peripheral nervous system, where they regulate the release of various neurotransmitters (Hill et al. 1997). In particular, prejunctional $H_3$ receptors involved in the negative modulation of cholinergic neurotransmission have been identified in mammalian brain, airways and digestive system (Trzeciakowski 1987; Ichinose et al. 1989; Clapham and Kilpatrick 1992). In the gastrointestinal tract, after initial observations suggesting a role for prejunctional histamine receptors in the modulation of cholinergic neurotransmission (Trzeciakowski 1987), evidence was provided that histamine $H_3$ receptors are implicated in the regulation of several digestive functions (Bertaccini and Coruzzi 1995), including gastric acid secretion (Soldani et al. 1993) and the contractile activity of small and large intestine (Hew et al. 1990; Leurs et al. 1991). Most of these effects have been ascribed to a negative control exerted by presynaptic $H_3$ receptors on the release of acetylcholine and non-adrenergic non-cholinergic mediators from nerve endings of myenteric plexus (Poli et al. 1991; Taylor and Kilpatrick 1992).

In previous studies, the pharmacological profile of intestinal $H_3$ receptors has been characterized by indirect functional techniques, based on the effects of histamine receptor ligands on the contractile activity of smooth muscle elicited by electrical stimulation of myenteric neurons (Menkveld and Timmerman 1990; Bertaccini and Coruzzi 1995). Nevertheless, several aspects regarding the mechanisms through which the histaminergic pathways regulate the release of acetylcholine in the intestine remain to be elucidated. For instance, the electrically induced [$^3$H]acetylcholine release was enhanced by the $H_3$ receptor antagonist thioperamide, suggesting the existence of a tonic inhibitory control by endogenous histamine on intestinal cholinergic transmission (Poli et al. 1991). However, this observation was not confirmed in functional experiments dealing with the assessment of electrically induced cholinergic motor responses (Schlicker et al. 1994a).

Little is currently known about the intracellular signal transduction pathways coupled to $H_3$ receptors at either presynaptic or postsynaptic locations (Bertaccini and Coruzzi 1995; Hill et al. 1997). Previous studies suggested that $H_3$ receptor activation reduces the entry of $Ca^{2+}$ into cholinergic nerve endings of guinea pig duodenum (Poli et al. 1994), but the molecular mechanisms through which $H_3$ receptors interact with intracellular effector systems remain to be clarified. In addition, experiments performed on cell lines transfected with human or rat $H_3$ receptor cDNA indicated a negative coupling of these receptors with adenylyl cyclase in both species (Lovenberg et al. 2000). However, studies dealing with histamine-mediated modulation of noradrenergic neurotransmission failed to demonstrate an involvement of cyclic AMP in the signaling pathway of prejunctional $H_3$ receptors at both central and peripheral level (Schlicker et al. 1994b; Celuch 1995; Blandizzi et al. 2000).

The present study was designed to pursue a dual purpose: (1) to examine the mechanisms through which prejunctional histamine receptors modulate the release of acetylcholine from intestinal cholinergic nerves; (2) to gain further insight into the pharmacological profile of histamine receptors involved in the regulation of intestinal cholinergic neurotransmission. In order to assess the effects of drugs directly on cholinergic nerve endings, longitudinal muscle-myenteric plexus preparations of guinea pig ileum were incubated with [$^3$H]choline, and the outflow of radioactivity elicited by electrical field stimulation was used as an index of endogenous acetylcholine release.

## Materials and methods

*Animals.* Male albino guinea pigs (Harlan Italy, Udine, Italy), 300–350 g body weight, were used throughout this study. The animals were fed standard laboratory chow and tap water ad libitum and were not used for at least 1 week after their delivery to the laboratory. They were housed, four in a cage, in temperature-controlled rooms on a 12-h light cycle at 22°–24°C and 50%–60% humidity. Their care and handling were in accordance with the provisions of the European Community Council Directive 86-609, recognized and adopted by the Italian Government.

*Preparations.* At the time of the experiment, the whole ileum was excised from the small intestine with the exception of the distal 10 cm, and longitudinal muscle strips with myenteric plexus attached were prepared as previously reported (Colucci et al. 1998).

*Measurement of [$^3$H]acetylcholine release.* The measurement of [$^3$H]acetylcholine release from isolated guinea pig ileum was carried out according to the procedure previously described (Colucci et al. 1998), with minor modifications. Longitudinal muscle strips of ileum, 3–4 cm long, were incubated for 30 min in Krebs solution at 37°C, aerated with 95% $O_2$ + 5% $CO_2$ (preincubation period), and then loaded with methyl-[$^3$H]choline (3 μCi/ml) for 45 min in 3 ml of Krebs solution. The Krebs solution had the following composition (mM): NaCl 113, KCl 4.7, $CaCl_2$ 2.5, $KH_2PO_4$ 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11.5 (pH 7.4±0.1). Electrical pulses (0.1 ms duration) were applied for 30 min at a frequency of 1 Hz during the incubation period. At the end of the loading period, the ileal strips were washed five times with Krebs solution, transferred to another organ bath (5-ml capacity), and superfused at a flow rate of 1 ml/min with Krebs solution at 37°C, aerated with 95% $O_2$ + 5% $CO_2$. The superfusing Krebs solution contained 3 μM hemicholinium-3 in order to inhibit the reuptake of [$^3$H]choline generated by the hydrolysis of released [$^3$H]acetylcholine. The first 60-min collection of effluent was discarded (preperfusion), after which 3-min fractions were collected for 90 min. During the superfusion period, the ileal preparations were subjected to electrical field stimulation, delivered as square wave pulses (10 V/cm voltage drop between the electrodes of each organ bath) of 1 ms duration at 1 Hz (180 pulses) in the 3rd ($S_1$) and 20th ($S_2$) collection periods. At the end of superfusion, the radioactivity of fractions was determined by liquid scintillation counting (Betamatic; Kontron Instruments, Milan, Italy), and the radioactive con-

tent of ileal strips was also measured. Each preparation was weighed, incubated in 1 ml of 10% trichloroacetic acid at room temperature for 30 min, homogenized, and then centrifuged (1000 *g*) at room temperature for 10 min. An aliquot of the supernatant (50 µl) was added to 5 ml of scintillator and the tritium content of the tissue was measured by liquid scintillation spectrometry.

*Experimental procedures.* In the first series of experiments, the mechanisms underlying the increase in tritium outflow, induced by electrical field stimulation, were examined. For this purpose, the ileal preparations were exposed to tetrodotoxin, ω-conotoxin (a selective blocker of N-type $Ca^{2+}$ channels; Dolphin 1995), or low $Ca^{2+}$ concentrations in the superfusion solution, from the 12th collection period onward.

In the second set of experiments the effects of histamine, histamine receptor agonists or histamine receptor antagonists on [$^3$H]acetylcholine release were determined. Histamine or histamine receptor agonists were added to the superfusing Krebs solution in the 12th collection period. Histamine receptor antagonists were also added to the superfusion solution in the 12th collection period when tested alone, but they were present from the beginning of superfusion in agonist-antagonist interaction experiments. Exposure to each drug continued until the end of the experiment.

