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Histamine-induced Ca^{2+} Entry in Human Astrocytoma U373 MG Cells: Evidence for Involvement of Store-operated Channels

Margarita Barajas,[1] Arturo Andrade,[1] Oscar Hernandez-Hernandez,[2] Ricardo Felix,[2] and Jose-Antonio Arias-Montaño[1]★

[1]Departamento de Fisiología, Biofísica y Neurociencias, México, D.F., México
[2]Departamento de Biología Celular, Centro de Investigación y de Estudios Avanzados (Cinvestav) del IPN, México, D.F., México

Glial and glia-derived cells express a variety of receptors for neurotransmitters and hormones, the majority of which evoke both Ca^{2+} release from intracellular stores and Ca^{2+} entry across the plasma membrane. We investigated the links between histamine H_1 receptor activation, Ca^{2+} release from intracellular stores and Ca^{2+} influx in human astrocytoma U373 MG cells. Histamine, through a H_1 receptor-mediated effect, evoked an increase in cytoplasmic free calcium concentration ($[Ca^{2+}]_i$) that occurred in two phases: an initial, transient, increase owing to Ca^{2+} mobilization from intracellular pools, and a second, sustained increase dependent on both Ca^{2+} influx and continuous receptor occupancy. The characteristics of histamine-induced increases in $[Ca^{2+}]_i$ were similar to the capacitative entry evoked by emptying of the Ca^{2+} stores with thapsigargine, and different from that observed when Ca^{2+} influx was activated with OAG (1-oleoyl-2-acetyl-*sn*-glycerol), a diacylglycerol (DAG) analog. OAG application or increased endogenous DAG, resulting from DAG kinase inhibition, reduced the histamine-induced response. Furthermore, activation of the DAG target, protein kinase C (PKC), by TPA (12-O-tetradecanoyl 4β-phorbol 13α-acetate) resulted in inhibition of the histamine-induced Ca^{2+} response, an action prevented by PKC inhibitors. By using reverse transcriptase–polymerase chain reaction analysis, mRNAs for transient receptor potential channels (TRPCs) 1, 4, and 6 as well as for STIM1 (stromal-interacting molecule) and Orai1 were found to be expressed in the U373 MG cells, and confocal microscopy using specific antibodies revealed the presence of the corresponding proteins. Therefore, TRPCs may be candidate proteins forming store-operated channels in the U373 MG cell line. Further, our results confirm the involvement of PKC in the regulation of H_1 receptor-induced responses and point out to the existence of a feedback mechanism acting via PKC to limit the increase in $[Ca^{2+}]_i$. **© 2008 Wiley-Liss, Inc.**

Key words: Ca^{2+} channels; H_1 receptor; histamine; TRP channels; STIM; Orai

Changes in the cytoplasmic free calcium concentration ($[Ca^{2+}]_i$) regulate a number of relevant physiological processes and have a major impact on cell development and function. These changes in $[Ca^{2+}]_i$ are mediated by two general mechanisms: Ca^{2+} release from intracellular stores and Ca^{2+} influx across the plasma membrane through specialized channels (Streb et al., 1983; Berridge, 1997; Hille, 2001). In nonexcitable cells Ca^{2+}-activated signaling by chemical messengers (agonists) occurs in two phases, an initial depletion of intracellular Ca^{2+} stores and a subsequent entry of Ca^{2+} from the extracellular space. This process is generally initiated by the interaction of an agonist with a $G\alpha_{q/11}$ protein-coupled receptor that triggers the activation of phospholipase C (PLC), which in turn breaks down phosphatidylinositol 4,5-biphosphate into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 induces the first phase of this signaling mechanism by interacting with its receptor channel (IP_3R) localized in the membrane of Ca^{2+} storing organelles. After a short delay, the second phase of this process is initiated (Parekh and Penner, 1977; Meldolesi et al., 1991).

Ca^{2+} influx induced by the activation of the PLC pathway may involve the opening of channels in the plasma membrane by second messengers such as DAG, protein kinase C (PKC), arachidonic acid and Ca^{2+} itself (Hille, 2001; Bird et al., 2004). These channels are known as receptor-operated channels. A second mechanism by which Ca^{2+} can enter the cells in response to the activation of the PLC pathway is triggered by depletion of Ca^{2+} stores. This process originates Ca^{2+} influx

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★Correspondence to: José A. Arias-Montaño, Departamento de Neurociencias, Cinvestav-IPN, Apdo. postal 14-740, 07000 México, D.F., México. E-mail: jaarias@fisio.cinvestav.mx

