UC Irvine
UC Irvine Previously Published Works

Title
Expression of a cloned rat histamine H2 receptor mediating inhibition of arachidonate release and activation of cAMP accumulation.

Permalink
https://escholarship.org/uc/item/7rg0t7d2

Journal
Proceedings of the National Academy of Sciences of the United States of America, 89(7)

ISSN
0027-8424

Authors
Traiffort, E
Ruat, M
Arrang, JM
et al.

Publication Date
1992-04-01

DOI
10.1073/pnas.89.7.2649

Copyright Information
This work is made available under the terms of a Creative Commons Attribution License, availalbe at https://creativecommons.org/licenses/by/4.0/

Peer reviewed

eScholarship.org
Powered by the California Digital Library
University of California

Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 2649–2653, April 1992
Pharmacology

Expression of a cloned rat histamine H_2 receptor mediating inhibition of arachidonate release and activation of cAMP accumulation

(second messengers/phospholipase A_2/[^{125}I]iodoaminopotentidine/Chinese hamster ovary cells)

ELISABETH TRAIFFORT*, MARTIAL RUAT, JEAN-MICHEL ARRANG, ROB LEURS, DANIELE PIOMELLI, AND JEAN-CHARLES SCHWARTZ

Unité de Neurobiologie et Pharmacologie, U. 109, Institut National de la Santé et de la Recherche Médicale, Centre Paul Broca, 2 ter rue d'Alésia, 75014 Paris, France

Communicated by James Black, December 2, 1991

ABSTRACT **A DNA, cloned after screening a rat genomic bank with probes derived from the sequence of a putative dog histamine H_2 receptor [Gantz, I., Schäffer, M., Delvalle, J., Logsdon, C., Campbell, V., Uhler, M. & Yamada, T. (1991) *Proc. Natl. Acad. Sci. USA* 88, 429–433], was used to prepare a probe for Northern blot analysis and to transfect Chinese hamster ovary (CHO) cells. Distribution of the gene transcripts in guinea pig tissues was consistent with that of H_2 receptors. Transfected CHO cells expressed a high density of sites binding [^{125}I]iodoaminopotentidine, a selective H_2-receptor ligand. These sites were characterized as typical H_2 receptors by using a series of competing agents that displayed apparent dissociation constants closely similar to corresponding values at a reference biological system. In transfected cells, histamine stimulated, with high potency and large receptor reserve, the accumulation of cAMP. In addition, in the same cells, histamine potently inhibited the release of arachidonic acid induced either by stimulation of constitutive purinergic receptors or by application of a Ca^{2+} ionophore. This inhibition was independent of either cAMP or Ca^{2+} levels. The results suggest that a single H_2 receptor may be linked not only to adenylyl cyclase activation but also to reduction of phospholipase A_2 activity. Because H_1 receptors have been reported to stimulate arachidonic acid release, inhibition of this release, an unexpected signaling pathway for H_2 receptors, may account for the opposing physiological responses elicited in many tissues by stimulation of these two receptor subtypes.**

Histamine (HA), a ubiquitous cell-to-cell messenger, exerts its numerous actions in the nervous, endocrine, and immune systems through interaction with three pharmacologically distinct receptor subtypes, termed H_1, H_2, and H_3 (1, 2). H_1 and H_2 receptors are positively coupled to phospholipase C and adenylyl cyclase, respectively, whereas the intracellular signaling system of the H_3 receptor is still unknown (3–5).

In contrast to membrane receptors for other monoamines, molecular studies of HA receptors have just begun (6). For instance, H_2-receptor peptides were identified in guinea pig brain by photoaffinity labeling (7) and, recently, canine (8) and human (9) H_2-receptor genes have been cloned. Transfection of mammalian cells with these intronless genes resulted in the expression of both [^{3}H]tiotidine binding sites and HA-sensitive adenylyl cyclase activity. Preliminary pharmacological characterizations of these receptors have been reported (8, 9).

Because molecular biological studies progressively reveal a much greater heterogeneity among receptor subtypes than previously thought (10, 11), we felt it of interest to identify and characterize receptors related to those described by Gantz *et al.* (8). We have cloned (12), from a rat genomic bank, a DNA encoding a 358-amino acid protein displaying 82% homology with canine H_2 receptor.