The third group of experiments was designed to examine the effects of histamine on tritium efflux after blockade of regulatory G proteins. For this purpose, ileal preparations were exposed to pertussis toxin or *N*-ethylmaleimide, two pharmacological agents able to block $G_i/G_o$ proteins through the ADP-ribosylation or alkylation of their sulfhydryl groups, respectively (Asano and Ogasawara 1986; Dolphin 1995). Some ileal strips were incubated with pertussis toxin for 6 h and then washed repeatedly with fresh Krebs solution for at least 1 h before loading with methyl-[$^3$H]choline. In this series, ileal preparations, to be used as controls or to assay the effects of histamine alone on [$^3$H]acetylcholine release, were maintained in the absence of pertussis toxin for 6 h and were then subjected to repeated washings for at least 1 h before incubation with methyl-[$^3$H]choline. In separate experiments, the ileal tissues were incubated with *N*-ethylmaleimide for 45 min, either in the absence or in the presence of dithiothreitol, which prevents the alkylation of sulfhydryl groups. The preparations were then washed at least five times with fresh Krebs solution before incubation with methyl-[$^3$H]choline. In both cases, the ileal tissues were challenged with histamine from the 12th collection period onward.

In the fourth set of experiments, histamine was tested on ileal preparations superfused with Krebs solution containing tetraethylammonium or 4-aminopyridine in order to induce a blockade of voltage-dependent $K^+$ channels (Pongs 1992). Tetraethylammonium or 4-aminopyridine were present in the solution, either alone or in combination with reduced concentration of $Ca^{2+}$, from the 9th collection period onward, whereas histamine was added to the superfusion solution from the 12th collection period onward. In all these experiments, after 60-min preperfusion, 3-min fractions of effluent were collected for 120 min and $S_2$ was delivered to the ileal tissues in the 30th collection period, since simultaneous reduction of the $Ca^{2+}$ concentration in the superfusing solution and addition of tetraethylammonium or 4-aminopyridine caused an increase in basal tritium efflux if the interval elapsing until application of $S_2$ was not long enough.

The fifth group of experiments was designed to assess whether extracellular $Ca^{2+}$ plays a role in the effects exerted by histamine on [$^3$H]acetylcholine release. For this purpose, ileal preparations were superfused, from the 9th collection period onward, with Krebs solution containing ω-conotoxin, nifedipine (an antagonist of L-type $Ca^{2+}$ channels; Dolphin 1995), or decreasing $Ca^{2+}$ concentrations. Histamine was present in the superfusion solution from the 12th collection period onward.

In the sixth series, we evaluated the possibility that adenylyl cyclase, serine/threonine protein kinase or tyrosine kinase pathways were involved in the effects of histamine on acetylcholine release. Groups of ileal preparations were exposed, in separate experiments, to the following drugs: forskolin, an activator of adenylyl cyclase (Daly et al. 1982), 8-bromo-cyclic AMP (8-Br-cAMP), a stable analog of cyclic AMP (Chen et al. 1998), rolipram, a selective blocker of type IV phosphodiesterase (Müller et al. 1996), phorbol myristate acetate (PMA), an activator of protein kinase C (Swartz 1993), H-89, an inhibitor of protein kinase A (Hidaka and Kobayashi 1992), Ro-31-8220, an inhibitor of protein kinase C (Beltman et al. 1996), KT5823, an inhibitor of protein kinase G (Kase et al. 1987), and lavendustin A, a blocker of tyrosine kinase (O'Dell et al. 1991). Each drug was added to the solution at the beginning of superfusion and remained in the superfusate until the end of experiment. Histamine was present in the superfusion solution from the 12th collection period onward.

*Calculations.* The amount of radioactivity released during superfusion was dependent upon the amount of radioactivity accumulated by the strips. In order to correct for this, the quantity of released radioactivity had to be normalized. This was achieved by dividing radioactivity in the first fraction by the total radioactivity present in the ileal strip at the start of the collection period, namely, by the sum of the radioactivity found in all fractions plus that remaining in the strip at the end of the experiment. A similar procedure was used for determination of tritium efflux in the subsequent fractions. In these determinations, however, the denominator was reduced by the amounts released into the prior fractions. Thus, the outflow of tritium was calculated as fraction of the tritium content of the ileal strip at the onset of the respective collection period (fractional rate; $min^{-1}$). The increase in tritium outflow evoked by electrical stimulation was calculated as the difference of 'total tritium outflow during the four collection periods subsequent to the onset of stimulation' minus 'estimated basal outflow.' The basal outflow was estimated as mean value of the tritium efflux determined during four collection periods: two immediately before the onset of electrical stimulation and two subsequent to the 12-min period in which the tritium outflow was increased by electrical stimulation. The evoked outflow was then expressed as a percentage of the tritium content of the tissue at the onset of electrical stimulation (Colucci et al. 1998). When test drugs were added to the superfusion solution between $S_1$ and $S_2$, their effects on the evoked tritium outflow were expressed as a ratio of the percentage release during the second ($S_2$) and the first ($S_1$) stimulation ($S_2/S_1$), namely, in the presence and absence of drug. For quantification of the effects of pertussis toxin, *N*-ethylmaleimide, dithiothreitol, forskolin, 8-Br-cAMP, rolipram, PMA, H-89, Ro-31-8220, KT5823 and lavendustin A, the $S_1$ value was used.

Potencies of histamine and *R*-α-methylhistamine were expressed as $EC_{50}$ (concentration of the agonist that produces 50% of the maximal response for the agonist); the percent maximum inhibition of tritium outflow, evoked by electrical stimulation in control experiments ($E_{max}$), was also evaluated. Antagonist potencies were expressed as p$K_d$ values from the equation:

$$K_d=[B]/(DR-1) \quad (1)$$

where *B* is the molar concentration of the antagonist and *DR* is the ratio of equally effective concentrations of the agonist ($EC_{50}$) in the presence and absence of the antagonist (Furchgott 1972).

*Drugs.* The following drugs were used: methyl-[$^3$H]choline chloride (specific activity: 75 Ci/mmol; Amersham Laboratories, Des Plaines, Ill., USA); tetrodotoxin, hemicholinium-3 bromide, histamine dihydrochloride, pyrilamine maleate, *N*-ethylmaleimide, (-)-dithiothreitol, tetraethylammonium chloride, 4-aminopyridine, ω-conotoxin GVIA, nifedipine, pertussis toxin, forskolin, 8-bromocyclic AMP, rolipram, Ro-31-8220 (2-{1-[3-(amidinothio)propyl]-1*H*-indol-3-yl}-3-(1-methylindol-3-yl)-maleimide methanesulfonate), H-89 (*N*-(2-[*p*-bromocinnamylamino]ethyl)-5-isoquinolinesulfonamide hydrochloride) (Sigma Chemicals, St. Louis, Mo., USA); *R*-(-)-α-methylhistamine, phorbol 12-myristate 13-acetate (Research Biomedicals International, Natick, Mass., USA); thioperamide maleate (Tocris Cookson, Bristol, UK); cimetidine (Italfarmaco, Milan, Italy); KT5823 (*N*-methyl-(8*R**,9*S**,11*S**)-(-)-9-methoxy-9-methoxycarbonyl-8-methyl-2,3,9,10-tetrahydro-8,11-epoxy-1*H*,8*H*,11*H*-2,7b,11a-triazadibenzo[a,g]cycloocta[c,d,e]-trinden-1-one;

Biomol Research Laboratories, Plymouth Meeting, Pa., USA); clobenpropit dihydrobromide (synthesized by Professor H. Timmerman, Vrije Universiteit, Amsterdam, The Netherlands); 2-pyridylethylamine, impromidine trihydrochloride (kindly provided by Smith Kline Beecham, King of Prussia, Pa., USA); lavendustin A (Alexis Biochemicals, Läufelfingen, Switzerland). Other reagents were of analytical grade. Pertussis toxin was dissolved in 0.1 M sodium phosphate buffer (pH 7) with 0.5 M sodium chloride. Thioperamide maleate, forskolin and lavendustin A were dissolved in dimethylsulfoxide and further dilutions were made with distilled water; the final concentration of dimethylsulfoxide in the superfusion solution (0.1%) had no effects on the basal or evoked tritium outflow.