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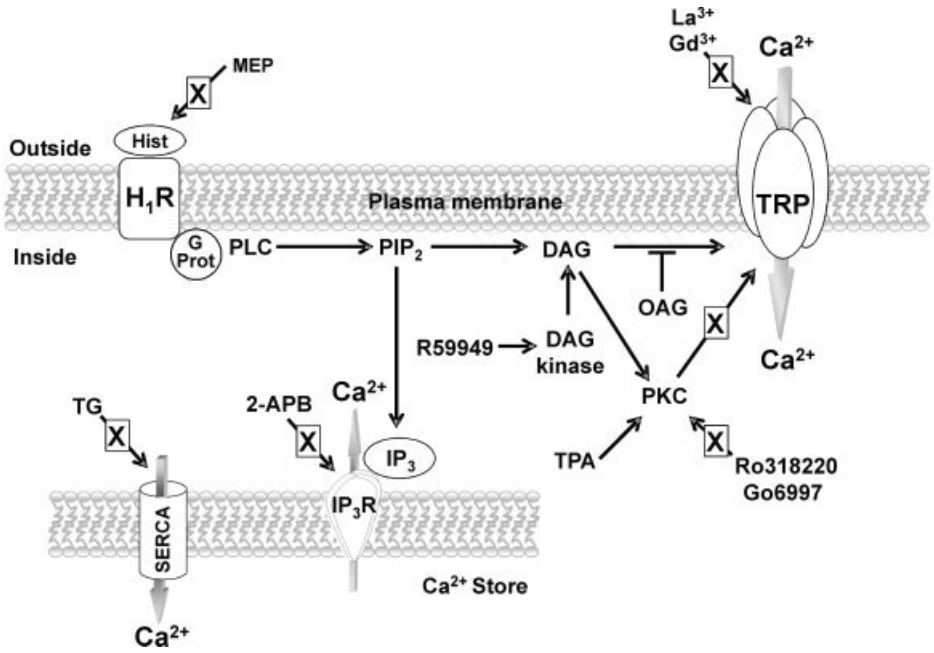

Fig. 1. Schematic overview of the protein channels and mechanisms involved in histamine-induced Ca^{2+} entry in human astrocytoma U373 MG cells. Hist, histamine; H_1R, histamine H1 receptor; MEP, mepyramine; G prot, G protein; PLC, phospholipase C; PIP_2, phosphatidylinositol 4,5 bisphosphate; DAG, diacylglycerol; TG, thapsigargine; SERCA, sarco(endo)plasmic reticulum calcium ATPase; 2-APB, 2-aminoethoxydiphenyl borate; IP_3, inositol 1,4,5-trisphosphate; IP_3R, IP_3 receptor; OAG, 1-oleoyl-2-acetyl-*sn*-glycerol; TPA, 12-O-tetradecanoyl 4β-phorbol 13α-acetate; PKC, protein kinase C; TRP, transient receptor potential channel.

by a mechanism named capacitative Ca^{2+} entry, which is mediated by proteins known as store-operated channels (SOCs) (Putney, 1986, 2001, 2005; Takemura and Putney, 1989). At present, it is thought that activation of multiple Ca^{2+} influx channels converge in the PLC-coupled receptor pathway (Jung et al., 2000).

In the central nervous system, one interesting property of nonexcitable glial cells is the expression of a wide variety of receptors for neurotransmitters and hormones, which gives them the ability to act as dynamic elements capable to integrate neuronal inputs and modulate neighboring cells (Araque et al., 2001). Although for glial cells it is well established that increases in $[Ca^{2+}]_i$ depend on the PLC activation, the precise molecular elements that participate in the Ca^{2+} entry and its regulation have not been elucidated.

Histamine is a widely distributed neurotransmitter in the mammalian central and peripheral nervous systems, where its actions are mediated mainly by four G-protein coupled receptors (H_1 to H_4) (Haas and Panula, 2003; Breunig et al., 2007). At the cellular level, H_1 and H_2 receptors have been identified not only in neurons, but also in astrocytes and blood vessel cells (Inagaki and Wada, 1994). Histamine-induced Ca^{2+} entry in glial cells and glioma-derived cell lines has been described, and the presence of store-operated and receptor-operated channels has been previously suggested (Arias-Montaño et al., 1994; Weiger et al., 1997; Jung et al., 2000; Mauban et al., 2006). However, very little is known regarding the molecular nature of the channels that participate in this physiological process and their regulation. In the current report, we explore the role of DAG and PKC in the regulation of the histamine-induced Ca^{2+} increase and the involvement of different proteins including the canonical transient receptor potential (TRPC) as possible intermediaries of the Ca^{2+} entry induced by the activation of the H_1 receptors expressed in the human astrocytoma cell line U373 MG (Fig. 1). Further, we show what is to our knowledge the first evidence for the expression in U373 MG cells of STIM1 (stromal-interacting molecule) and Orai1, two proteins that have been recently proposed to underlie the well-described Ca^{2+}-release-activated current (I_{CRAC}) in nonexcitable cells (Prakriya et al., 2006; Yeromin et al., 2006).