We report here that this protein represents a typical H_2 receptor, as indicated by both tissue localization of the gene transcripts and detailed pharmacological analysis in transfected CHO-K1 cells. In addition, we show that this H_2 receptor mediates not only the expected activation of cAMP formation but also a marked inhibition of arachidonic acid (Δ_4Ach) release, which is independent of both cAMP and Ca^{2+} levels.

MATERIALS AND METHODS

Materials. $Na^{125}I$ (2000 Ci/mmol; 1 Ci = 37 GBq), [^{3}H]Δ_4Ach (202 Ci/mmol), [^{3}H]choline (76 Ci/mmol), and *myo*-[2-^{3}H]inositol (17.4 Ci/mmol) were from Amersham. [^{125}I]Iodoaminopotentidine (^{125}I-APT) was synthesized as described (7). The drugs and their sources were as follows: cimetidine, burimamide, metiamide, and dimaprit (Smith Kline & French); famotidine (Merck Sharpe & Dohme); tiotidine (ICI); and ranitidine (Glaxo). 5-Amino-2-(3-{3-[1-(1-pyrrolidinyl)ethyl]phenoxy}propyl)amino-1,3,4-thiadiazole (PPAT) stereoisomers (13) and APT were generous gifts from W. Schunack (Institute of Pharmacy, Berlin). ATP was from Boehringer Mannheim and other reagents from Sigma.

Northern Blot Analysis. Poly(A)$^+$ mRNAs, isolated from tissues of male Hartley guinea pigs (14), were subjected to agarose gel electrophoresis (1% agarose containing 1 M HCHO), blotted onto nitrocellulose, and immobilized by heating at 80°C for 2 h. Prehybridization was at 42°C for 2 h in 40% (vol/vol) formamide/2× Denhardt's solution/50 mM Tris·HCl, pH 7.4/4× standard saline citrate (SSC)/0.1% sodium pyrophosphate/1% SDS/denaturated salmon sperm DNA (100 μg/ml)/yeast tRNA (50 μg/ml). Hybridization was carried out overnight at 42°C, in prehybridization solution containing a probe (15 × 10^6 dpm/ml) corresponding to nucleotides 10–644 of the NuA_2 clone (12) ^{32}P-labeled by nick-translation. Blots were washed three times in 2× SSC/0.1% SDS at 42°C for 10 min, once in 0.2× SSC/0.1% SDS at 42°C for 25 min, and once in 0.2× SSC/0.1% SDS at 55°C for 20 min.

Expression in CHO Cells. The expression vector $pSVH_2$ was derived from the $pSVD_2$ plasmid (15) in which a *Hin*dIII–*Bgl* II restriction fragment was replaced by an ≈1.3-kilobase *Apa* I–*Bgl* II fragment of the NuA_2 clone, comprising the full

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: APT, aminopotentidine; ^{125}I-APT, [^{125}I]iodoaminopotentidine; HA, histamine; Δ_4Ach, arachidonic acid; PLA_2, phospholipase A_2; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; K_B, apparent dissociation constant.

*To whom reprint requests should be addressed.

1074-base coding sequence of the putative rat H_2 receptor (12); a *Hin*dIII–*Apa* I oligonucleotide adaptor was used. CHO-K1 cells, deficient in dihydrofolate reductase, were transfected with this plasmid using Lipofectin (Bethesda Research Laboratories). Stable transfectants were selected in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (vol/vol) fetal calf serum, without hypoxanthine and thymidine, and tested for H_2-receptor binding.

H_2-Receptor Binding. Membranes were incubated for 90 min at 25°C with ^{125}I-APT alone (total binding) or in presence of 1 μM tiotidine (nonspecific binding) or with various drugs, and incubations were terminated by filtration (7).

cAMP Accumulation. Cells (96-well plates) were washed twice (10 min at 37°C) and incubated with the appropriate drugs in DMEM for 10 min at 37°C. cAMP was extracted and analyzed by radioimmunoassay (New England Nuclear).

[^{3}H]Δ_4Ach Release. Cells (24-well plates) were labeled by incubation for 2 h at 37°C with 0.5 μCi of [^{3}H]Δ_4Ach in 1 ml of DMEM supplemented with 0.2% bovine serum albumin to trap the released radioactivity. After washing twice with 0.75 ml of DMEM plus bovine serum albumin, cells were preincubated for 10 min in 0.5 ml of the same medium containing appropriate drugs. After addition of 0.5 ml of DMEM plus bovine serum albumin containing ATP (200 μM) or A23187 (4 μM), incubations were performed at 37°C for 30 min. [^{3}H]Δ_4Ach release was determined by liquid scintillation counting. Thin-layer chromatography revealed that free [^{3}H]Δ_4Ach constituted more than 90% of the released radioactivity (16).