*Statistical analysis.* Results are given as means ± standard error of the mean (SEM). The significance of differences was evaluated by Student's *t*-test. When more than one group was compared with a control, significance was assessed by one-way analysis of variance followed by Dunnett or Student-Newman-Keuls test. *P*-values lower than 0.05 were considered significant; *n* indicates the number of experiments. The ileal preparations included in each test group were obtained from distinct animals, and therefore, in the present study, *n* refers also to the number of animals used per experimental group. $EC_{50}$ values were interpolated from concentration-response curves. All statistical procedures and curve fitting were performed by means of personal computer programs (GraphPad Prism, software package version 2.01 for Windows 95, and InStat, software package version 2.01 for MacIntosh; both from GraphPad Software, San Diego, Calif., USA).

## Results

### Effects of tetrodotoxin, ω-conotoxin or low $Ca^{2+}$ concentrations in the superfusion solution

In control experiments (*n*=12), after a 60-min initial preperfusion period, the spontaneous tritium overflow approached a rate of 0.0020±0.00014 $min^{-1}$ and did not vary significantly throughout the experiments. When the superfused ileum strips were subjected to electrical field stimulation, the tritium efflux increased significantly from 0.0019±0.00012 $min^{-1}$ to 0.0057±0.00025 $min^{-1}$ ($P<0.05$). The increase in [$^3$H]acetylcholine release evoked by electrical stimulation was observed usually in four consecutive 3-min fractions; the release reached a peak during this time and then declined to the prestimulation value. The evoked tritium efflux was 2.94±0.27% for $S_1$ and 2.79±0.23% for $S_2$, not significantly different from each other; the calculated ratio $S_2/S_1$ was 0.95±0.04. Under these conditions, the tritium outflow, induced by electrical stimulation, was significantly decreased by tetrodotoxin 1 μM ($S_2/S_1$=0.05±0.03, $P<0.05$) or ω-conotoxin 0.01 μM ($S_2/S_1$=0.29±0.04, $P<0.05$) as well as by exposure of ileal preparations to low $Ca^{2+}$ concentrations in the superfusion solution (1.5 mM: $S_2/S_1$=0.68±0.05, $P<0.05$; 0.8 mM: $S_2/S_1$=0.21±0.03, $P<0.05$). Therefore, it was assumed that, following the application of electrical stimuli, radiolabelled acetylcholine undergoes an exocytotic release operated by extracellular $Ca^{2+}$ influx into cholinergic axon terminals through voltage-dependent N-type $Ca^{2+}$ channels. Neither the pharmacological agents nor lowering of $Ca^{2+}$ concentration modified the resting overflow of tritium (not shown).

**Table 1** Effects of histamine, histamine receptor agonists and histamine receptor antagonists on electrically evoked [$^3$H]acetylcholine release. Longitudinal muscle strips of guinea pig ileum were preincubated with [$^3$H]choline, superfused with Krebs solution and subjected twice ($S_1$ and $S_2$) to electrical field stimulation. The effects of test drugs on tritium outflow, evoked by electrical stimulation, are expressed as $S_2/S_1$ values (ratio of the percentage release during the second stimulation over that obtained during the first stimulation). Each value represents the mean ± SEM of 5–12 experiments

| | Electrically evoked tritium overflow ($S_2/S_1$) |
|---|---|
| Control | 0.95±0.04 |
| Histamine (1 μM) | 0.42±0.03* |
| Pyridylethylamine (1 μM) | 0.99±0.05 |
| Impromidine (1 μM) | 0.97±0.06 |
| *R*-α-methylhistamine (1 μM) | 0.48±0.04* |
| Pyrilamine (1 μM) | 0.96±0.08 |
| Cimetidine (10 μM) | 1.04±0.05 |
| Thioperamide (1 μM) | 0.99±0.06 |
| Clobenpropit (1 μM) | 1.03±0.06 |

*$P<0.05$: significant difference from control value

### Effects of histamine, histamine receptor agonists and histamine receptor antagonists

Histamine, applied at the concentration of 1 μM, inhibited the evoked tritium outflow by 55.8% (Table 1). The inhibitory effect of histamine was mimicked by *R*-α-methylhistamine ($H_3$ receptor agonist), but not by pyridylethylamine ($H_1$ receptor agonist) or impromidine ($H_2$ receptor agonist). Under the same experimental conditions, the histamine receptor antagonists pyrilamine ($H_1$), cimetidine ($H_2$), thioperamide ($H_3$) or clobenpropit ($H_3$) did not significantly modify the electrically induced tritium output (Table 1).

When tested at increasing concentrations (from 0.0001 μM to 100 μM), both histamine and *R*-α-methylhistamine yielded inhibitory concentration-response curves, with $EC_{50}$ values of 33.5 nM and 41.6 nM, and $E_{max}$ values of 59.7% and 54.8%, respectively. The concentration-response curves obtained with histamine or *R*-α-methylhistamine were shifted to the right in the presence of thioperamide (1 μM) or clobenpropit (0.01 μM; Fig. 1). The following $pK_d$ values were estimated for the antagonism of thioperamide and clobenpropit against histamine or *R*-α-methylhistamine: 8.31 (thioperamide vs. histamine); 8.53 (thioperamide vs. *R*-α-methylhistamine); 9.44 (clobenpropit vs. histamine); 9.32 (clobenpropit vs. *R*-α-methylhistamine). By contrast, pyrilamine (1 μM) or cimetidine (10 μM) did not modify the inhibitory effects of histamine or *R*-α-methylhistamine on the electrically induced tritium outflow (not shown). Histamine, as well as all the histamine receptor agonists and antagonists tested, did not affect the resting tritium outflow (not shown).

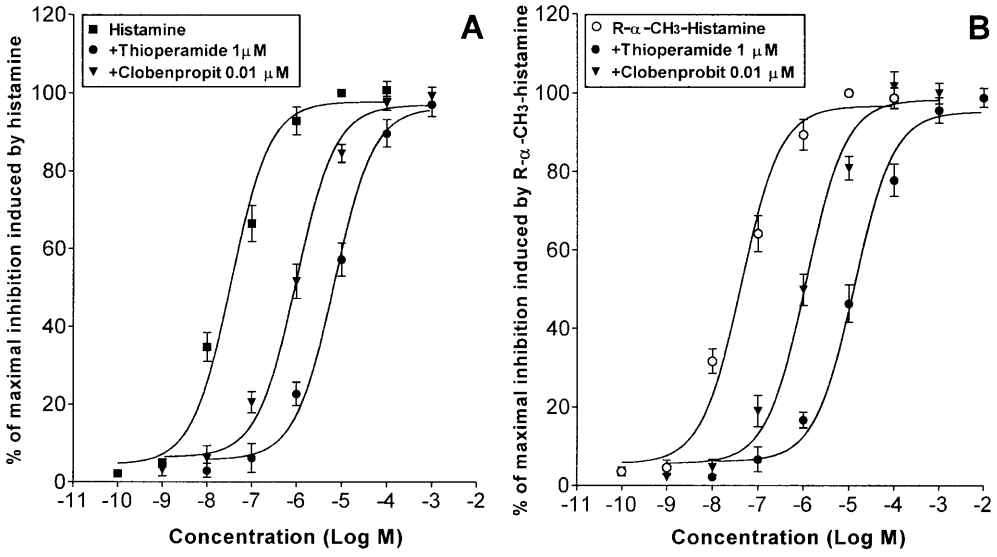


**Fig. 1** Effects of increasing concentrations of **A** histamine or **B** *R*-α-methyl-histamine, in the absence and in the presence of thioperamide (1 μM) or clobenpropit (0.01 μM), on tritium outflow evoked by electrical field stimulation of guinea pig ileum longitudinal muscle strips preincubated with [$^3$H]choline. Each point represents the mean of 4–6 experiments ± SEM (*vertical bars*)

