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Histamine H_1 receptor activation stimulates [^{3}H]GABA release from human astrocytoma U373 MG cells

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Abstract

In U373 MG cells, a line derived from a human astrocytoma, histamine stimulated the release of [^{3}H]γ-aminobutyric acid ([^{3}H]GABA) in a concentration-dependent manner (286 ± 23% of basal release at 1 mM histamine). Neither Ca^{2+} removal nor Cd^{2+} (100 μM) affected [^{3}H]GABA release evoked by 100 μM histamine but the response was significantly reduced by 10 μM U-73122 ({1-[6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)-amino)-hexyl]-1*H*-pyrrole-2,5-dione}), an inhibitor of phospholipase C activation (79 ± 8% inhibition) and by 10 μM dimethylbenzamil, a selective blocker of plasma membrane Na^+/Ca^{2+} exchange (58 ± 6% inhibition). In [^{3}H]inositol-labelled cells histamine stimulated [^{3}H]inositol phosphate accumulation (EC_{50}, 17 ± 2 μM; maximum effect, 203 ± 4% of basal). Histamine-evoked Ca^{2+} mobilisation yielded an EC_{50} of 12 ± 2 μM and maximum $\Delta[Ca^{2+}]_i$ of 337 ± 23 nM. Thapsigargin (1 nM) increased $[Ca^{2+}]_i$ ($\Delta[Ca^{2+}]_i$ 164 ± 12 nM) and prevented any further increase by histamine (100 μM). The effects of histamine on [^{3}H]GABA release, [^{3}H]inositol phosphate accumulation and Ca^{2+} mobilisation were blocked by the selective histamine H_1 receptor antagonist mepyramine. Taken together, these results indicate that histamine stimulates [^{3}H]GABA release by increasing $[Ca^{2+}]_i$. The mechanism of release may be related to changes in transmembranal Na^+ gradients and reversal of GABA carrier transport due to stimulation of plasma membrane Na^+/Ca^{2+} exchange.

Keywords: Histamine; Histamine H_1 receptor; Astrocytoma; GABA (γ-aminobutyric acid); Glia; Ca^{2+}

1. Introduction

Histamine is a neuromodulator in mammalian central nervous system (CNS) where it regulates pre- and post-synaptically functions such as awareness, wakefulness, pain and motor activity, among others (Schwartz et al., 1991). On the basis of their pharmacology and signal transduction mechanisms histamine receptors have been subdivided into H_1, H_2 and H_3 subtypes (Hill, 1990). Histamine H_1 receptors are among those receptors whose activation leads to breakdown of phosphatidylinositol 4,5-biphosphate (PIP_2) into inositol 1,4,5-triphosphate (IP_3) and diacylglicerol. IP_3 mobilises Ca^{2+} ions from intracellular stores while diacylglycerol activates protein kinase C (Berridge, 1993).

Besides being located on neurones, histamine H_1 receptors are also present on glial cells, namely on astrocytes (Arbonés et al., 1988, 1990; Inagaki et al., 1989, 1991; Kondu et al., 1991; Inagaki and Wada, 1994). Among other functions, glial cells are involved in terminating the action of inhibitory and excitatory amino acids, by taking up and metabolising such amino-acid transmitters (Barres, 1991; Attwell and Mobbs, 1994). However, it has been suggested that glial cells may have a more active role in synaptic transmission by releasing neuroactive substances (Martin, 1992), and recently it was reported that astrocytes are able to release glutamate following bradykinin-mediated increases in intracellular Ca^{2+} ($[Ca^{2+}]_i$) (Parpura et al., 1994).

The cell line U373 MG, derived from a human astrocytoma, expresses histamine H_1 receptors whose binding properties are similar to those present in mammalian brain (Arias-Montaño et al., 1994). Thus this astrocyte-derived cell line appears to be a useful model on which to study glial functional mechanisms coupled to H_1 receptor activation and we report herein that activation of H_1 receptors is followed by Ca^{2+} mobilisation and release of [^{3}H]γ-

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aminobutyric acid ([^{3}H]GABA) previously taken up. A preliminary report of this work appeared elsewhere in abstract form (Soria and Arias-Montaño, 1995).

2. Materials and methods

2.1. Cell culture

Human astrocytoma cells (U373 MG) were obtained from National Culture Collection (Porton Down, UK). Cells were grown in Dulbecco's modified Eagle's Medium (DMEM)/nutrient mixture F-12 (1:1, v/v) supplemented with 10% bovine foetal serum, penicillin (50 UI ml^{-1}) and streptomycin (0.1 mg ml^{-1}). Cells were grown as monolayers in either flasks or 12-well plates in a humidified atmosphere (5% CO_2) at 37°C.

2.2. [^{3}H]GABA release

Cells grown in 12-well plates were incubated for 30 min in Krebs-Hepes (KH) buffer (0.5 ml/well) containing 80 nM [^{3}H]GABA (3.6 μCi/well). After this period the buffer was removed and cells were washed five times with 1 ml of warmed (37°C) KH buffer. Seven successive 2 min incubations (1 ml each) were then carried out with warmed KH buffer and samples (fractions 1–7) were individually collected into 4-ml insert vials. Histamine was present in fraction 4. Where required the selective H_1 receptor antagonist mepyramine or the inhibitor of plasma membrane Na^+/Ca^{2+} exchange dimethylbenzamil, were present from fraction 1. Scintillation liquid (2 ml) was added to each vial and the tritium content determined by liquid scintillation counting. To determine the total amount of [^{3}H]GABA remaining in the cells, 1 ml NaOH was added to each well and allowed to stand for 30 min at room temperature before being collected. The radioactivity present in each well was determined as above. KH buffer used for [^{3}H]GABA uptake and release contained 10 μM aminooxyacetic acid, an inhibitor of GABA deamination.

2.3. Accumulation of [^{3}H]inositol phosphates ([^{3}H]IPs)

Cells were grown to near confluence in 12-well plates. The culture medium was removed and each well washed with 1 ml inositol-free DMEM before addition of 1 ml inositol-free DMEM containing 10% dialysed calf serum, 10 μM *myo*-inositol and 2.5 μCi [^{3}H]inositol (0.2 μM). After a 24 h incubation the [^{3}H]inositol-labelled cells were washed twice with 1 ml KH buffer before adding to each well 240 μl of KH buffer containing 20 mM LiCl. Plates were then incubated for 10 min at 37°C. At this stage histamine (1–1000 μM) or water (for basal formation) were added in a 10 μl volume and cells were incubated for a further 2 min. When required mepyiramine (1 μM) was added from the beginning of the incubation in KH buffer.