MATERIALS AND METHODS

Chemicals

Histamine, mepyramine (MEP), thapsigargine (TG), 2-aminoethoxydiphenyl borate (2-APB), flufenamic acid, TPA (12-O-tetradecanoyl 4β-phorbol 13α-acetate), La^{3+}, and Gd^{3+} were purchased from Sigma Chemical Co. (St. Louis, MO). OAG (1-oleoyl-2-acetyl-*sn*-glycerol), R59949, Ro 31-8220, and Gö 6967 were from Calbiochem (San Diego, CA).

Cell Culture

U373 MG cells (National Culture Collection, Porton Down, United Kingdom) were grown to confluence in

TABLE I. Sets of Primers Used to Amplify β-actin and the Distinct Ca^{2+} Channel Subunit Genes From U373MG Cells

Target gene	GenBank accession no.	Primer sequence
TRPC1	NM_003304	F: ATTTTGGAAAATTTCTTGGGATGT
		R: TTTGTCTTCATGATTTGCTATCA
TRPC3	NM_003305	F:GACATATTCAAGTTCATGGTCCTC
		R: ACATCACTGTCATCCTCAATTTC
TRPC4	NM_016179	F: CTGCAAATATCTCTGGGAAGA
		R: GCTTTGTTCGTGCAAATTTCC
TRPC5	NM_012471	F: AAGCACTCTTCGCAATATC
		R: TCCTTGCAAACTTCCACTC
TRPC6	NM_004621	F: GACATCTTCAAGTTCATGGTCATA
		R: ATCAGCGTCATCCTCAATTTC
TRPC7	NM_020389	F: TGCTGCTCAAGGGTGC
		R: CTGCTGACAGTTAGGGT
β-actin	NM_001101	F: CCAAGGCCAACCGCGAGAAGATGAC
		R: AGGGTACATGGTGGTGCCGCCAGAC
STIM	NM_003156	F: CAGAAGTACACAATTGGACC
		R: ATATAGGCAAACCAGCAGCC
Orai-1	NM_032790	F: AGAGTTACTCCGAGGTGATG
		R: TGACCGAGTTGAGATTGTGC

Dulbecco's modified Eagle medium and nutrient mixture HAM F-12 (1:1 v/v; Invitrogen; Carlsbad, CA), containing 10% of fetal bovine serum and supplemented with penicillin (100 IU/mL), streptomycin (0.1 mg/mL), and amphotericin B (0.25 μg/mL). Cells were grown as monolayers at 37°C in a 5% CO_2–95% air humidified atmosphere.

Measurements of Intracellular $[Ca^{2+}]$

Once the U373 MG cells had reached ~80% confluence, the culture medium was removed and the cells were washed with Dulbecco's phosphate-buffered saline (PBS; Sigma-Aldrich, St. Louis, MO) without Ca^{2+}. After 3 min of incubation in a trypsin/EDTA solution (Sigma-Aldrich), fresh culture medium was added, and the cellular suspension was spun down (100*g*, 3 min). The pellet was resuspended in 5 mL of Krebs-HEPES (KH) solution, supplemented with bovine serum albumin (5 mg/mL) 2 μM of the Ca^{2+} indicator fura-2 acetoxymethyl ester AM. After 90 min of incubation at room temperature in the dark, cells were washed by centrifugation (100*g*, 5 min) and resuspended in KH solution. The cell suspension was divided into aliquots and kept on ice until required.

Fluorometric analysis was performed in a Perkin-Elmer LS 50B spectrophotometer. Fluorescence was monitored at an emission wavelength of 510 nm, after excitation at 340 and 380 nm, as described by Grykiewicz et al. (1985). $[Ca^{2+}]_i$ was calculated from the following equation: $[Ca^{2+}]_i = K_d \times [(R - R_{max})/R_{max} - R)] \times [F_{min}\ (380\ nm)/F_{max}\ (380\ nm)]$, where K_d is the dissociation constant of Ca^{2+} for fura-2 (224 nM at 22°C), R_{max} is the maximal fluorescent ratio obtained after cell lysis with Triton X-100, and R_{min} is the minimal fluorescent ratio obtained from buffering Ca^{2+} with 5 mM EGTA. $F_{min}(380)$ and $F_{max}(380)$ are the fluorescence intensities, measured at 380 nm, in the absence and in the presence of saturating concentrations of Ca^{2+}, respectively.