## Effects of histamine on longitudinal muscle strips of ileum pretreated with pertussis toxin or *N*-ethylmaleimide

In control experiments, where ileal strips were incubated for 6 h in the absence of pertussis toxin, the electrically induced tritium efflux ($S_1$=3.14±0.31%; $n$=8) was not significantly different from that measured under standard conditions ($S_1$=2.94±0.27%; $n$=12). In addition, tritium outflow evoked by $S_1$ was not significantly affected by pretreatment of ileal preparations with pertussis toxin (3 μg/ml), *N*-ethylmaleimide (30 μM), dithiothreitol (100 μM) or dithiothreitol plus *N*-ethylmaleimide ($S_1$=3.42±0.48%, 3.49±0.55%, 2.89±0.36% and 3.17±0.51%, respectively; $n$=6 for each treatment), and in all cases $S_2/S_1$ values did not differ significantly from those estimated in control experiments, indicating that the viability of ileal tissues and their responsiveness to electrical stimulation were not affected by exposure to the above drugs.

The inhibitory effect of histamine (1 μM) on tritium overflow, elicited by electrical stimulation, was attenuated, but not abolished, by pretreatment with pertussis toxin (Fig. 2), and it was completely prevented by *N*-ethylmaleimide (Fig. 3). Dithiothreitol did not interfere with the inhibitory action of histamine on the evoked [$^3$H]acetyl-

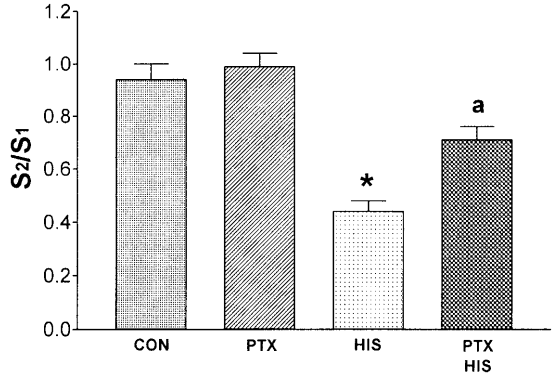


**Fig. 2** Effect of pertussis toxin (*PTX*, 3 μg/ml) on the inhibitory action exerted by histamine (*HIS*, 1 μM) on the electrically evoked tritium outflow from longitudinal muscle strips of guinea pig ileum preincubated with [$^3$H]choline. Ileal preparations were exposed to pertussis toxin for 6 h and were then subjected to repeated washings for 1 h before incubation with [$^3$H]choline. Tissues used for control experiments or exposed to histamine alone were incubated for 6 h in the absence of pertussis toxin and underwent repeated washings for 1 h before loading with [$^3$H]choline. Histamine was added to the superfusion solution before $S_2$, from the 12th collection period onward. Each *column* represents the mean of 5–8 experiments ± SEM (*vertical bars*). **P*<0.05: significant difference from control values (*CON*); $^a$*P*<0.05: significant difference from histamine alone (*HIS*)

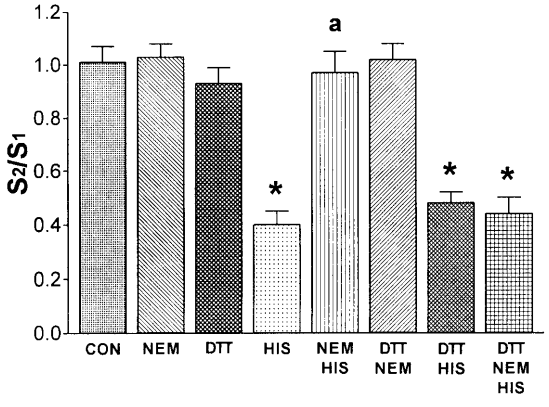


**Fig. 3** Effects of *N*-ethylmaleimide (*NEM*, 30 μM), either alone or in the presence of dithiothreitol (*DTT*, 100 μM), on the inhibitory action exerted by histamine (*HIS*, 1 μM) on the electrically evoked tritium outflow from longitudinal muscle strips of guinea pig ileum preincubated with [$^3$H]choline. Ileal preparations were exposed to *N*-ethylmaleimide and dithiothreitol for 45 min and 50 min, respectively, before incubation with [$^3$H]choline. Histamine was added to the superfusion solution before $S_2$, from the 12th collection period onward. Each *column* represents the mean of 5–7 experiments ± SEM (*vertical bars*). **P*<0.05: significant difference from control values (*CON*); $^a$*P*<0.05: significant difference from histamine alone (*HIS*)

choline release, but totally counteracted the blocking action exerted by *N*-ethylmaleimide against the inhibitory effect of histamine (Fig. 3). The spontaneous outflow of radioactivity was not modified by pertussis toxin or dithiothreitol, whereas it was slightly increased by *N*-ethylmaleimide (not shown).

### Effects of histamine in the presence of tetraethylammonium and 4-aminopyridine

Similar results were obtained in experiments designed to investigate the influence of tetraethylammonium or 4-aminopyridine on histamine-induced inhibition of [$^3$H]acetylcholine release. When tested alone, both tetraethylammonium (1 mM) and 4-aminopyridine (1 mM) significantly enhanced the evoked tritium outflow (+54.2% and +98.7%, respectively), these effects being counterbalanced by a reduction of $Ca^{2+}$ concentration in the superfusion solution (from 2.5 mM to 1.5 mM or 0.8 mM, respectively; Fig. 4). Since tetraethylammonium and 4-aminopyridine exerted excitatory effects on [$^3$H]acetylcholine release, the inhibitory action of histamine (1 μM) on the electrically induced tritium outflow was apparently counteracted by these $K^+$ channel blockers in experiments where the superfusion solution contained 2.5 mM $Ca^{2+}$ (Fig. 4). However, in the presence of tetraethylammonium or 4-aminopyridine, the inhibitory effect of histamine was again evident when the ileal preparations were exposed to low $Ca^{2+}$ concentration (1.5 mM or 0.8 mM, respectively) in order to counterbalance the increase in the evoked tritium efflux produced by these $K^+$ channel blockers (Fig. 4). The resting outflow was moderately increased by both tetraethylammonium and 4-aminopyridine (+9.2% and +17.8%, respectively).