Incubations were terminated by addition of 250 μl of ice-cold perchloric acid (10%) containing 1 mM EGTA and 1 mg · ml^{-1} phytic acid. Plates were allowed to stand at 4°C for 30 min, an aliquot (450 μl) was transferred to a microcentrifuge tube (1.5 ml) and inositol phosphates were extracted by the trioctylamine-freon method (Sharpes and McCarl, 1982; Downes et al., 1986). Trioctylamine/1,1,2 trichlorotrifluoroethane (1:1, v/v) (0.5 ml) was added to the sample, the mixture was vortex mixed, and the resulting three phases separated by centrifugation at $900 \times g$ for 5 min. A portion (0.4 ml) of the upper, aqueous, phase was transferred to an insert vial, 3 ml 50 mM Tris buffer, pH 7.4, added and the mixture applied to chromatography columns (Bio-Rad Poly-Prep) containing 2 ml of a 1:1 slurry of Dowex AG 1-X8 (formate form, 100–200 mesh; Bio-Rad)/H_2O.

[^{3}H]Inositol and [^{3}H]glycerophosphoinositol were removed with 10 ml water and 10 ml 60 mM ammonium formate/5 mM sodium tetraborate, respectively. Total [^{3}H]IPs ([^{3}H]IP_1 + [^{3}H]IP_2 + [^{3}H]IP_3) were then eluted into scintillation vials with 8 ml 800 mM ammonium formate/100 mM formic acid. Scintillation liquid (10 ml) was added to each eluted sample and the tritium content determined by liquid scintillation counting.

2.4. Measurements of $[Ca^{2+}]_i$

Cells were grown to confluence in flasks. The culture medium was removed and the cells washed in 15 ml phosphate-buffered saline (in mM: NaCl 137, KCl 2.7, Na_2HPO_4 8.1 and KH_2PO_4 1.5) containing 0.6 mM EDTA before dissociation with 10 ml trypsin/EDTA (500–750 units · ml^{-1}/0.6 mM). After centrifugation at $100 \times g$ for 5 min the cells were resuspended in 5 ml KH buffer containing 2 μM Fura 2-acetomethylester (Fura 2-AM) and 10 mg · ml^{-1} bovine serum albumin and then incubated at 37°C for 60 min. After centrifugation as above cells were resuspended in KH buffer, aliquoted and allowed to stand on ice until use.

Aliquots ($2–3 \times 10^6$ cells) were diluted in 1.5 ml warmed (37°C) KH buffer in a plastic cuvette placed into the warmed cell of a Perkin Elmer LB-50 fluorometer. UV excitation was set at 340 and 380 nm and the emission light was measured at 510 nm. Due to rapid desensitisation of the response (data not shown) only a single concentration of histamine was tested in each cell sample.

Values for intracellular free $[Ca^{2+}]_i$ were estimated from the equation (Grynkiewicz et al., 1985): $[Ca^{2+}]_i = K_D \times f \times (R - R_{min}/R_{max} - R)$, where R is the fluorescent ratio (F_1/F_2) at 340 and 380 nm excitation wavelengths, f is the ratio of fluorescence at 380 nm of free Fura-2 over that of Ca^{2+}-bound dye and K_D is the dissociation constant of Fura-2 for Ca^{2+} ions (224 nM). For each cell sample the parameters R_{max} and R_{min} were determined. R_{max} is the fluorescence when cells were lysed with Triton X-100 (1% in phosphate buffered saline, pH 7.4) and R_{min} is the fluorescence remaining after the addition of 5 mM EGTA in 0.44 M Tris buffer, pH 7.4.

Typical values for R_{max} and R_{min} were 6.1 ± 0.3 and 0.91 ± 0.01, respectively ($n = 11$ determinations).

2.5. Analysis of data

All data are expressed as means ± S.E.M. Release of [^{3}H]GABA was initially expressed as a fraction of the total amount of tritium (the sum of all fraction plus that remaining in the cells). [^{3}H]GABA taken up by the cells was usually 8×10^4 dpm/well and basal release per 2 min (1 ml) fraction corresponded to 1–2% of total tritium. To allow for differences between experiments the data were normalised by expressing the amount of [^{3}H]GABA present in each fraction as a percentage of the amount of [^{3}H]GABA present in the fraction collected immediately before exposition to the drug under test (i.e. the release in fraction 3 was set to 100%). At least 3 replicate determinations were made for each experimental condition within an experiment.

Concentration-response curves were fitted by non-linear regression to a four-parameter logistic (Hill) equation using the program Prism (GraphPad Software, San Diego, CA, USA). Receptor occupancy (DR) by histamine was calculated from the equation: $DR = K_A[A]/K_A[A] + 1$; where K_A is the agonist apparent affinity constant ($1/EC_{50}$, see Results) and [A] is the agonist concentration. Receptor occupancy by histamine in the presence of the H_1 receptor antagonist mepyramine was estimated from the equation: $DR = K_A[A]/K_A[A] + K_B[B] + 1$; where [B] is the concentration of antagonist and K_B its affinity constant ($1/K_d$).

2.6. Drugs

[^{3}H]GABA (specific activity 86 Ci · mmol^{-1}) and *myo*-[^{3}H]inositol (17.2 Ci · mmol^{-1}) were from Amersham International (Little Chalfont, UK). Aminooxyacetic acid (hemihydrochloride), Fura 2-AM and monensin (sodium salt) were purchased from Sigma (St. Louis, MO, USA). Mepyramine (pyrilamine maleate), histamine dihydrochloride, thapsigargin and [Sar^9,Met(O$_2$)^{11}]substance P were from RBI (Natick, MA, USA). Dimethylbenzamil (2′,4′-dimethylbenzamil hydrochloride) was from Molecular Probes (Eugene, OR, USA). The compounds U-73122 ({1-[6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)-amino)-hexyl]-1*H*-pyrrole-2,5-dione}) and U-73343 ({1-[6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)-amino)-hexyl]-2,5-pyrrolidine-dione}) were kindly provided by Dr. Agustín Guerrero (CINVESTAV, México).