[^{3}H]Δ_4Ach Incorporation. Cells (35-mm dishes) were incubated in 3 ml of DMEM containing 0.2% bovine serum albumin and [^{3}H]Δ_4Ach at 0.05 μCi/ml for 30 min at 37°C. The medium was removed and cells were washed. Lipids were extracted into hexane/isopropanol, 3:2 (vol/vol), and, after drying the organic phase under vacuum, fractionated by thin-layer chromatography [hexane/ethyl ether/MeOH/AcOH, 60:30:4:1 (vol/vol)]. After visualization with I_2 vapors, bands corresponding to phospholipids and free fatty acids were scraped and radioactivity was determined.

[^{3}H]Inositol Phosphate Formation. Cells (24-well plates) were labeled by incubation with *myo*-[^{3}H]inositol (0.5 μCi/ml) for 20 h in selective culture medium. After washings and preincubation (15 min at 37°C) in DMEM containing 20 mM LiCl, cells were incubated for 30 min in 0.5 ml of the same medium containing the appropriate drugs. Reactions were stopped by addition of 0.5 ml of 10% (vol/vol) $HClO_4$ and sonication. After a 1-h incubation at 4°C, the medium was neutralized, extracted with chloroform/methanol/water, 2:1:1 (vol/vol), and [^{3}H]inositol phosphates were isolated by chromatography (17).

[^{3}H]Choline Release. Cells (24-well plates) were labeled by incubation with [^{3}H]choline (1 μCi per well) for 15 h at 37°C in 1 ml of DMEM supplemented with 10% fetal calf serum. The medium was replaced with DMEM containing choline chloride (1 mM), and cells were further incubated for 2 h. After washing, cells were preincubated for 10 min with or without HA and, finally, incubated for 30 min with HA with or without A23187 (2 μM). Released radioactivity was measured in a 0.5-ml sample of incubation medium (18).

Intracellular Free Ca^{2+} Concentration ([Ca^{2+}]$_i$). Cells were trypsinized, washed twice with DMEM plus bovine serum albumin (0.2%), and incubated for 90 min at 37°C in 2 ml of the same medium containing fura-2 acetoxymethyl ester (2 μM). Cells were washed and resuspended in Tyrode's solution, and [Ca^{2+}]$_i$ was measured by using a Hitachi F-2000 fluorescence spectrophotometer (19).

RESULTS

Northern Blot Analysis. The probe revealed a single band of ≈4.5 kilobases in various guinea pig tissues. The hybridization signal was strongest in striatum, heart, and stomach, whereas it was hardly detectable in thalamus, lung, and adrenals (Fig. 1).

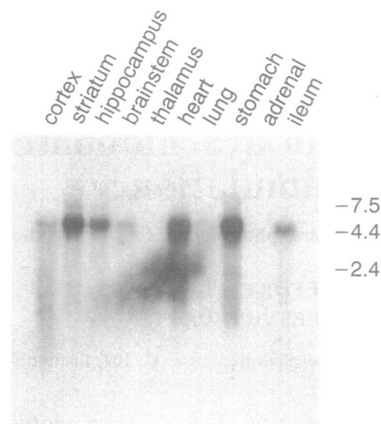

FIG. 1. Northern blot analysis of NuA$_2$ transcripts in guinea pig tissues. Poly(A)$^+$ RNA samples (8 μg per lane) were used. Blots were exposed to x-ray films for 6 days at −80°C with intensifying screens. Molecular sizes in kilobases are shown.

Expression and Pharmacological Characterization of H_2 Receptors in Transfected CHO Cells. Stably transfected cells expressed ^{125}I-APT binding, whereas wild-type CHO cells did not. Among several clones that were isolated on the basis of high H_2-receptor binding (0.05–2 pmol of sites per mg of membrane proteins), one termed CHO(H_2) was selected for further studies.

Binding of ^{125}I-APT to CHO(H_2) cell membranes was saturable and monophasic (n_H = 0.98) with a K_d of 0.45 ± 0.10 nM and a B_{max} of 1.5 ± 0.5 pmol/mg of protein (mean ± SEM derived from Scatchard analysis of three experiments with triplicate samples and 8–12 concentrations studied; data not shown).