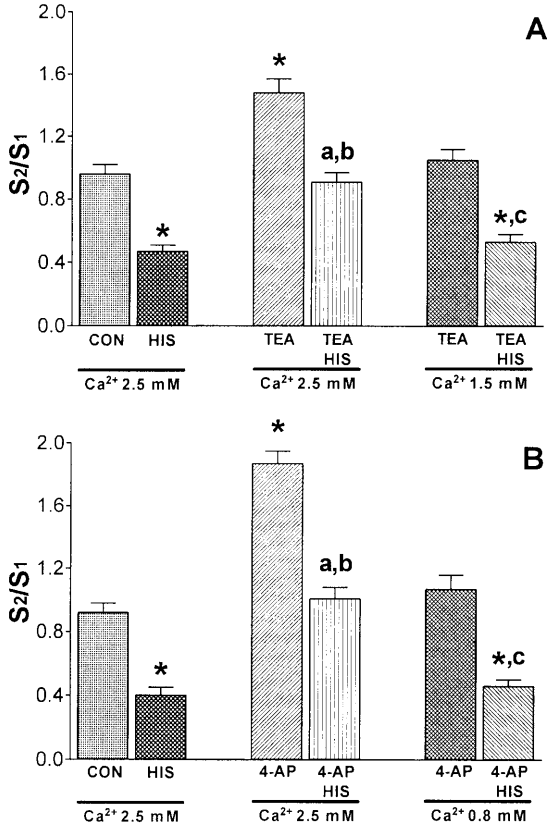


**Fig. 4** Effects of **A** tetraethylammonium (*TEA*, 1 mM), tested in the presence of 2.5 mM or 1.5 mM $Ca^{2+}$ in the superfusion solution, and **B** 4-aminopyridine (*4-AP*, 1 mM), tested in the presence of 2.5 mM or 0.8 mM $Ca^{2+}$ in the superfusion solution, on the inhibitory action exerted by histamine (*HIS*, 1 μM) on the electrically evoked tritium outflow from guinea pig ileum longitudinal muscle strips preincubated with [$^3$H]choline. Each *column* represents the mean of 5–8 experiments ± SEM (*vertical bars*). **P*<0.05: significant difference from control values (*CON*); $^a$*P*<0.05: significant difference from histamine alone (*HIS*); $^b$*P*<0.05: significant difference from TEA or 4-AP in the presence of 2.5 mM $Ca^{2+}$; $^c$*P*<0.05: significant difference from TEA or 4-AP in the presence of 1.5 mM or 0.8 mM $Ca^{2+}$, respectively

### Effects of histamine in the presence of ω-conotoxin, nifedipine or reduced $Ca^{2+}$ concentration in the superfusion solution

When tested at the concentration of 0.001 μM, ω-conotoxin reduced the increase in tritium efflux elicited by electrical stimulation (–26.6% vs. control) and significantly enhanced the histamine-induced inhibition of the evoked tritium output (histamine vs. control: –52.1%; histamine plus ω-conotoxin vs. ω-conotoxin alone: –82.6%; Fig. 5). By contrast, nifedipine (0.01 μM) had no effect on the evoked tritium outflow and did not interfere with the inhibitory action of histamine (Fig. 5). Similar results were obtained when nifedipine was assayed at 0.1 μM or 1 μM (not shown). Additional experiments showed that the extent of the inhibitory action, exerted by histamine on the electrically induced [$^3$H]acetylcholine release, increased as the $Ca^{2+}$ concentration in the superfusing Krebs solution was lowered from the 9th collection period onward.

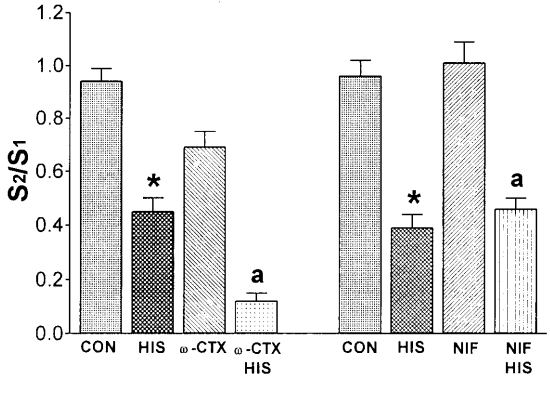


**Fig. 5** Effects of ω-conotoxin (ω-*CTX*, 0.001 μM) or nifedipine (*NIF*, 0.01 μM) on the inhibitory action exerted by histamine (*HIS*, 1 μM) on the electrically evoked tritium outflow from longitudinal muscle strips of guinea pig ileum preincubated with [$^3$H]choline. Each *column* represents the mean of 5–7 experiments ± SEM (*vertical bars*). **P*<0.05: significant difference from control values (*CON*); $^a$*P*<0.05: significant difference from ω-conotoxin or nifedipine alone

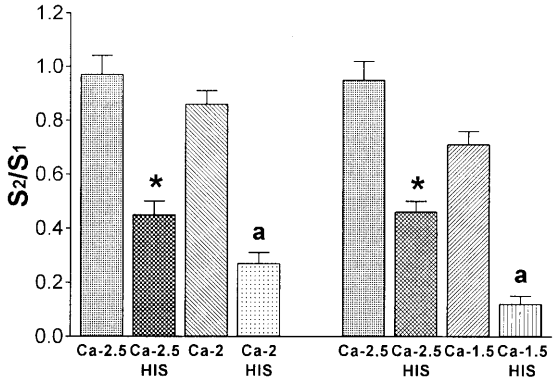


**Fig. 6** Effects of the reduction of $Ca^{2+}$ concentration in the superfusion solution from 2.5 mM to 2 mM (*Ca-2*) or 1.5 mM (*Ca-1.5*) on the inhibitory action exerted by histamine (*HIS*, 1 μM) on the electrically evoked tritium outflow from longitudinal muscle strips of guinea pig ileum preincubated with [$^3$H]choline. Each *column* represents the mean of 5–9 experiments ± SEM (*vertical bars*). **P*<0.05: significant difference from control values obtained with $Ca^{2+}$ at 2.5 mM (*Ca-2.5*); $^a$*P*<0.05: significant difference from control values obtained with $Ca^{2+}$ at 2 mM (*Ca-2*) or 1.5 mM (*Ca-1.5*)

In particular, the degree of histamine-induced inhibition of [$^3$H]acetylcholine release accounted for –68.6% and –83.1% when the ileal preparations were exposed to 2 mM and 1.5 mM $Ca^{2+}$, respectively (Fig. 6). ω-Conotoxin, nifedipine and reduction of $Ca^{2+}$ concentration in the superfusion solution did not significantly alter the resting tritium outflow (not shown).

### Effects of histamine in the presence of drugs acting on adenylyl cyclase, serine/threonine protein kinase and tyrosine kinase pathways

Tritium efflux induced by $S_1$ was not modified by exposure of ileal strips to rolipram (100 μM), PMA (1 μM), H-89 (10 μM), Ro-31-8220 (1 μM), KT5823 (1 μM) or lavendustin A (10 μM), whereas it was increased by 52.7% and 46.2% in the presence of forskolin (10 μM) and 8-Br-cAMP (1 mM), respectively (Table 2). In all cases $S_2/S_1$ values were not significantly different from those obtained in control experiments (Table 2). No relevant changes in the resting tritium output were detected in the presence of PMA, Ro-31-8220, KT5823 or lavendustin A (not shown). However, forskolin, 8-Br-cAMP or rolipram moderately enhanced (+21.6%, +16.3% and +11.4%, respectively) and H-89 reduced (–13.8%) the spontaneous tritium outflow. Under these conditions, none of the above drugs were able to significantly interfere with the inhibitory action exerted by histamine (1 μM) on the electrically evoked release of [$^3$H]acetylcholine (Table 2).