The composition of the KH solution was (mM): NaCl 127, KCl 3.73, $MgSO_4$ 1.18, KH_2PO_4 1.18, $CaCl_2$ 1.8, HEPES 20, glucose 11, pH 7.4 with NaOH. For experiments with La^{3+} and Gd^{2+}, $MgCl_2$ and KCl were equimolarly substituted for $MgSO_4$ and KH_2PO_4, respectively.

Concentration–response data for agonist-induced increases in $[Ca^{2+}]_i$ were fitted by nonlinear regression to a Hill equation (logistic equation). The actual equation fitted was: $\text{Response} = [Resp_{max}\ C^n/(C^n + EC_{50}{}^n)]$, where $Resp_{max}$ is the maximum response, C is the agonist concentration, n is the Hill coefficient, and EC_{50} is the concentration giving the half-maximal response. The statistical significance of differences between sample means was established by the Student's *t*-test or ANOVA followed by a *post hoc*-test (Dunnett or Student-Newman-Keuls).

RNA Extraction and Reverse Transcription–Polymerase Chain Reaction

Total RNA was extracted from U373 MG cells, rat, and human brain with the TriZol reagent (Invitrogen). Isolated RNA was quantified by measuring its absorbance at 260 nm. The SuperScript First Strand system (Invitrogen) was used for reverse transcription polymerase chain reaction (RT-PCR) as recommended by the manufacturer, and 2 μg of total RNA was reverse transcribed by using oligo-dT primer. The resulting cDNA was used for amplification of the six human genes for *Trp* homologs, STM1, Orai1, and β-actin. The PCR cycling conditions included an initial denaturation step at 94°C for 5 min followed by 30–40 cycles at 94°C for 30 sec, 50–55°C for 1 min (depending on the set of primers used, see Table I), and 72°C for 30 sec. The final elongation step was at 72°C for 5 min. PCR reactions were then chilled at 4°C. Negative control reactions for RT-PCR were performed by omitting the use of retrotranscriptase in the reaction. All cDNAs amplified by PCR were sequenced on an automated sequencer (ABIPrism310; Perkin-Elmer Applied Biosystems, Foster City, CA).

TABLE II. Antibodies Used to Detect Distinct TRPC Channel Subunits in U373MG Cells

Antibody	Source	Specificity	Supplier	Dilution
TRPC1	Rabbit	Human	Alomone Labs (Jerusalem, Israel)	1:80
TRPC4	Rabbit	Mouse[a]	Alomone Labs (Jerusalem, Israel)	1:100
TRPC5	Rabbit	Human	Alomone Labs (Jerusalem, Israel)	1:120
TRPC6	Rabbit	Mouse[a]	Alomone Labs (Jerusalem, Israel)	1:120
Orai-1	Rabbit	Human	Alomone Labs (Jerusalem, Israel)	1:100
STIM-1	Mouse	Human	BD-Bioscience (San Jose, CA)	1:100
FITC-conjugated	Goat	—	Zymed Labs (San Francisco, CA)	1:200

[a]According to the BLAST results, the epitope sequence has an identity of 93% with respect to the human sequence.

Immunocytochemistry and Confocal Microscopy

U373 MG cells were seeded on glass coverslips and grown for 48 hr. Adhering cells were washed twice with PBS and fixed with 4% paraformaldehyde for 5 min at room temperature, rinsed three times with PBS containing 0.2% Triton X-100 (PBS-T), and blocked with 3% bovine serum albumin (in PBS) for 30 min. Samples were then incubated overnight at room temperature with primary antibodies directed against intracellular epitopes of TRPC (Table II). After rinsing with PBS (3 × 5 min), samples were incubated 1 hr at room temperature with fluorescein isothiocyanate–conjugated secondary antibodies (Table II) in the dark. Coverslips were then washed twice with PBS-T before nucleus staining with 10 μg/mL propidium iodide for 5 min at room temperature. Last, the coverslips were washed three times with PBS-T and twice with PBS before mounting with Vectashield (Vector Laboratories, Burlingame, CA). As negative controls, cell samples were incubated either with the secondary antibody alone or with the primary antibody preadsorbed with a specific blocking peptide (1:5 w/w).