Specific binding at 0.3 nM ^{125}I-APT represented 90% of the total and was monophasically inhibited by H_2 antagonists with K_i values highly correlated (r = 0.98) with their apparent dissociation constant (K_B) values at a reference system (Figs. 2 and 3). HA inhibited ^{125}I-APT binding biphasically (n_H = 0.43 ± 0.03) with IC_{50} values of 1.1 ± 0.5 and 133 ± 49 μM. In presence of 0.1 mM guanosine 5′-[γ-imido]triphosphate, the inhibition curve became nearly monophasic (n_H = 0.80 ± 0.08) and a K_i value of 75 ± 24 μM was obtained (Fig. 2).

HA-Induced Responses in CHO(H_2) Cells. HA stimulated cAMP accumulation in CHO(H_2) cells up to 15-fold, and the response was antagonized in a surmountable manner by ranitidine (Fig. 4). Dimaprit (0.1 mM) or impromidine (10 μM), two H_2-receptor agonists, also enhanced cAMP accu-

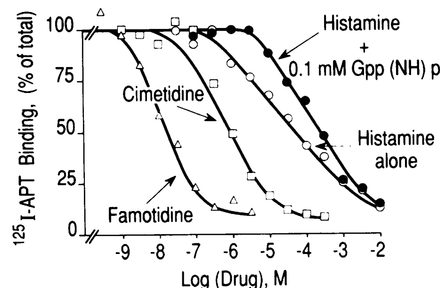

FIG. 2. Pharmacological characterization of ^{125}I-APT binding to membranes prepared from CHO(H_2) cells. Membranes were incubated with 0.3 nM ^{125}I-APT, alone or in presence of competing agents. A representative experiment is shown in which control binding was 1200 ± 50 cpm/μg of protein. Gpp(NH)p, guanosine 5′-[γ-imido]triphosphate.

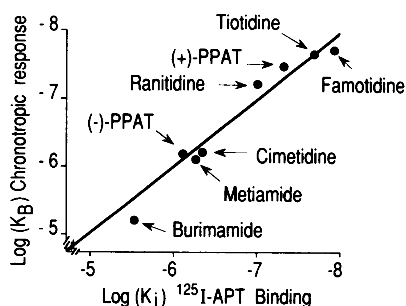

FIG. 3. Compared potencies of H_2-receptor antagonists as inhibitors of ^{125}I-APT binding to membranes from CHO(H_2) cells and as antagonists of the H_2-receptor-mediated chronotropic response at the isolated guinea pig right atrium. K_i values were calculated (20) from IC_{50} values derived from two to four experiments as those depicted in Fig. 2. K_B values are apparent dissociation constants from refs. 13 and 21. PPAT, 5-Amino-2-(3-{3-[1-(1-pyrrolidinyl)ethyl]phenoxy}propyl)-amino-1,3,4-thiadiazole.

mulation in CHO(H_2). Stimulation corresponded to 92 ± 4% and 88 ± 5% of the maximal response to HA (n = 3).

In CHO(H_2) cells labeled by incubation with [^{3}H]Δ_4Ach, HA inhibited [^{3}H]Δ_4Ach release evoked by stimulating constitutive purinergic receptors with 0.1 mM ATP (22); inhibition was maximal at 0.5 μM HA (38 ± 3%) and competitively antagonized by ranitidine (1 μM) (Fig. 5). Dimaprit (10 μM) inhibited ATP-induced release of [^{3}H]Δ_4Ach by 36 ± 5%, an effect blocked by 10 μM ranitidine. In addition, HA inhibited [^{3}H]Δ_4Ach release evoked by the Ca^{2+} ionophore A23187 (Table 1), to a maximal extent of 40 ± 6% and with an EC_{50} value of 0.03 ± 0.02 μM (data not shown). In contrast, HA did not affect either basal [^{3}H]Δ_4Ach release from CHO(H_2) cells (Table 1) or stimulated release in wild-type CHO (Table 1). Forskolin and prostaglandin E_1, which raised cAMP levels in CHO (data not shown), or 8-bromo-cAMP did not mimic the effect of HA on Δ_4Ach release (Table 1).