3. Results

3.1. Histamine-induced [^{3}H]GABA release

Values for basal [^{3}H]GABA release were usually in the range 400–600 dpm per 1 ml samples and corresponded to 1–2% of [^{3}H]GABA captured (i.e., tritium remaining in the cells plus tritium present in all fractions collected). The addition of histamine (1–1000 μM) for 2 min to the incubation medium resulted in augmented [^{3}H]GABA release from U373 MG cells as compared to basal efflux (Fig. 1A). Histamine-stimulated release was concentration-dependent (Fig. 1A,B) with 1 mM histamine (the highest concentration tested) evoking a release of $286 \pm 23\%$ of basal ($n = 3$ experiments).

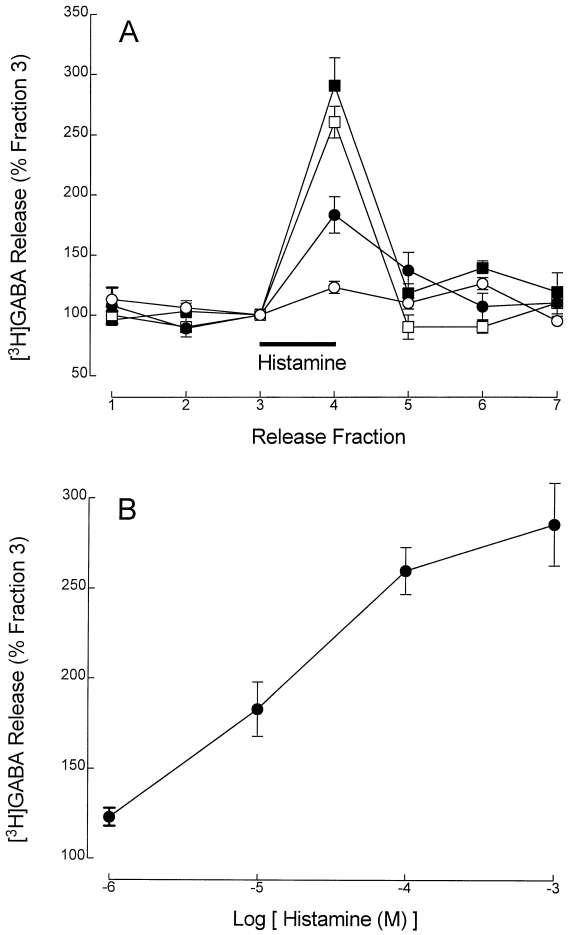

Fig. 1. Histamine-induced [^{3}H]GABA release. Cells grown in 12-well plates were incubated in KH buffer containing [^{3}H]GABA as described under Materials and methods. Non-captured [^{3}H]GABA was washed out and successive 2 min incubations (1 ml each) were then carried out. Samples were collected and the tritium content determined. [^{3}H]GABA release is expressed as a percentage of [^{3}H]GABA present in fraction 3. Histamine was present in fraction 4 as indicated by the bar in the graph. Values are means ± S.E.M. from 3 experiments with four replicates for each condition. (A) Time course for [^{3}H]GABA release evoked by histamine (○, 1 μM; ●, 10 μM; □, 100 μM; ■, 1 mM). (B) Concentration-response curve. Points (means ± S.E.M.) correspond to [^{3}H]GABA released in fraction 4.

[^{3}H]GABA release stimulated by 100 μM histamine ($211 \pm 18\%$ of basal) was fully blocked by the selective H_1 receptor antagonist mepyramine (1 μM; $97 \pm 5\%$ of basal, $n = 3$). On the basis of binding experiments the K_d of mepyramine for H_1 receptors present on U373 MG cells

was determined to be 2.5 nM (Arias-Montaño et al., 1994). An apparent K_A for the endogenous agonist can be estimated from the EC_{50} (apparent $K_A = 1/EC_{50}$) for either histamine-induced inositol phosphate formation, 17 ± 2 μM, or histamine-induced Ca^{2+} mobilisation, 12 ± 3 μM (see below). Thus the theoretical inhibition by 1 μM mepyramine of the H_1 receptor-mediated effect was estimated to be 97–98%, in good agreement with the experimental value found (100% inhibition).

In order to test whether [^{3}H]GABA release was dependent on extracellular Ca^{2+} in a series of experiments ($n = 4$) the effect of histamine was assayed in KH buffer with no added Ca^{2+} or in the presence of 100 μM Cd^{2+}, a non-selective Ca^{2+} channel blocker. Under these conditions [^{3}H]GABA release induced by 100 μM histamine (220 ± 22% of basal) was neither affected by Ca^{2+} removal (229 ± 30% of basal) nor by Cd^{2+} (231 ± 11% of basal).

Substitution of choline chloride for sodium chloride resulted in a significant increase of basal [^{3}H]GABA efflux (3–5-fold of that in normal KH buffer). Under these conditions the addition of histamine (100 μM) had no further effect on [^{3}H]GABA release (data not shown). In sister cultures (i.e., wells within the same plate) incubated with normal KH buffer histamine-induced [^{3}H]GABA release was 242 ± 23% of basal ($n = 3$).

In normal KH buffer the Na^+ ionophore monensin significantly increased [^{3}H]GABA release. Concentrations of 1, 3 and 10 μM monensin augmented [^{3}H]GABA efflux to 127 ± 2% ($P < 0.05$), 185 ± 19% ($P < 0.01$) and 227 ± 8% ($P < 0.01$) of control values respectively (ANOVA and post hoc Dunnett's test, $n = 3$).

[^{3}H]GABA release elicited by 100 μM histamine (227 ± 9% of basal, $n = 3$ experiments) was significantly reduced by 10 μM of dimethylbenzamil, an amiloride derivative which selectively blocks plasma membrane Na^+/Ca^{2+} exchange (Fig. 2). The mean inhibition from 3 experiments was 58 ± 6% ($P < 0.1$, Student's *t*-test). The concentration of dimethylbenzamil chosen (10 μM) is the IC_{50} reported by Kaczorowski et al. (1985) for blockade of the plasma membrane Na^+/Ca^{2+} exchanger.