Images were acquired with a Leica TCS SP2 confocal microscope by the Leica confocal software (LCS) 2.61 (Leica Microsystems, Wetzlar, Germany). An Ar/Kr laser (488 nm) was used for the excitation of fluorescein isothiocyanate, and the 543-nm line of a Ne laser was used to image the red fluorochrome. The section thickness was estimated to be 1 μm (pinhole aperture half open) with a 20× HC PL Fluotar numeric aperture 0.50 objective lens. Images were analyzed by LCS Lite (Leica) software. To enable comparison, all images were recorded using the same parameters of laser power and photomultiplier sensitivity. Images shown are representative of at least three separate experiments in each condition and were processed by using identical values for contrast and brightness.

RESULTS

Activation of the H_1 Receptor Induces Capacitative Ca^{2+} Entry in U373 MG Cells

In U373 MG cells in suspension, the resting mean $[Ca^{2+}]_i$ was 78 ± 4 nM (mean ± standard error from 40 determinations). Previous work showed the presence of H_1 receptors in this cell line (Arias-Montaño et al., 1994), and exposure to histamine (30 μM) in the presence of Ca^{2+} (1.8 mM) in the extracellular medium induced an increase in $[Ca^{2+}]_i$ that occurred in two temporally distinct phases: a fast transitory response with a peak of 413 ± 73 nM followed by a much slower increase in $[Ca^{2+}]_i$, which remained while histamine was present (Fig. 2A).

The histamine-induced in $[Ca^{2+}]_i$ (peak response) was concentration-dependent in the 1–100 μM concentration range (Fig. 2B), with EC_{50} 4.96 μM (pEC_{50} 5.30 ± 0.02) and Hill coefficient (n_H) 0.96 ± 0.12 not statistically different from unity (Student's *t*-test).

To determine whether histamine is required for the development of the two phases of Ca^{2+} mobilization, we tested the effect of MEP, a highly selective H_1 receptor antagonist (Fig. 1), applied at the onset and at the end of the transient phase. As can be seen in Figures 2C and 2D, MEP (3 μM) abolished the $[Ca^{2+}]_i$ responses, indicating that both the transient and the plateau phases of Ca^{2+} mobilization depend on the activation of H_1 receptors.

In order to delineate the Ca^{2+} pathways activated by histamine, the effects of the agonist (30 μM) were compared in Ca^{2+} (1.8 mM)-containing and nominally Ca^{2+}-free solutions (Fig. 3). Elimination of extracellular Ca^{2+} had only a minor effect on the initial increase in $[Ca^{2+}]_i$ induced by histamine application, but abolished the secondary component of the response (Fig. 3A,B). These results suggest that the Ca^{2+} response elicited by histamine in U373 MG cells consist of an intracellular Ca^{2+} release component, which is the major component of the initial increase, and a Ca^{2+} influx component, which sustained the secondary increase in $[Ca^{2+}]_i$.

Store-operated Ca^{2+} influx, originally called capacitative Ca^{2+} entry (CCE), is a ubiquitous phenomenon found in both electrically excitable and nonexcitable cells (Putney, 1986, 2001, 2005), and is important for refilling the intracellular Ca^{2+} stores after they are depleted after metabotropic receptor activation (Putney, 1986, 2007). The term *capacitative* in this particular type of Ca^{2+} influx was originally meant to imply the continuous loading and discharge of a Ca^{2+} store, much as in an electrical circuit, in which charge must load a capacitator before current can flow through it (Putney, 2007).

To determine whether CCE participates in the response to histamine, we used an "add-back" protocol that consists in the removal and readdition of Ca^{2+} to the extracellular medium after depletion of the intracellular Ca^{2+} stores by H_1 receptor activation. As it can be