The effect of HA on [^{3}H]Δ_4Ach release was likely produced by inhibition of liberation of [^{3}H]Δ_4Ach rather than by enhancement of [^{3}H]Δ_4Ach reincorporation into phospholipids. In agreement, HA (10 μM) did not affect [^{3}H]Δ_4Ach incorporation into phospholipids. After a 30-min incubation in the presence of [^{3}H]Δ_4Ach, CHO(H_2) cells incorporated into phospholipids 3715 ± 76 cpm per well. No significant change in labeling was seen when cells were exposed to 10 μM HA (4020 ± 109 cpm per well), 4 μM A23187 (3325 ± 79 cpm per well), or HA plus A23187 (3370 ± 94 cpm per well) (data from one experiment performed in triplicate; similar results were obtained in one additional experiment).

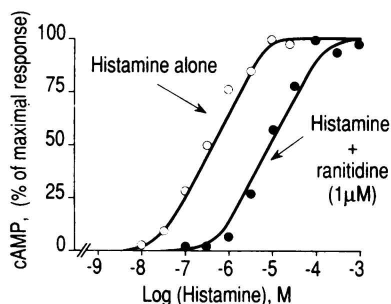

FIG. 4. HA-induced stimulation of cAMP accumulation in CHO(H_2) cells and its inhibition by ranitidine. cAMP levels in absence and presence of 10 μM HA alone were 0.61 ± 0.07 and 9.7 ± 1.0 pmol per well, respectively (mean ± SEM of quadruplicates in a single experiment, which was repeated three times). The EC_{50} values of HA were 0.2 ± 0.1 and 5.6 ± 2.4 μM in the absence and presence of 1 μM ranitidine, respectively, leading (20) to an apparent K_i value of 0.04 μM.

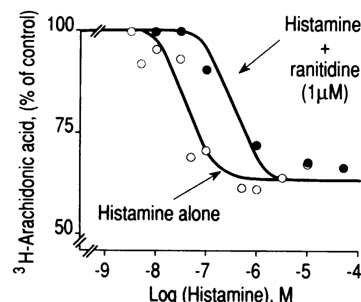

FIG. 5. HA-induced inhibition of evoked [^{3}H]Δ_4Ach release from CHO(H_2) cells. [^{3}H]Δ_4Ach release, triggered by stimulation of purinergic receptors with 0.1 mM ATP (30-min incubation), was 2229 ± 77 cpm per well. Basal release was 226 ± 10 cpm per well (mean ± SEM of quadruplicate determinations from at least three experiments). EC_{50} values for HA were 0.04 ± 0.02 μM and 0.3 ± 0.1 μM in absence and presence of 1 μM ranitidine, respectively, leading (20) to an apparent K_i value of 0.16 μM.

HA (1 μM) did not significantly modify either basal [Ca^{2+}]$_i$ or [Ca^{2+}]$_i$ transients induced by 0.1 mM ATP ([Ca^{2+}]$_i$ was 388 ± 30 nM and 376 ± 48 nM, in the absence and presence of HA, respectively; n = 8). HA also failed to affect the basal formation of [^{3}H]inositol phosphates (178 ± 4 cpm and 183 ± 6 cpm in the absence and presence of 3 μM HA, respectively; n = 4–8). As reported (22), ATP (0.4 mM) stimulated [^{3}H]inositol phosphate formation; the effect of ATP was slightly, but significantly (+15%; $P < 0.01$), enhanced by 20 μM HA (643 ± 14 cpm and 736 ± 16 cpm, respectively; n = 4–8).

[^{3}H]Choline release evoked by A23187 (2 μM) remained unaltered in the presence of 10 μM HA (3836 ± 97 cpm and 4254 ± 167 cpm, respectively), as well as basal release (2103 ± 193 cpm and 2393 ± 94 cpm, respectively; n = 4).

DISCUSSION

The cloned gene encodes a typical H_2 receptor as shown by both tissue distribution of its transcripts and detailed pharmacological characterization.