To test whether other agonists coupled to IP_3 formation and Ca^{2+} mobilisation had similar actions on [^{3}H]GABA release to that found for histamine, we tested the effect of [Sar^9,Met(O$_2$)^{11}]substance P, a selective agonist on tachykinin NK_1 receptors which have been shown to couple to PIP_2 hydrolysis and Ca^{2+} mobilisation in the U373 MG cell line (Eistetter et al., 1992; Pradier et al., 1993; Bordey et al., 1994). The addition of [Sar^9,Met(O$_2$)^{11}]substance P for 2 min to the incubation medium increased [^{3}H]GABA release in a concentration-dependent manner, with values of 198 ± 11%, 221 ± 4% and 296 ± 31% of basal for 0.01, 0.1 and 1 μM, respectively. All these values were significantly different from basal release ($P < 0.01$, ANOVA and post hoc Dunnett's test, $n = 3$) and the effect of the highest concentration tested (1 μM) was similar to that found for 1 mM histamine (286 ± 23% of basal).

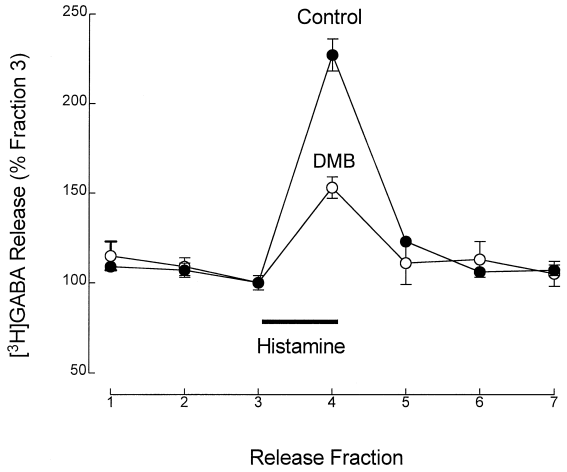

Fig. 2. Inhibition by dimethylbenzamyl (DMB) of histamine-induced [^{3}H]GABA release. Cells grown in 12-well plates were incubated in KH buffer containing [^{3}H]GABA as described under Materials and methods. Non-captured [^{3}H]GABA was washed out and successive 2 min incubations (1 ml each) were then carried out. Samples were collected and the tritium content determined. [^{3}H]GABA release is expressed as a percentage of [^{3}H]GABA present in fraction 3. Histamine was present in fraction 4 as indicated by the bar in the graph. Where required, the selective blocker of plasma membrane Na^+/Ca^{2+} exchange dimethylbenzamyl (10 μM) was present from fraction 1. Values are means ± S.E.M. from 3 experiments with six replicates for each condition.

3.2. Histamine-induced [^{3}H]IP accumulation

In [^{3}H]inositol-labelled cells and in the presence of 20 mM LiCl, histamine (present for 2 min) stimulated total [^{3}H]IP accumulation in a concentration-dependent manner (Fig. 3). Best-fit values for the concentration-response curve yielded an EC_{50} of 17 ± 2 μM, maximum effect of 203 ± 4% of basal accumulation and Hill coefficient (n_H) 1.3 ± 0.2. In agreement with a H_1 receptor mediated effect, [^{3}H]IP accumulation induced by 1 mM histamine (199 ± 8% of basal) was blocked (99% inhibition) by 5 μM mepyramine (theoretical inhibition, 96–98%).

In order to test whether inositol phosphate formation was related to histamine-induced [^{3}H]GABA release, the action of U-73122, an inhibitor of agonist-induced phospholipase C activation (Thompson et al., 1991), was tested on the [^{3}H]GABA release stimulated by 100 μM histamine. Table 1 shows the effect of 10 μM U-73122, present for 30 min before exposure to histamine. Whereas U-7312 significantly inhibited the response to histamine, the inactive analogue U-73343 had no effect. The concentration of U-73122 chosen (10 μM) is that shown to inhibit more than 80% of phosphoinositide hydrolysis in SK-N-SH neuroblastoma cells (Thompson et al., 1991).

3.3. Histamine-induced Ca^{2+} mobilisation

Overall basal $[Ca^{2+}]_i$ in cell suspensions was 118 ± 6 nM ($n = 24$ experiments). As is shown in Fig. 4A his-

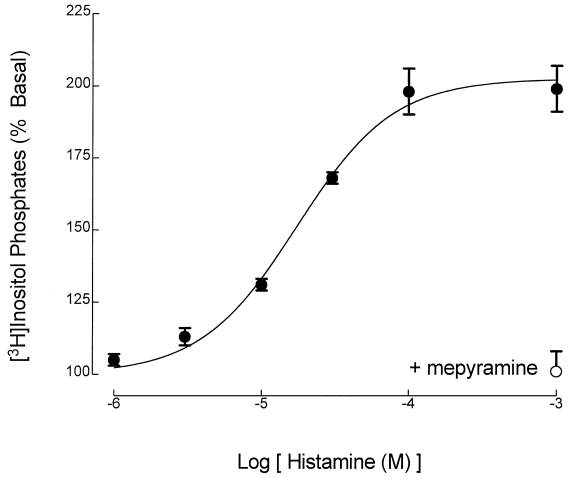

Fig. 3. Concentration-response curve for histamine-induced [^{3}H]IP accumulation. Cells grown in 12-well plates were labelled with [^{3}H]inositol as described in Materials and methods. After washing out the excess of [^{3}H]inositol cells were incubated for 10 min in KH buffer containing 20 mM LiCl before being exposed to histamine (1–1000 μM) for 2 min. [^{3}H]IPs were extracted and separated as described under Materials and methods. [^{3}H]IP accumulation is expressed as a percentage of basal accumulation (688 ± 21 dpm). Values are means ± S.E.M. from 3 experiments with four replicates for each condition. Where required, the selective H_1 receptor antagonist mepyramine (5 μM) was present 10 min before histamine. The curve drawn is the best-fit line to a logistic (Hill) equation. Best-fit values for the concentration-response are given in the text.

tamine (100 μM) evoked a rapid increase in $[Ca^{2+}]_i$ within the next few seconds after drug application with a peak $[Ca^{2+}]_i$ of 462 ± 21 nM. After this peak $[Ca^{2+}]_i$ declined rapidly to reach a second, sustained phase which remained throughout the experiment (up to 300 s). For the second, sustained phase $[Ca^{2+}]_i$ was 225 ± 8 nM ($\Delta[Ca^{2+}]_i = 107 \pm 10$ nM). Fig. 4A also shows that the rise in $[Ca^{2+}]_i$ induced by 100 μM histamine was fully