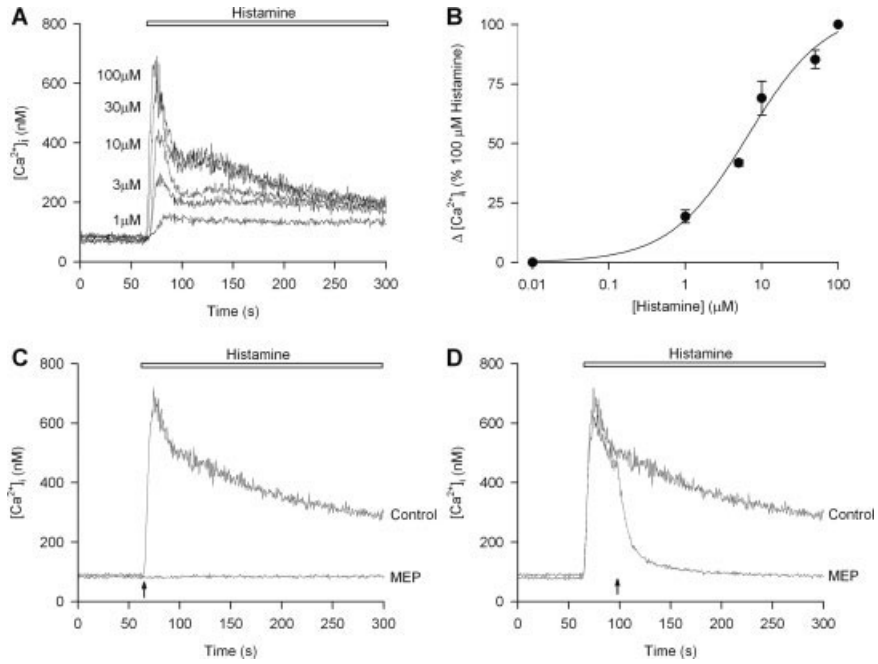

Fig. 2. Histamine-induced Ca^{2+} responses in U373 MG cells. **A:** Representative traces of Ca^{2+} responses from fura 2–loaded cells induced by increasing concentrations of histamine. The cytosolic Ca^{2+} concentration changes show the typical biphasic response induced by histamine (30 μM). Agonist application is indicated by the horizontal bar. **B:** Concentration-dependence of the histamine-induced increases in $[Ca^{2+}]_i$. Peak $[Ca^{2+}]_i$ responses in response to 0.01–100 μM histamine were normalized to the response evoked by 100 μM of the agonist. Values are mean ± SEM from four independent determinations for each concentration. The line is the best fit to a logistic (Hill) equation. Best-fit parameters are given in the text. **C:** Blockade by the H_1 receptor antagonist mepyramine (MEP, 3 μM) of the Ca^{2+} response induced by 30 μM histamine. **D:** Effect of mepyramine (MEP, 3 μM) on the plateau phase of the histamine-induced Ca^{2+} response. The arrow indicates the time of the application of the H_1 receptor antagonist.

seen in Figure 3, removal of extracellular Ca^{2+} prevented the plateau phase/secondary increase in the histamine-induced Ca^{2+} transient (Fig. 3C), and the increase in $[Ca^{2+}]_i$ upon readmission of extracellular Ca^{2+} was markedly reduced by the H_1 receptor antagonist MEP (Fig. 3C,D). Taken together, these observations suggest the presence of CCE in the U373 MG cells triggered by H_1 receptor activation.

To obtain an initial assessment of the channels involved in the histamine-induced Ca^{2+} entry, the effects of several nonselective cation channel blockers were examined by the add-back protocol. The pharmacological agents used have been shown to inhibit CCE acting at different levels (Fig. 1) (Trebak et al., 2003). In the presence of histamine (30 μM), application of 1 μM La^{3+} or Gd^{3+} as well as 30 μM 2-APB caused a significant inhibition of the agonist-induced Ca^{2+} transient in the range 70%–92% (Table III). Interestingly, very similar results were observed in a separate set of experiments, in which the same blockers were used to evaluate the Ca^{2+} entry triggered by TG, a classical CCE inductor that prevents the reuptake of Ca^{2+} into the endoplasmic reticulum and gradually depletes the intracellular Ca^{2+} stores. In contrast, application of La^{3+}, Gd^{3+} and 2-APB did not block the Ca^{2+} entry via receptor-operated channels activated by the membrane-permeant DAG analog, 1-oleoyl-2-acetyl-*sn*-glycerol (OAG). Furthermore, the ionic selectivity determined for the histamine-induced response showed a similar profile to that obtained for the TG-induced response, and was different from that observed when Ca^{2+} influx was activated with OAG (Table III). Together, these data strongly suggest that the Ca^{2+} influx triggered by H_1 receptor activation in U373 MG cells is related to the opening of SOCs.

Histamine-induced Ca^{2+} Mobilization Is Inhibited by DAG

Evidence that DAG activates native receptor-driven Ca^{2+} influx has been presented for different cell types (Chakrabarti and Chakrabarti, 2006). Therefore, to explore a possible role of DAG-activated channels in the histamine-induced Ca^{2+} entry, we analyzed the effect of OAG on Ca^{2+} mobilization in U373 MG cells by the add-back protocol. Upon application of OAG, a concentration-dependent $[Ca^{2+}]_i$ increase was observed (Fig. 4A,B), with an apparent threshold of 10 μM. Interestingly, when applied under free-Ca^{2+} conditions, OAG did not produce a significant change in basal $[Ca^{2+}]_i$, whereas in the add-back protocol, OAG induced significant Ca^{2+} entry upon readdition of Ca^{2+} to the extracellular medium (data not shown). We then proceeded to

characterize the OAG-induced Ca^{2+} entry by using the same channel blockers mentioned above and found that although La^{3+} induced a small inhibition, neither Gd^{3+} nor flufenamic acid exerted a significant action, and 2-APB even increased the magnitude of Ca^{2+} entry. Because this pharmacological profile is different from that observed for histamine- and TG-induced CCE (Table III), we speculated that the Ca^{2+} influx activated by OAG is mediated by DAG-activated channels. Notably, when OAG was applied concurrently with histamine, an inhibition of the Ca^{2+} response was observed (Fig. 4C). This inhibition was observed at concentrations as low as 3 μM (Fig. 4D), a value below that needed to activate DAG-dependent channels (Fig. 4A,B).