Northern blot analysis of guinea pig tissues revealed a single band of size similar to that obtained in rat tissues (12). Labeling of this transcript was stronger than in rat, in agreement with the higher density of H_2 receptors in guinea pig revealed with ^{125}I-APT, a highly selective and sensitive probe (7, 23). In brain, regional distribution of the transcript was consistent with that obtained in membrane binding or

Table 1. Effects of HA on [^{3}H]Δ_4Ach release induced by ATP or A23187 in CHO(H_2) cells

Agent(s)	[^{3}H]Δ_4Ach, cpm per well
None	266 ± 10
HA (3 μM)	231 ± 29
ATP (100 μM)	2616 ± 73
ATP (100 μM) + HA (3 μM)	1844 ± 53**
ATP (100 μM) + 8-bromo-cAMP (0.2 mM)	2926 ± 100
ATP (100 μM) + forskolin (0.5 μM)	2652 ± 100
ATP (100 μM) + prostaglandin E_1 (0.2 μg/ml)	3279 ± 146*
A23187 (2 μM)	4997 ± 201
A23187 (2 μM) + HA (3 μM)	3098 ± 201**

Cells were preincubated for 10 min in the presence of various agents before the addition of A23187 or ATP and incubated for 30 min. Data are the mean ± SEM from at least three experiments. *, $P < 0.05$; **, $P < 0.001$ as compared to A23187 or ATP alone (Student's t test). On wild-type CHO, HA did not affect A23187-induced [^{3}H]Δ_4Ach release (3809 ± 89 vs. 4010 ± 112 cpm per well).

autoradiographic studies with ^{125}I-APT (7). mRNA was detected in all peripheral tissues where H_2-receptor-mediated responses occur (1, 3, 24). Interestingly, the same mRNA was found in both stomach and heart, suggesting that the same molecular species mediates the actions of HA in these tissues. This is consistent with the hypothesis that the marked differences in potency of some H_2-receptor antagonists in stomach and heart are related to bioavailability factors rather than to receptor heterogeneity (25, 26). Hence, the absence of corresponding mRNA in dog (8) and rat heart (12) is presumably not due to the existence of a distinct cardiac isoreceptor but rather to the absence of expression of H_2 receptors in the heart of these two species, as suggested by the lack of HA-induced stimulation of adenylyl cyclase (24).

The pharmacological characterization of ^{125}I-APT binding sites expressed by CHO cells transfected with pSVH$_2$ also leaves little doubt about this gene representing that of a typical H_2 receptor. Thus the K_d of ^{125}I-APT at equilibrium and the K_i values of HA and various antagonists were closely similar to their K_B values at the reference biological system (1) defining the H_2 receptor (13, 21) or K_i values at cerebral H_2 receptors (7). In agreement, HA and selective H_2-receptor agonists markedly enhanced cAMP accumulation in transfected cells, a response blocked competitively by ranitidine with the expected potency. The association of H_2 receptors with cAMP accumulation (27, 28) and adenylyl cyclase (24, 29, 30) is well established.

The cAMP response elicited by HA in CHO(H$_2$) cells occurred with an EC_{50} value about 1000-fold lower than the K_i value for inhibition of ^{125}I-APT binding. This large receptor reserve, which likely reflects a high level of expression of H_2 receptors in these cells, may account for the intrinsic activities displayed by the partial agonists dimaprit and impromidine, which were higher than in brain slices (31). Presence of spare H_2 receptors has been demonstrated in guinea pig brain slices (7, 32, 33).

Stimulation of cAMP formation is thought to mediate most physiological responses produced by H_2-receptor activation (24). We found, unexpectedly, that H_2-receptor occupation also resulted in a reduction of evoked Δ_4Ach release from CHO(H$_2$) cells. Maximal inhibition by HA was 40% when [^{3}H]Δ_4Ach release was evoked either by stimulating constitutive purinergic receptors (22) or by increasing $[Ca^{2+}]_i$ with the Ca^{2+} ionophore A23187.

Liberation of Δ_4Ach from membrane phospholipids mainly occurs through either activation of intracellular phospholipases A_2 (PLA$_2$) (34) or by hydrolysis of 1,2-diacylglycerol, produced by activation of phosphatidylinositol-specific phospholipase C (35). Transfected H_2 receptors may reduce evoked Δ_4Ach release by inhibiting PLA$_2$ activity. In agreement, we found that HA did not affect the incorporation of [^{3}H]Δ_4Ach into phospholipids or the activation of either phosphatidylinositol-specific or phosphatidylcholine-specific phospholipase C (18).

The mechanism of H_2-dependent inhibition of Δ_4Ach release, described here, remains unknown. The results suggest, however, that the response was independent of changes in cAMP because it could not be mimicked by agents known to enhance cAMP levels. Furthermore, activation of dopamine D_1 receptors enhances cAMP levels in transfected CHO cells without inhibiting ATP- or A23187-induced [^{3}H]Δ_4Ach release (36).