**Table 2** Effects of drugs active on cyclic AMP, serine/threonine protein kinase or tyrosine kinase pathways on the histamine-induced inhibition of electrically evoked [$^3$H]acetylcholine release. Longitudinal muscle strips of guinea pig ileum were preincubated with [$^3$H]choline, superfused with Krebs solution and subjected twice ($S_1$ and $S_2$) to electrical field stimulation. The effects of test drugs on tritium outflow, evoked by electrical stimulation, are expressed as $S_1$ (percentage of the tritium content of the tissue at the onset of the first electrical stimulation) or $S_2/S_1$ values (ratio of the percentage release during the second stimulation over that obtained during the first stimulation). Each value represents the mean ± SEM of 5–7 experiments

| | Electrically evoked tritium overflow | | |
|---|---|---|---|
| | $S_1$ | $S_2/S_1$ | |
| | | Histamine (μM) | |
| | | 0 | 1 |
| Control | 2.79±0.34 | 0.97±0.05 | 0.45±0.03** |
| Forskolin (10 μM) | 4.26±0.49* | 0.93±0.06 | 0.41±0.05** |
| 8-Br-cAMP (1 mM) | 4.08±0.38* | 0.95±0.08 | 0.43±0.04** |
| Rolipram (100 μM) | 2.93±0.22 | 1.04±0.07 | 0.46±0.05** |
| PMA (1 μM) | 3.12±0.38 | 0.98±0.05 | 0.42±0.04** |
| H-89 (10 μM) | 2.64±0.33 | 0.94±0.06 | 0.47±0.06** |
| Ro-31-8220 (1 μM) | 2.88±0.45 | 0.96±0.06 | 0.42±0.06** |
| KT5823 (1 μM) | 3.11±0.41 | 1.03±0.05 | 0.48±0.05** |
| Lavendustin A (10 μM) | 3.05±0.28 | 1.01±0.04 | 0.39±0.07** |

**P*<0.05: significant difference from control value
***P*<0.05: significant difference from $S_2/S_1$ values obtained in the absence of histamine

## Discussion

The present study provides evidence that histamine $H_3$ receptors, involved in the regulation of intestinal cholinergic neurotransmission, are coupled to $G_i/G_o$ proteins. Therefore, taking into account the mechanisms currently proposed for the regulation of neurotransmitter release by G proteins (Miller 1998), efforts were made to elucidate the possible role played by $K^+$ or $Ca^{2+}$ channels as well as by various signal transduction pathways in the modulation of acetylcholine release by $H_3$ receptors.

The pharmacological profile of histamine receptors characterized in the present study conforms to the criteria for classification of $H_3$ receptors (Hill et al. 1997): (1) the inhibitory effect of histamine on tritium outflow was mimicked by *R*-α-methylhistamine, but not by pyridylethylamine or impromidine; (2) the inhibitory actions of both histamine and *R*-α-methylhistamine were antagonized by thioperamide or clobenpropit, whereas they were insensitive to antagonists for $H_1$ or $H_2$ receptors. These findings agree with previous studies dealing with presynaptic modulation of cholinergic neurotransmission by $H_3$ receptors (Fuder and Muscholl 1995; Hill et al. 1997). For instance, the potencies calculated for thioperamide and clobenpropit are comparable with those found by other authors in functional assays on isolated intestinal preparations (Leurs et al. 1991; Schlicker et al. 1994a). In addition, our estimated potencies for thioperamide and clobenpropit are consistent with values obtained for the same drugs in [$^3$H]acetylcholine or [$^3$H]noradrenaline release assays from central nervous system tissues (Clapham and Kilpatrick 1992; Alves-Rodriguez et al. 1998).

It is remarkable that, in the present study, very similar $EC_{50}$ values were obtained for histamine (33.5 nM) and *R*-α-methylhistamine (41.6 nM), whereas in other reports *R*-α-methylhistamine was more potent than histamine (for review, see Leurs et al. 1995; Hill et al. 1997). At present, we do not have a clear explanation for these differences. However, in previous studies performed on guinea pig intestine, $EC_{50}$ values of approximately 40–770 nM and 5–25 nM have been estimated for histamine and *R*-α-methylhistamine, respectively (Trzeciakowski 1987; Menkveld and Timmerman 1990; Poli et al. 1991). On this basis, although the $EC_{50}$ values obtained in the present study do not fall exactly within the above ranges, it is conceivable that the discrepancies between our data and those reported by other authors might be due to experimental variability.

Binding experiments have suggested the existence of multiple $H_3$ receptor subtypes, termed $H_{3A}$ and $H_{3B}$, characterized by high and low affinity for thioperamide, respectively (West et al. 1990). Subsequent studies confirmed this subclassification and showed that other antagonists, including clobenpropit, are not able to differentiate between $H_{3A}$ and $H_{3B}$ receptor subtypes (Schlicker et al. 1994a). These studies also demonstrated that noradrenaline release in mouse brain cortex is modulated by $H_{3A}$ receptor subtypes. In spite of these findings, the heterogeneity of $H_3$ receptors is still a matter of debate (Hill et al. 1997) and no conclusive evidence of functional $H_{3B}$ receptors has been obtained. In the present study, the potencies of thioperamide and clobenpropit against histamine or *R*-α-methylhistamine would suggest that intestinal cholinergic neurotransmission is modulated by $H_3$ receptors resembling the $H_{3A}$ subtype.

In the present series of experiments, neither thioperamide nor clobenpropit affected the resting or electrically induced tritium outflow, indicating that endogenous histamine does not exert a tonic inhibitory effect on intestinal cholinergic neurotransmission. Conflicting data regarding an effect of endogenous histamine on cholinergic transmission have been previously reported. In agreement with our findings, Schlicker et al. (1994a) showed that thioperamide and clobenpropit did not alter the cholinergic twitch responses of guinea pig ileum. However, thioperamide enhanced the evoked [$^3$H]acetylcholine release from guinea pig ileum (Poli et al. 1991) or rat brain cortex (Clapham and Kilpatrick 1992). The discrepancy between our results and those reported by Poli et al. (1991) may be due to the different experimental conditions used in these studies. Indeed, the electrical stimulation applied to ileal preparations during incubation with [$^3$H]choline in our studies might deplete endogenous histamine stores, thus causing an underestimation of the enhancing action of thioperamide on [$^3$H]acetylcholine release.

Previous binding studies provided evidence that $H_3$ receptors belong to the superfamily of G protein-coupled receptors (West et al. 1990; Hill et al. 1997). In keeping with this view, the present results, showing that the inhibitory action of histamine on [$^3$H]acetylcholine release was attenuated by pertussis toxin and abolished by *N*-ethylmaleimide, suggest that the $H_3$ receptors, located on cholinergic nerve endings of guinea pig ileum, are coupled to G proteins belonging to $G_i/G_o$ subtypes. This finding is consistent with the results of previous studies where the involvement of $G_i/G_o$ proteins was demonstrated for the $H_3$ receptor-mediated modulation of noradrenergic neurotransmission (Nozaki and Sperelakis 1989; Endou et al. 1994; Schlicker et al. 1994b; Blandizzi et al. 2000).

In the case of presynaptic receptors linked to G proteins, three major mechanisms have been proposed for the modulation of neurotransmitter release: (1) activation of $K^+$ channels, resulting in a reduction of action potential efficacy; (2) inhibition of $Ca^{2+}$ channels, leading to an impairment of exocytotic machinery; (3) direct modulation of the vesicle release apparatus (Miller 1998).

As far as $K^+$ channels are concerned, in the present study care was taken to assay the effects of tetraethylammonium and 4-aminopyridine on the evoked tritium outflow, since it is known that the blockade of voltage-dependent $K^+$ channels prolongs the duration of action potential, thus causing an increment of $Ca^{2+}$ entry into axon terminals, with a consequent enhancement of neurotransmitter release (Starke et al. 1989). As expected, both tetraethylammonium and 4-aminopyridine enhanced the electrically induced [$^3$H]acetylcholine release, and this effect could be counterbalanced by an appropriate reduction of $Ca^{2+}$ concentration in the superfusion solution, confirming that the potentiating actions of these $K^+$ channel blockers are indirect in nature and depend on an increased $Ca^{2+}$ influx. These findings allowed a correct interpretation of data obtained from experiments where the inhibitory effect of histamine on [$^3$H]acetylcholine release was examined in the presence of $K^+$ channel blockade. Indeed, under these conditions, tetraethylammonium or 4-aminopyridine caused an apparent attenuation of the histamine-induced inhibitory effect, which was ascribed to an increment of intraneuronal $Ca^{2+}$ concentration induced by the prolongation of action potential. A consistent support to this view came from experiments in which the increment of tritium outflow, produced by tetraethylammonium or 4-aminopyridine, was counterbalanced by a decrease in $Ca^{2+}$ concentration in the superfusion solution and, as a consequence, the modulatory action of histamine on [$^3$H]acetylcholine release was again well evident. On this basis, since neither tetraethylammonium nor 4-aminopyridine inhibited the modulatory effect of histamine, a coupling of $H_3$ receptors to voltage-dependent $K^+$ channels appears to be unlikely.