Table 1
Effect of U-73122 and U-73343 on histamine-induced [^{3}H]GABA release

	[^{3}H]GABA release (% basal)	% response to histamine (basal subtracted)
100 μM histamine	203 ± 7	100 ± 7
100 μM histamine + 10 μM U-73122	122 ± 5 [a]	21 ± 8 [a]
100 μM histamine + 10 μM U-73343	198 ± 5	95 ± 8

Cells grown in 12-well plates were incubated in KH buffer containing [^{3}H]GABA as described under Materials and methods. Non-captured [^{3}H]GABA was washed out and successive 2 min incubations (1 ml each) were then carried out. Histamine was added after collecting 3 fractions and [^{3}H]GABA release was expressed as a percentage of [^{3}H]GABA present in fraction 3 (478 ± 39 dpm, 1.7 ± 0.2% of [^{3}H]GABA captured). The inhibitor of phosphoplipase C activation U-73122 or its inactive analogue U-73343 were present for 30 min before testing the effect of histamine. Values are means ± S.E.M. from 3 experiments with four replicates for each condition. [a] Significantly different from histamine alone ($P < 0.01$, ANOVA and post hoc Dunnett's test).

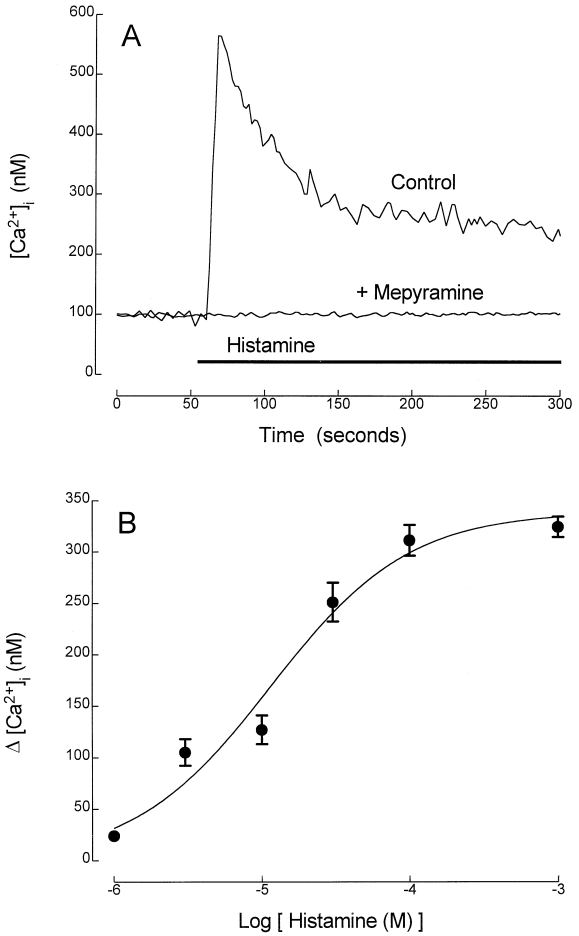

Fig. 4. Histamine-induced increases in $[Ca^{2+}]_i$. Cell suspensions were loaded with Fura 2-AM as described in Materials and methods and aliquots ($(2–3)\times10^6$ cells) were used for determination of $[Ca^{2+}]_i$ by fluorometry. (A) The effect of histamine (100 μM) was tested either in the presence or absence of the selective H_1 receptor antagonist mepyramine (1 μM) added at the beginning of the experiment. The traces are representative of 4 experiments carried out with different cell preparations. (B) Concentration-response curve. The histamine-induced increases in $[Ca^{2+}]_i$ ($\Delta[Ca^{2+}]_i$) were calculated at the peak of the response by subtracting the corresponding basal $[Ca^{2+}]_i$. Values are means ± S.E.M. from 4 experiments with three or four determinations for each concentration. The curve drawn is the best-fit line to a logistic (Hill) equation. Best-fit values for the concentration-response are given in the text.

blocked by the H_1 receptor antagonist mepyramine (1 μM; theoretical inhibition 98%). Histamine-induced increase in $[Ca^{2+}]_i$ was concentration-dependent (Fig. 4B). Best-fit values for the concentration-response curve yielded an EC_{50} of 12 ± 3 μM, maximum $\Delta[Ca^{2+}]_i$ of 339 ± 29 nM and Hill coefficient (n_H) 0.93 ± 0.19.

To study the dependence on extracellular Ca^{2+} the effect of histamine (100 μM) was assayed either in normal KH buffer or in KH buffer without $CaCl_2$ added. When tested in Ca^{2+}-free buffer, the initial peak remained unchanged but the second, sustained phase was not observed (Fig. 5).

The source of intracellular Ca^{2+} mobilised by H_1 re-

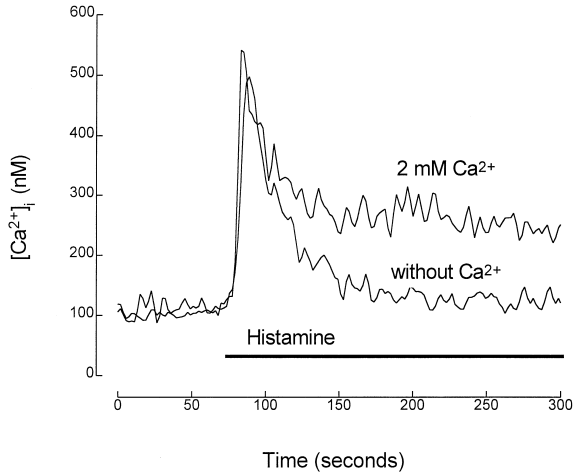

Fig. 5. Ca^{2+} dependence of histamine-induced increase in $[Ca^{2+}]_i$. Cell suspensions were loaded with Fura 2-AM as described in Materials and methods and $[Ca^{2+}]_i$ determined by fluorometry. The effect of histamine (100 μM) was tested in either normal KH buffer or KH buffer without added $CaCl_2$. The traces are representative of 5 experiments carried out in different cell preparations.