Receptor-dependent activation of PLA$_2$ is often associated with elevation of $[Ca^{2+}]_i$, even though these two events may be independent (22). H_2 receptors do not appear to modulate Δ_4Ach release by reducing agonist-induced $[Ca^{2+}]_i$ increases. (*i*) HA failed to inhibit Ca^{2+} transients evoked by stimulating purinergic receptors. (*ii*) HA-induced inhibition was also observed when [^{3}H]Δ_4Ach release was evoked by the Ca^{2+} ionophore A23187, which increases $[Ca^{2+}]_i$ bypassing receptor stimulation. Activation of transfected H_2 receptors may reduce Δ_4Ach release through a direct inhibition of PLA$_2$—i.e., by a mechanism (perhaps mediated by a guanine nucleotide binding protein) similar to that responsible for receptor-linked inhibition of adenylyl cyclase. Thus, the results support the hypothesis that PLA$_2$ activity may be subjected to both stimulatory and inhibitory control by membrane receptors, as earlier suggested by *in vitro* studies on retinal tissue (37).

The physiological role of H_2-receptor-mediated inhibition of Δ_4Ach release remains to be established. The potential importance of this reponse is indicated by its associated receptor occupancy, which is similar to or lower than that required to affect cAMP levels. Because Δ_4Ach release is the limiting step in eicosanoid biosynthesis, its inhibition by HA is expected to result in decreased formation of these biologically active lipids.

The existence of a signaling pathway for H_2 receptors, independent of cAMP, might account for a variety of unexplained observations. (*i*) In brain, the regional distribution of ^{125}I-APT binding sites does not parallel that of H_2-receptor-linked adenylyl cyclase (7). (*ii*) Denervation hypersensitivity to HA is observed on electrophysiological but not on cAMP responses (38). (*iii*) The opposing effects of H_1- and H_2-receptor stimulation observed in many systems are not likely to result from interactions at the level of cAMP generation where, instead, a synergism occurs (32). These opposing actions may well be accounted for by interactions at the level of Δ_4Ach release since, contrary to H_2 receptors, H_1 receptors may be positively coupled to release of this fatty acid (39, 40).

In conclusion, the present study does not substantiate the hypothesis of the existence of multiple H_2 receptors but underscores the emerging view that a single receptor subtype can control several signaling pathways in the same cell.

We thank A. Galtier for secretarial assistance and V. Mignon for technical assistance. We thank also P. Sokoloff, C. Pilon, B. Giros, and M. P. Martres for helpful discussions. This work was supported in part by a grant from the Direction des Recherches et Etudes Techniques.

1. Black, J. W., Duncan, W. A. M., Durant, C. J., Ganellin, C. R. & Parsons, M. E. (1972) *Nature (London)* **236**, 385–390.
2. Arrang, J. M., Garbarg, M. & Schwartz, J. C. (1983) *Nature (London)* **302**, 832–837.
3. Hill, S. J. (1990) *Pharmacol. Rev.* **42**, 46–83.
4. Schwartz, J. C., Arrang, J. M., Garbarg, M., Pollard, H. & Ruat, M. (1991) *Physiol. Rev.* **71**, 1–51.
5. Leurs, R., Van der Goot, H. & Timmerman, H. (1991) *Adv. Drug Res.* **20**, 217–304.
6. Ruat, M., Traiffort, E. & Schwartz, J. C. (1992) in *The Histamine Receptors*, eds. Schwartz, J. C. & Haas, H. L. (Liss, New York), 97–107.
7. Ruat, M., Traiffort, E., Bouthenet, M. L., Schwartz, J. C., Hirschfeld, J., Buschauer, A. & Schunack, W. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1658–1662.
8. Gantz, I., Schäffer, M., Delvalle, J., Logsdon, C., Campbell, V., Uhler, M. & Yamada, T. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 429–433.
9. Gantz, I., Munzert, G., Tashiro, T., Schäffer, M., Wang, L., Delvalle, J. & Yamada, T. (1991) *Biochem. Biophys. Res. Commun.* **178**, 1386–1392.
10. Caron, M. G. (1989) *Curr. Opinion Cell Biol.* **1**, 159–166.
11. Schofield, P. R., Shivers, B. D. & Seeburg, P. H. (1990) *Trends Neurosci.* **13**, 8–11.
12. Ruat, M., Traiffort, E., Arrang, J. M., Leurs, R. & Schwartz, J. C. (1991) *Biochem. Biophys. Res. Commun.* **179**, 1470–1478.
13. Keller, F., Buschauer, A. & Schunack, W. (1988) *Pharm. Ztg.* **1**, 48–55.
14. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
15. Sokoloff, P., Giros, B., Martres, M. P., Bouthenet, M. L. & Schwartz, J. C. (1990) *Nature (London)* **347**, 146–151.