In the present study, at least two lines of evidence support the view that an inhibition of $Ca^{2+}$ channels, with subsequent reduction of extracellular $Ca^{2+}$ influx into the cholinergic nerve endings, may account for the $H_3$ receptor-mediated inhibition of evoked [$^3$H]acetylcholine release from ileal preparations: (1) the inhibitory effect of histamine was markedly enhanced by ω-conotoxin, a selective blocker of N-type $Ca^{2+}$ channels, which play a pivotal role in the regulation of exocytotic neurotransmitter release (Dolphin 1995), whereas the blockade of L-type $Ca^{2+}$ channels by nifedipine was without consequences; (2) lowering of $Ca^{2+}$ concentration in the superfusion so-

lution significantly enhanced the inhibitory action of histamine. These findings, taken together with data reported from previous studies on duodenal motility (Poli et al. 1994), indicate that presynaptic $H_3$ receptors inhibit N-type $Ca^{2+}$ channels, causing a reduced availability of extracellular $Ca^{2+}$ ions for stimulus-release coupling at the level of intestinal cholinergic terminals.

In general, receptors coupled to $G_i/G_o$ proteins regulate neuronal $Ca^{2+}$ currents by interference with adenylyl cyclase, phospholipase C or tyrosine kinase pathways, as well as by direct interaction with voltage-dependent $Ca^{2+}$ channels (Dolphin 1995; Diverse Pierluissi et al. 1997). In previous studies, histamine decreased forskolin-stimulated cyclic AMP formation in cell lines transfected with human or rat $H_3$ receptor cDNA (Lovenberg et al. 1999, 2000), but other authors failed to observe an inhibition of adenylyl cyclase activity in studies on native $H_3$ receptors (Garbarg et al. 1989; Marley et al. 1991; Schlicker et al. 1994b; Celuch 1995). Cherifi et al. (1992) described a negative coupling of $H_3$ receptors to phospholipase C in human HGT-1 cells, but no evidence of protein kinase C involvement was obtained for $H_3$ receptors modulating glutamate release at a central level (Brown and Haas 1999). More recently, the molecular cloning of guinea pig $H_3$ receptor has led to the identification of two receptor isoforms, differing by a 30-amino-acid stretch within the third intracellular loop, the molecular domain that in G protein-dependent receptors is thought to be responsible for coupling to the signal transduction pathway (Tardivel-Lacombe et al. 2000).

The above findings prompted us to assay various pharmacological tools to explore the possibility that the activation of presynaptic $H_3$ receptors interferes with the activity of adenylyl cyclase (forskolin, 8-Br-cAMP, rolipram), serine-threonine protein kinases (PMA, H-89, Ro-31-8220, KT5823) or tyrosine kinase (lavendustin A). However, none of these drugs was able to modify the inhibitory effect of histamine on the evoked [$^3$H]acetylcholine release. Therefore, since the inhibition of $Ca^{2+}$ channels by $G_i/G_o$ proteins may involve a direct action of the $\beta\gamma$-subunits of the G protein on the $\beta$-subunit of voltage-dependent $Ca^{2+}$ channels (Dolphin 1998), it is conceivable that in enteric cholinergic neurons $G_i/G_o$ proteins, coupled to $H_3$ receptors, inhibit $Ca^{2+}$ influx into nerve endings through a direct interaction with N-type $Ca^{2+}$ channels. On these bases, the present study, as well as the results of previous reports (Marley et al. 1991; Cherifi et al. 1992; Schlicker et al. 1994b), argue against the possibility that $H_3$ receptors are coupled to adenylyl cyclase or phospholipase C pathways at the level of central or peripheral sites. It remains then to be established whether the results obtained with cloned $H_3$ receptors (Lovenberg et al. 1999, 2000) might be ascribed to transfection artifacts or rather reflect the existence of alternative transduction mechanisms for native $H_3$ receptors in different species and/or anatomical locations.

In conclusion, the present study identified the mechanisms through which the activation of presynaptic $H_3$ receptors, located on cholinergic nerve endings, caused an inhibitory modulation of intestinal cholinergic neurotransmission. According to our findings, it appears that these receptors are coupled to $G_i/G_o$ proteins which modulate the activity of N-type $Ca^{2+}$ channels through a direct link, thus causing a reduced availability of extracellular $Ca^{2+}$ at the level of ileal cholinergic nerve terminals.

## References


Alves-Rodriguez A, Timmerman H, Willems E, Lemstra S, Zuiderveld OP, Leurs R (1998) Pharmacological characterization of the histamine $H_3$ receptor in the rat hippocampus. Brain Res 788:179–186

Asano T, Ogasawara N (1986) Uncoupling of γ-aminobutyric acid B receptors from GTP-binding proteins by *N*-ethylmaleimide: effect of *N*-ethylmaleimide on purified GTP-binding proteins. Mol Pharmacol 29:244–249

Beltman J, McCormick F, Cook SJ (1996) The selective protein kinase C inhibitor, Ro-31-8220, inhibits mitogen-activated protein kinase phosphatase-1 (MKP0-1) expression, induces c-Jun expression, and activates Jun *N*-terminal kinase. J Biol Chem 271:27018–27024

Bertaccini G, Coruzzi G (1995) An update on histamine $H_3$ receptors and gastrointestinal functions. Dig Dis Sci 40:2052–2063

Blandizzi C, Tognetti M, Colucci R, Del Tacca M (2000) Histamine $H_3$ receptors mediate inhibition of noradrenaline release from intestinal sympathetic nerves. Br J Pharmacol 129:1387–1396

Brown RE, Haas HL (1999) On the mechanism of histaminergic inhibition of glutamate release in the rat dentate gyrus. J Physiol (Lond) 515.3:777–786

Celuch SM (1995) Possible participation of histamine $H_3$ receptors in the modulation of noradrenaline release from rat spinal cord slices. Eur J Pharmacol 287:127–133

Chen TC, Hinton DR, Zidovetzki R, Hofman FM (1998) Up-regulation of the cAMP/PKA pathway inhibits proliferation, induces differentiation, and leads to apoptosis in malignant gliomas. Lab Invest 78:165–174

Cherifi Y, Pigeon C, Le Romancer M, Bado A, Reyl-Desmars F, Lewin MJM (1992) Purification of a histamine $H_3$ receptor negatively coupled to phosphoinositide turnover in the human gastric cell line HGT1. J Biol Chem 267:25315–25320

Clapham J, Kilpatrick GJ (1992) Histamine $H_3$ receptors modulate the release of [$^3$H]acetylcholine from slices of rat entorhinal cortex: evidence for the possible existence of $H_3$ receptor subtypes. Br J Pharmacol 107:919–923

Colucci R, Blandizzi C, Carignani D, Placanica G, Lazzeri G, Del Tacca M (1998) Effects of imidazoline derivatives on cholinergic motility in guinea pig ileum: involvement of presynaptic $\alpha_2$-adrenoceptors or imidazoline receptors? Naunyn-Schmiedeberg's Arch Pharmacol 357:682–691

Daly JW, Padgett W, Seamon KB (1982) Activation of cyclic AMP-generating systems in brain membranes and slices by the diterpene forskolin: augmentation of receptor-mediated responses. J Neurochem 38:532–544