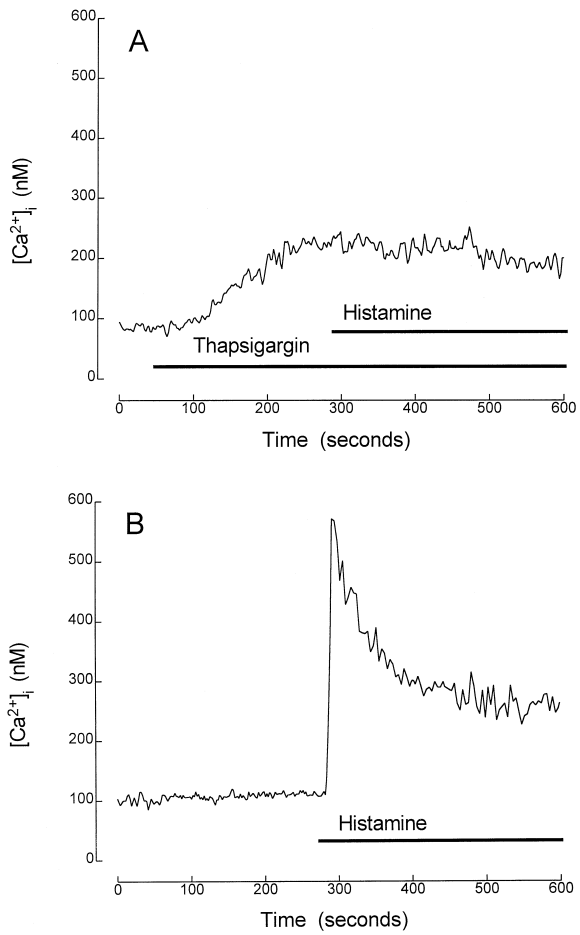

Fig. 6. Abolition of histamine-induced increase in $[Ca^{2+}]_i$ by thapsigargin. Cell suspensions were loaded with Fura 2-AM as described under Materials and methods and $[Ca^{2+}]_i$ determined by fluorometry. (A) Effect of thapsigargin (1 nM) applied before histamine (100 μM). (B) Control response to histamine. The traces are representative of 4 experiments carried out in different cell preparations.

ceptor activation was studied further by using thapsigargin, a drug which empties intracellular IP_3-sensitive Ca^{2+} stores (Simpson et al., 1995). Application of thapsigargin (1 nM) resulted in a increase of $[Ca^{2+}]_i$ ($\Delta[Ca^{2+}]_i$ 164 ± 12 nM, basal $[Ca^{2+}]_i = 94 \pm 8$ nM) which remained without change throughout the experiment (600 s). Once the effect of thapsigargin was established histamine (100 μM) produced no further increase in $[Ca^{2+}]_i$ (Fig. 6; $n = 6$ experiments). Thapsigargin was dissolved in dimethylsulfoxide (final concentration 0.1%) which had no effect by itself on basal $[Ca^{2+}]_i$. Since exposure to thapsigargin resulted in a sustained increase in $[Ca^{2+}]_i$ we tested whether thapsigargin by itself was able to stimulate [^{3}H]GABA release from U373 MG cells. Concentrations of 10 and 100 nM thapsigargin significantly stimulated [^{3}H]GABA release to values of $186 \pm 8\%$ and $260 \pm 30\%$ of basal respectively ($P < 0.01$, ANOVA and post hoc Dunnett's test, $n = 3$).

4. Discussion

We have shown herein that in U373 MG astrocytoma cells activation of histamine H_1 receptors is followed by inositol phosphate formation, Ca^{2+} mobilisation and release of [^{3}H]GABA previously taken up.

Termination of neurotransmission mediated by amino acids occurs through their removal from the synaptic cleft via carrier proteins present on both neuronal and glial cells (Barres, 1991; Attwell and Mobbs, 1994). Since glial carrier proteins usually have a higher affinity for their substrates than neuronal transport proteins do, they play an essential role in the inactivation of amino acid neurotransmitters. On the other hand, glial cells posses ionic channels (including voltage-activated channels) as well as receptors for several neurotransmitters, and recently there has been increasing evidence pointing out that glial cells may release the neurotransmitters previously taken up in response to depolarisation and to neurotransmitter-receptor activation (Murphy and Pearce, 1987; Barres, 1991; Martin, 1992; Sontheimer, 1994; Parpura et al., 1994).

The cell line U373 MG, derived from a human astrocytoma, possesses histamine H_1 receptors whose binding properties have been previously characterised (Arias-Montaño et al., 1994) providing evidence for such receptors being similar to those present in mammalian brain. Activation of H_1 receptors is coupled to inositol phosphate formation and Ca^{2+} mobilisation from intracellular stores (Hill, 1990) and our results showed that when [^{3}H]inositol-labelled U373 MG cells were exposed to histamine for 2 min (the same time the cells were assayed for histamine-induced [^{3}H]GABA release) [^{3}H]IP formation was significantly increased above basal levels in a concentration-dependent manner with this effect being blocked by mepyramine, a selective H_1 receptor antagonist.

In the U373 MG cells Ca^{2+} mobilisation stimulated by histamine showed a biphasic response, with an initial peak and a sustained secondary phase. This biphasic 'spike and

plateau' pattern has been often observed in response to histamine and other Ca^{2+}-mobilising transmitters in glial cells (Finkbeiner, 1993). Likewise histamine-induced [^{3}H]IPs formation, the increase in $[Ca^{2+}]_i$ was dependent on the agonist concentration (with an EC_{50} of 12 ± 3 μM resembling that for [^{3}H]IPs accumulation, 17 ± 2 μM) and was blocked by the H_1 receptor antagonist mepyramine. The potency of mepyramine to inhibit histamine-induced [^{3}H]IP formation, Ca^{2+} mobilisation and [^{3}H]GABA release was in good agreement with the K_D estimated (2.5 nM) for binding to H_1 receptors present on U373 MG cells (Arias-Montaño et al., 1994).

While histamine-induced rise in $[Ca^{2+}]_i$ as a whole is initiated by Ca^{2+} mobilisation from intracellular stores as evidenced by the abolition of the response by previous exposure to thapsigargin, a drug which empties intracellular IP_3-sensitive Ca^{2+} stores by selectively blocking ATP-dependent pumps (Simpson et al., 1995), the two phases seem to originate from different sources of Ca^{2+}. On the basis of their sensitivity to Ca^{2+} removal it appears that the first rapid and transient phase depends only on Ca^{2+} ions mobilised from intracellular stores whereas the second slow and sustained phase seems to be originated entirely by entry of Ca^{2+} ions from the extracellular medium since it was abolished in Ca^{2+}-free buffer. As stated above this biphasic pattern has been observed in several cell types and it has been suggested that the second phase is associated with Ca^{2+} entry triggered by the emptied intracellular stores with entering Ca^{2+} ions being required for refilling such stores (Putney, 1990; Putney and Bird, 1993).