16. Salmon, J. A. & Flower, R. J. (1982) *Methods Enzymol.* **86**, 477–511.
17. Berridge, M. J., Dawson, R. M. C., Downes, C. P., Heslop, J. P. & Irvine, P. F. (1983) *Biochem. J.* **212**, 437–482.
18. Exton, J. H. (1990) *J. Biol. Chem.* **265**, 1–4.
19. Grynkiewicz, G., Peonie, M. & Tsien, R. Y. (1985) *J. Biol. Chem.* **260**, 3440–3450.
20. Cheng, Y. & Prusoff, W. (1973) *Biochem. Pharmacol.* **22**, 3099–3108.
21. Cooper, D. G., Young, R. C., Durant, G. J. & Ganellin, C. R. (1990) in *Comprehensive Medicinal Chemistry*, eds. Sammes, P. G. & Taylor, J. B. (Pergamon, Oxford, U.K.), pp. 323–421.
22. Gupta, S. K., Diez, E., Heasley, L. E., Osawa, S. & Johnson, G. L. (1990) *Science* **249**, 662–666.
23. Traiffort, E., Ruat, M. & Schwartz, J. C. (1991) *Eur. J. Pharmacol.* **207**, 143–148.
24. Johnson, C. L. (1982) in *Pharmacology of Histamine Receptors*, eds. Ganellin, C. R. & Parsons, M. E. (Wright, Bristol, U.K.), pp. 146–216.
25. Shankley, N. P., Black, J. W., Ganellin, C. R. & Mitchell, R. C. (1988) *Br. J. Pharmacol.* **94**, 264–274.
26. Buschauer, A. & Baumann, G. (1991) in *New Perspectives in Histamine Research*, eds. Timmerman, H. & Van der Goot, H. (Birkhäuser, Basel), Vol. 33, pp. 231–256.
27. Nahorski, S. R., Rogers, K. J. & Smith, B. M. (1974) *Life Sci.* **15**, 1887–1894.
28. Baudry, M., Martres, M. P. & Schwartz, J. C. (1975) *Nature (London)* **253**, 362–363.
29. Green, J. P., Johnson, C. L., Weinstein, H. & Maayani, S. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5697–5701.
30. Hegstrand, L. R., Kanof, P. D. & Greengard, P. (1976) *Nature (London)* **260**, 163–165.
31. Dam Trung Tuong, M., Garbarg, M. & Schwartz, J. C. (1980) *Nature (London)* **287**, 548–551.
32. Palacios, J. M., Garbarg, M., Barbin, G. & Schwartz, J. C. (1978) *Mol. Pharmacol.* **14**, 971–982.
33. Gajtkowski, G. A., Norris, D. B., Rising, T. J. & Wood, T. P. (1983) *Nature (London)* **304**, 65–67.
34. Waite, M. (1987) in *Handbook of Lipid Research*, ed. Hanahan, D. J. (Plenum, New York), Vol. 5, pp. 111–134.
35. Crooke, S. T. & Bennett, C. F. (1989) *Cell Calcium* **10**, 309–323.
36. Piomelli, D., Pilon, C., Giros, B., Sokoloff, P., Martres, M. P. & Schwartz, J. C. (1991) *Nature (London)* **353**, 164–167.
37. Jelsema, C. L. & Axelrod, J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3625–3627.
38. Haas, H. L., Wolf, P., Palacios, J. M., Garbarg, M., Barbin, G. & Schwartz, J. C. (1978) *Brain Res.* **156**, 275–291.
39. Juan, H. & Sametz, W. (1980) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **314**, 183–190.
40. Murayama, T., Kajiyama, Y. & Nomura, Y. (1990) *J. Biol. Chem.* **265**, 4290–4295.