Diverse Pierluissi M, Remmers AE, Neubig RR, Dunlap K (1997) Novel form of crosstalk between G protein and tyrosine kinase pathways. Proc Natl Acad Sci USA 94:5417–5421

Dolphin AC (1995) Voltage-dependent calcium channels and their modulation by neurotransmitters and G proteins. Exp Physiol 80:1–36

Dolphin AC (1998) Mechanisms of modulation of voltage-dependent calcium channels by G proteins. J Physiol (Lond) 506: 3–11

Endou M, Poli E, Levi R (1994) Histamine $H_3$-receptor signaling in the heart: possible involvement of Gi/Go proteins and N-type $Ca^{2+}$ channels. J Pharmacol Exp Ther 269:221–229

Fuder H, Muscholl E (1995) Heteroreceptor-mediated modulation of noradrenaline and acetylcholine release from peripheral nerves. Rev Physiol Biochem Pharmacol 126:265–412

Furchgott RF (1972) The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint theory. In: Blascho H, Muscholl E (eds) Catecholamines, handbook of experimental pharmacology, vol 33. Springer, Berlin Heidelberg New York, pp 283–335

Garbarg M, Tuong MD, Gros C, Schwartz JC (1989) Effects of histamine $H_3$-receptor ligands on various biochemical indices of histaminergic neuron activity in rat brain. Eur J Pharmacol 164:1–11

Hew RWS, Hodgkinson CR, Hill SJ (1990) Characterization of histamine $H_3$-receptors in guinea pig ileum with $H_3$-selective ligands. Br J Pharmacol 101:621–624

Hidaka H, Kobayashi R (1992) Pharmacology of protein kinase inhibitors. Annu Rev Pharmacol Toxicol 32:377–397

Hill SJ, Ganellin CR, Timmerman H, Schwartz JC, Shankley NP, Young JM, Schunack W, Levi R, Haas HL (1997) International Union of Pharmacology. XIII. Classification of histamine receptors. Pharmacol Rev 49:253–278

Ichinose M, Stretton CD, Schwartz J-C, Barnes PJ (1989) Histamine $H_3$-receptors inhibit cholinergic neurotransmission in guinea-pig airways. Br J Pharmacol 97:13–15

Kase H, Iwahashi K, Nakanishi S, Matsuda Y, Yamada K, Takahashi M, Murakata C, Sato A, Kaneko M (1987) K-252 compounds, novel and potent inhibitors of protein kinase C and cyclic nucleotide-dependent protein kinases. Biochem Biophys Res Commun 142:436–440

Leurs R, Brozius MM, Smit MJ, Bast A, Timmerman H (1991) Effects of histamine $H_1$-, $H_2$- and $H_3$-receptor selective drugs on the mechanical activity of guinea pig small and large intestine. Br J Pharmacol 102:179–185

Leurs R, Vollinga RC, Timmerman H (1995) The medicinal chemistry and therapeutic potentials of ligands of the histamine $H_3$ receptor. Prog Drug Res 45:107–165

Lovenberg TW, Roland BL, Wilson SJ, Jiang X, Pyati J, Huvar A, Jackson MR, Erlander MG (1999) Cloning and functional expression of the human histamine $H_3$ receptor. Mol Pharmacol 55:1101–1107

Lovenberg TW, Pyati J, Chang H, Wilson SJ, Erlander MG (2000) Cloning of rat histamine $H_3$ receptor reveals distinct species pharmacological profiles. J Pharmacol Exp Ther 293:771–778

Marley PD, Thomson KA, Jachno K, Johnston MJ (1991) Histamine-induced increases in cyclic AMP levels in bovine adrenal medullary cells. Br J Pharmacol 104:839–846

Menkveld GJ, Timmerman H (1990) Inhibition of electrically evoked contractions of guinea pig ileum preparations mediated by the histamine $H_3$ receptor. Eur J Pharmacol 186:343–347

Miller RJ (1998) Presynaptic receptors. Annu Rev Pharmacol Toxicol 38:201–227

Müller T, Engels P, Fozard JR (1996) Subtypes of the type 4 cAMP phosphodiesterases: structure, regulation and selective inhibition. Trends Pharmacol Sci 17:294–298

Nozaki M, Sperelakis N (1989) Pertussis toxin effects on transmitter release from perivascular nerve terminals. Am J Physiol 256:H455–H459

O'Dell TJ, Kandel ER, Grant SG (1991) Long-term potentiation in the hippocampus is blocked by tyrosine kinase inhibitors. Nature 353:558–560

Pattichis K, Louca LL (1995) Histamine, histamine $H_2$-receptor antagonists, gastric acid secretion and ulcers: an overview. Drug Metabol Drug Interact 12:1–36

Poli E, Coruzzi G, Bertaccini G (1991) Histamine $H_3$ receptors regulate acetylcholine release from the guinea pig ileum myenteric plexus. Life Sci 48:63–68

Poli E, Pozzoli C, Coruzzi G, Bertaccini G (1994) Signal transducing mechanisms coupled to histamine $H_3$ receptors and alpha-2 adrenoceptors in the guinea pig duodenum: possible involvement of N-type $Ca^{2+}$ channels. J Pharmacol Exp Ther 270:788–794

Pongs O (1992) Structural basis of voltage-gated $K^+$ channel pharmacology. Trends Pharmacol Sci 13:359–365

Raithel M, Matek M, Baenkler HW, Jorde W, Hahn EG (1995) Mucosal histamine content and histamine secretion in Crohn's disease, ulcerative colitis and allergic enteropathy. Int Arch Allergy Immunol 108:127–133

Schlicker E, Kathmann M, Reidemeister S, Stark H, Schunack W (1994a) Novel histamine $H_3$ receptor antagonists: affinities in an $H_3$ receptor binding assay and potencies in two functional $H_3$ receptor models. Br J Pharmacol 112:1043–1048

Schlicker E, Kathmann M, Detzner M, Exner HJ, Göthert M (1994b) $H_3$ receptor-mediated inhibition of noradrenaline release: an investigation into the involvement of $Ca^{2+}$ and $K^+$ ions, G protein and adenylate cyclase. Naunyn-Schmiedeberg's Arch Pharmacol 350:34–41

Soldani G, Mengozzi G, Intorre L, De Giorgi G, Coruzzi G, Bertaccini G (1993) Histamine $H_3$ receptor-mediated inhibition of gastric acid secretion in conscious dogs. Naunyn-Schmiedeberg's Arch Pharmacol 347:61–65

Starke K, Göthert M, Kilbinger H (1989) Modulation of neurotransmitter release by presynaptic autoreceptors. Physiol Rev 69:864–989

Swartz KJ (1993) Modulation of $Ca^{2+}$ channels by protein kinase C in rat central and peripheral neurons: disruption of G protein-mediated inhibition. Neuron 11:305–320

Tardivel-Lacombe J, Rouleau A, Heron A, Morisset S, Pillot C, Cochois V, Schwartz JC, Arrang JM (2000) Cloning and cerebral expression of the guinea pig histamine H3 receptor: evidence for two isoforms. Neuroreport 11:755–759

Taylor SJ, Kilpatrick GJ (1992) Characterization of histamine-$H_3$ receptors controlling non-adrenergic non-cholinergic contractions of the guinea pig isolated ileum. Br J Pharmacol 105: 667–674

Trzeciakowski JP (1987) Inhibition of guinea pig ileum contractions mediated by a class of histamine receptor resembling the $H_3$ subtype. J Pharmacol Exp Ther 243:874–880

West RE Jr, Zweig A, Shih NY, Siegel MI, Egan RW, Clark MA (1990) Identification of two $H_3$ receptor subtypes. Mol Pharmacol 38:610–613