Despite the presence of a component dependent on extracellular Ca^{2+} in H_1 receptor-mediated increases in $[Ca^{2+}]_i$, histamine-induced [^{3}H]GABA release does not appear to relay on Ca^{2+} entry since the effect remained unchanged when Ca^{2+} ions were removed from the KH buffer or when the experiment was carried out in the presence of Cd^{2+}, a non-selective blocker of Ca^{2+} channels. Furthermore glial or glial-derived cells are not known to have neurotransmitter-containing vesicles which could account for exocytotic GABA release. Thus, it can be assumed that [^{3}H]GABA release following H_1 receptor activation is related to IP_3 formation and Ca^{2+} mobilisation from intracellular stores. Further support for this supposition is provided by experiments showing that activation of tachykinin NK_1 receptors, which are also coupled to IP_3 formation and Ca^{2+} mobilisation (Tung and Lee, 1991; Eistetter et al., 1992; Pradier et al., 1993; Bordey et al., 1994) also stimulate [^{3}H]GABA release from U373 MG cells. Moreover, that an increase in $[Ca^{2+}]_i$ may lead to GABA release is supported by the fact that thapsigargin, which rises $[Ca^{2+}]_i$ by empting intracellular IP_3-sensitive Ca^{2+} stores, is able to stimulate [^{3}H]GABA independently of receptor stimulation. More direct evidence for the existence of a functional link between histamine-induced inositol phosphate formation and [^{3}H]GABA release is provided by our results showing that inhibition of phospholipase C activation due to the compound U-73122 (Thompson et al., 1991) results in a marked reduction of histamine-induced [^{3}H]GABA release whereas the inactive analogue U-73343 had no effect.

The mechanism by which cells buffer Ca^{2+} ions released from internal stores is 2-fold: Ca^{2+} ions are pumped back into intracellular stores by sarcoplasmic-endoplasmic reticulum Ca^{2+}-ATPases, or are extruded by plasma membrane Na^+/Ca^{2+} exchange and ATP-dependent pumps (Finkbeiner, 1993; Kostyuk and Verkhratsky, 1994). The extrusion of Ca^{2+} ions by Na^+/Ca^{2+} exchange would result in increased intracellular Na^+ concentration ($[Na^+]_i$) and this process appears to bear special relevance to explain the histamine-induced [^{3}H]GABA release observed in our experiments. In some instances release of amino acid neurotransmitters from glial cells shows correlation with changes in membrane potential and/or $[Na^+]_i$ rather than changes in $[Ca^{2+}]_i$ (Bernath, 1992; Gemba et al., 1994) and it has been suggested that elevated $[Na^+]_i$ promotes the release of neurotransmitters by reversing the Na^+-dependent transport carrier (Bernath, 1992). In neuronal and glial cells an increased $[Na^+]_i$ may originate GABA efflux by two mechanisms: directly by changing the gradient of Na^+ on which depends the GABA transport system or by depolarising the cell membrane and reversing thus the GABA carrier (Bernath, 1992; Adam-Vizi, 1992). In cultured chick retina cells activation of NMDA and non-NMDA ionotropic glutamate receptors results in increased [^{3}H]GABA release and this event is related to entry of Na^+ ions, since removal of external Na^+ abolished both effects (Duarte et al., 1993).

On the basis of our results the sequence of events accounting for histamine-induced [^{3}H]GABA release may be as follows: H_1 receptor activation stimulates PIP_2 hydrolysis and IP_3 formation. IP_3 binds to specific receptors and releases Ca^{2+} ions from thapsigargin-sensitive intracellular stores. Along being taken up again by the same stores, Ca^{2+} ions are extruded via plasma membrane Na^+/Ca^{2+} exchange raising thus $[Na^+]_i$. Modification of the transmembranal Na^+ gradient caused by Na^+ entry would then lead to the reversal of GABA transport and release of the amino acid. A role for Ca^{2+}/Na^+ exchange in histamine-induced [^{3}H]GABA by raising $[Na^+]_i$ after H_1 receptor-mediated Ca^{2+} mobilisation is supported by the significant reduction in histamine-induced [^{3}H]GABA release observed in the presence of dimethylbenzamyl, a selective blocker of Na^+/Ca^{2+} exchange (Kaczorowski et al., 1985; Andreeva et al., 1991).

In line with a role for increased $[Na^+]_i$ in [^{3}H]GABA release from U373 MG cells the ionophore monensine, which allows the entry of Na^+ ions into the intracellular space, induced a significant increase in [^{3}H]GABA efflux above basal levels. Substitution of choline for Na^+ ions also increased greatly (3–5-fold of basal) basal [^{3}H]GABA efflux supporting that changes in Na^+ transmembranal

gradients are able to bring about [^{3}H]GABA release. In both cases [^{3}H]GABA release follows what is expected for a Na^+-dependent carrier, since when external Na^+ is strongly diminished or when $[Na^+]_i$ is increased the direction of the carrier is reversed producing release (rather than uptake) of GABA (Bernath, 1992). Although the increased efflux of [^{3}H]GABA in Na^+-free buffer does not allow for a direct comparison making the evidence not conclusive, the lack of effect of histamine observed in Na^+-free buffer suggests that the presence of Na^+ ions in the extracellular medium is required for histamine-induced [^{3}H]GABA release.

In summary the data presented herein indicate that in the astrocytoma U373 MG cells activation of histamine H_1 receptors results in inositol phosphate formation, Ca^{2+} mobilisation and release of [^{3}H]GABA. The release of the tritiated amino acid appears to be related to reversal of GABA carrier due to changes in transmembranal Na^+ gradients provoked by stimulation of plasma membrane Na^+/Ca^{2+} exchange by increasing $[Ca^{2+}]_i$.

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References

Adam-Vizi, V., 1992, External Ca^{2+}-independent release of neurotransmitters, J. Neurochem. 58, 395.

Andreeva, N., B. Khodorov, E. Stelmashook, E. Cragoe, Jr. and I. Victorov, 1991, Inhibition of Na^+/Ca^{2+} exchange enhances delayed neuronal death elicited by glutamate in cerebellar granule cell cultures, Brain Res. 548, 322.

Arbonés, L., F. Picatoste and A. García, 1988, Histamine H_1-receptors mediate phosphoinositide hydroysis in astrocyte-enriched primary cultures, Brain Res. 450, 144.