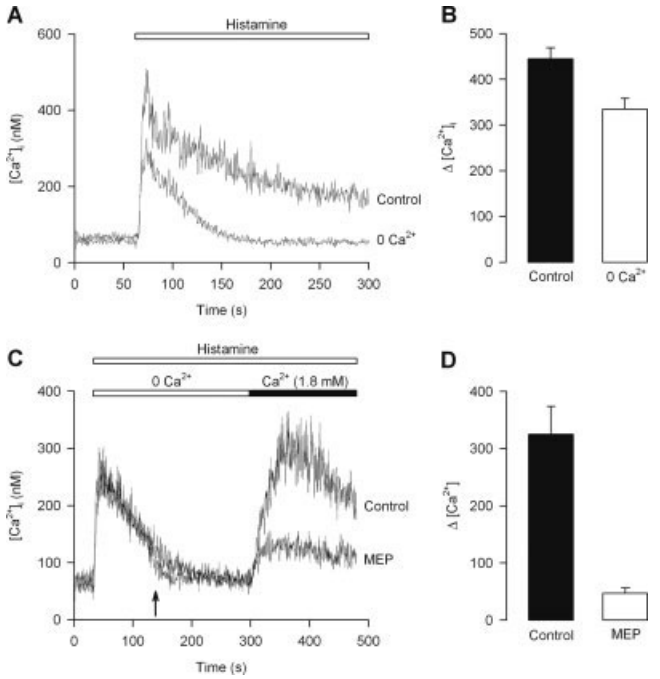

Fig. 3. Histamine induces capacitative Ca^{2+} entry. **A:** Representative traces showing the effect of extracellular Ca^{2+} removal on the Ca^{2+} response induced by histamine (30 μM). **B:** Analysis of the combined data from 5–7 independent determinations. Bars represent mean ± SEM. **C:** Representative traces of $[Ca^{2+}]_i$ measured by using the Ca^{2+} add-back protocol. Histamine (30 μM) application induces a second increase of $[Ca^{2+}]_i$ after the addition of extracellular Ca^{2+} that is significantly reduced by mepyramine (MEP, 3 μM). **D:** Analysis of the combined data from 5–7 independent determinations. Bars represent mean ± SEM. The response in 0 Ca^{2+} was statistically different from the control value ($P < 0.05$, Student's *t*-test).

PKC-dependent Desensitization of the H_1 Receptor: Implications for Histamine-induced Ca^{2+} Entry

In order to analyze in more detail the role of DAG in the histamine-induced Ca^{2+} entry, DAG intracellular levels were increased by preincubation with R59949, a selective inhibitor of DAG kinase (Fig. 1), one three major enzymes that normally metabolize DAG (Suh and Hille, 2006). As shown in Figure 5, incubation with R59949 (1 μM) resulted in about 80% decrease in Ca^{2+} entry promoted by H_1 receptor activation. Given that the main effector of DAG is PKC, we next explored the potential involvement of PKC in the DAG-induced inhibition of histamine-evoked Ca^{2+} entry. It is known that the PKC family consists of at least 12 isoforms, divided according to their mechanisms of activation in three groups: classical, novel and atypical, with the first two groups being activated by DAG (Way et al., 2000). To evaluate a possible involvement of PKC we used Ro 31-8220 (200 nM), a general PKC inhibitor (Keller and Niggli, 1993), and Gö 6967 (200 nM), a selective inhibitor of classical PKCs and PKCμ (Gschwendt et al., 1996) (Fig. 1). As can be seen in Figure 5, both PKC inhibitors partially prevented the inhibitory effect of R59949 on histamine-induced Ca^{2+} entry, suggesting DAG-mediated inhibition depends on PKC activity. Furthermore, when PKC was directly activated by 100 nM TPA, a strong inhibition in both phases of histamine-induced Ca^{2+} mobilization was observed, an effect antagonized by the PKC inhibitors Ro 31-8220 and Gö 6967 (Figs. 6A–D). In addition, activation of PKC with TPA significantly inhibited the Ca^{2+} entry induced by histamine in the add-back protocol (Figs. 6E,F).