Arbonés, L., F. Picatoste and A. García, 1990, Histamine stimulates glycogen breakdown and increases $^{45}Ca^{2+}$ permeability in rat astrocytes in primary cultures, Mol. Pharmacol. 37, 921.

Arias-Montaño, J.A., V. Berger and J.M. Young, 1994, Histamine- and carbachol-induced inositol phosphate formation in human U373 MG cells: receptor characteristics and Ca^{2+} dependence, Br. J. Pharmacol. 111, 598.

Attwell, D. and P. Mobbs, 1994, Neurotransmitter transporters, Curr. Opin. Neurobiol. 4, 353.

Barres, B.A., 1991, New roles for glia, J. Neurosci. 11, 3685.

Bernath, S., 1992, Calcium-independent release of amino acid neurotransmitters: fact or artifact? Prog. Neurobiol. 38, 57.

Berridge, M., 1993, Inositol triphosphate and calcium signalling, Nature 361, 315.

Bordey, A., P. Feltz and J. Trouslard, 1994, Mobilization of intracellular calcium by substance P in a human astrocytoma cell line (U-373 MG), Glia 11, 277.

Downes, C.P., P.T. Hawkins and R.F. Irving, l986, Ins(1,3,4,5)tetrakisphosphate and not PIP_2 is the probable precursor of Ins(1,3,4)triphosphate in stimulated parotid gland, Biochem J. 238, 501.

Duarte, C.B., I.L. Ferreira, P.F. Santos, C.R. Oliveira and A.P. Carvalho, 1993, Glutamate increases the $[Ca^{2+}]_i$ but stimulates Ca^{2+}-independent release of [^{3}H]GABA in cultured retina cells, Brain Res. 611, 130.

Eistetter, H.R., A. Mills, R. Brewster, S. Alouani, C. Rambosson and E. Kawashiwa, 1992, Functional characterization of neurokinin-1 receptors on human U373MG astrocytoma cells, Glia 6, 89.

Finkbeiner, S.M., 1993, Glial calcium, Glia 9, 83.

Gemba, T., T. Oshima and M. Ninomiya, 1994, Glutamate efflux via the reversal of the sodium-dependent glutamate transporter caused by glycolytic inhibition in rat cultured astrocytes, Neuroscience 63, 789.

Grynkiewicz, G., M. Ponie and R.Y. Tsien, 1985, A new generation of Ca^{2+} indicators with greatly improved fluorescence properties, J. Biol. Chem. 6, 3440.

Hill, S.J., 1990, Distribution, properties, and functional characteristics of three classes of histamine receptor, Pharmacol. Rev. 42, 46.

Inagaki, N. and H. Wada, 1994, Histamine and prostanoid receptors on glial cells, Glia 11, 101.

Inagaki, N., H. Fukui, Y. Taguchi, N.P. Wang, A. Yamatodani and H. Wada, 1989, Characterization of histamine H_1-receptor on astrocytes in primary culture: [^{3}H]-mepyramine binding studies, Eur. J. Pharmacol. 173, 43.

Inagaki, N., H. Fukui, Y. Taguchi, N.P. Wang, A. Yamatodani and H. Wada, 1991, Single type-2 astrocytes show multiple independent sites of Ca^{2+} signaling in response to histamine, Proc. Natl. Acad. Sci. USA 88, 4215.

Kaczorowski, G.J., F. Barros, J.K. Dethmers and M.J. Trumble, 1985, Inhibition of Na^+/Ca^{2+} exchange in pituitary plasma membrane vesicles by analogues of amiloride, Biochemistry 24, 1394.

Kondu, H., N. Inagaki, H. Fukui, Y. Koyama, A. Kanamura and H. Wada, 1991, Histamine-induced inositol phosphate accumulation in type-2 astrocytes, Biochem. Biophys. Res. Commun. 177, 734.

Kostyuk, P. and A. Verkhratsky, 1994, Calcium stores in neurons and glia, Neuroscience 63, 381.

Martin, D.L., 1992, Synthesis and release of neuroactive substances by glial cells, Glia 5, 81.

Murphy, S. and B. Pearce, 1987, Functional receptors for neurotransmitters on astroglial cells, Neuroscience 22, 381.

Pradier, L., E. Heuillet, J.P. Hubert, S. Laville, S. Le Guern and A. Doble, 1993, Substance P-evoked calcium mobilisation and ionic current activation in the human astrocytoma cell line U 373 MG: pharmacological characterization, J. Neurochem. 61, 1850.

Parpura, V., T.A. Basarsky, F. Liu, K. Jeftinija, S. Jeftinija and P.G. Haydon, 1994, Glutamate-mediated astrocyte-neuron signalling, Nature 369, 744.

Putney, J.W., 1990, Capacitative calcium entry revisited, Cell Calcium 11, 611.

Putney, J.W. and G.S. Bird, 1993, The inositol phosphate calcium signalling system in non-excitable cells, Endocrine Rev. 14, 610.

Schwartz, J.C., J.-M. Arrang, M. Garbarg, H. Pollard and M. Ruat, 1991, Histaminergic transmission in the mammalian brain, Physiol. Rev. 71, 1.

Sharpes, E.S. and R.L. McCarl, l982, A HPLC method to measure ^{32}P incorporation into phosphorilated metabolites in cultured cells, Anal. Biochem. 124, 421.

Simpson, P.B., R.A.J. Challis and S.R. Nahorski, 1995, Neuronal Ca^{2+} stores: activation and function, Trends Neurosci. 18, 299.

Sontheimer, H., 1994, Voltage-dependent ion channels in glial cells, Glia 11, 156.

Soria, L.E. and J.A. Arias-Montaño, 1995, Histamine H_1-receptor activation induces [^{3}H]GABA release from human U373 MG astrocytoma cells, Soc. Neurosci. Abstr. 21, 1130.

Thompson, A.K., S.P. Mostafapourt, L.C. Denlinger, J.E. Bleasdale and S.K. Fisher, 1991, The aminosteroid U-73122 inhibits muscarinic receptor sequestration and phosphoinositide hydrolysis in SK-N-SH neuroblastoma cells, J. Biol. Chem. 266, 23856.

Tung, W.L. and C.-M. Lee, 1991, Effects of tachykinins on [^{3}H]taurine release from human astrocytoma cells (U-373MG), Brain Res. 549, 171.