TABLE III. Effects of Various Antagonists on the Ca^{2+} Influx Elicited by Histamine, Thapsigargine (TG) and 1-Oleoyl-2-acetyl-*sn*-glycerol (OAG)

Condition	Histamine-induced Ca^{2+} entry	TG-induced Ca^{2+} entry	OAG-induced Ca^{2+} entry
Control	200 ± 27 (8)	302 ± 26 (9)	148 ± 12 (7)
La^{3+} (1 μM)	59 ± 13 (11)	35 ± 6 (11)	31 ± 8 (5)
Control	244 ± 26 (9)	231 ± 42 (5)	220 ± 10 (4)
Gd^{3+} (1 μM)	51 ± 11 (7)	53 ± 9 (5)	224 ± 14 (4)
Control	1.2 ± 0.1 (13)	2.2 ± 0.2 (7)	0. 6 ± 0.1 (11)
Flufenamic acid (30 μM)	0.7 ± 0.1 (10)	1.5 ± 0.1 (8)	0.7 ± 0.1 (8)
Control	251 ± 13 (4)	312 ± 30 (3)	294 ± 69 (4)
2-APB (25 μM)	21 ± 2 (5)	61 ± 3 (3)	592 ± 87 (5)
Ionic selectivity	$Ca^{2+} > Ba^{2+} > Sr^{2+}$	$Ca^{2+} > Ba^{2+} > Sr^{2+}$	$Ba^{2+} > Ca^{2+} > Sr^{2+}$

Values are expressed as mean of $\Delta[Ca^{2+}]_i$ (La^{3+}, Gd^{3+} and 2-APB) or Δ F_{340}/F_{380} ratio (flufenamic acid) ± SEM. The number of experiments is indicated in parentheses. Drug concentrations were as follows: histamine (30 μM), thapsigargin (100 nM), OAG (30 μM).

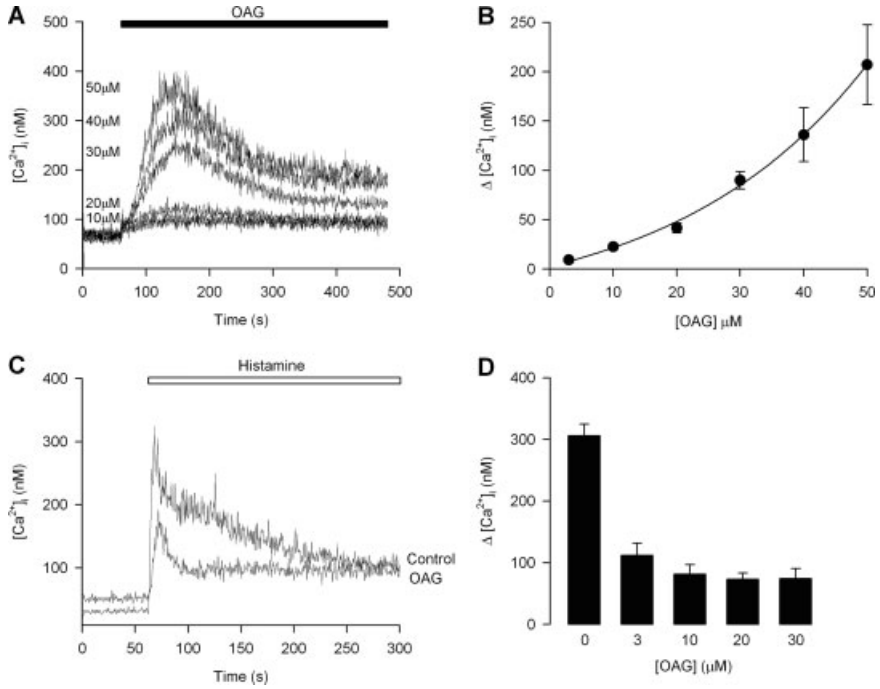

Fig. 4. OAG increases $[Ca^{2+}]_i$, but antagonizes the histamine-induced Ca^{2+} response. **A:** Representative traces of $[Ca^{2+}]_i$ changes induced by increasing concentrations of OAG. Drug application is indicated by the horizontal bar. **B:** Concentration dependence of the Ca^{2+} response induced by OAG application. **C:** Representative traces illustrating the inhibitory effect of OAG on the Ca^{2+} response induced by histamine (30 μM). **D:** Effect of increasing OAG concentrations on the histamine-induced cytosolic Ca^{2+} increase. Bars represent mean ± SEM from 3–6 independent determinations. All values in the presence of OAG were statistically different from histamine alone ($P < 0.001$, ANOVA and post hoc Dunnett test).

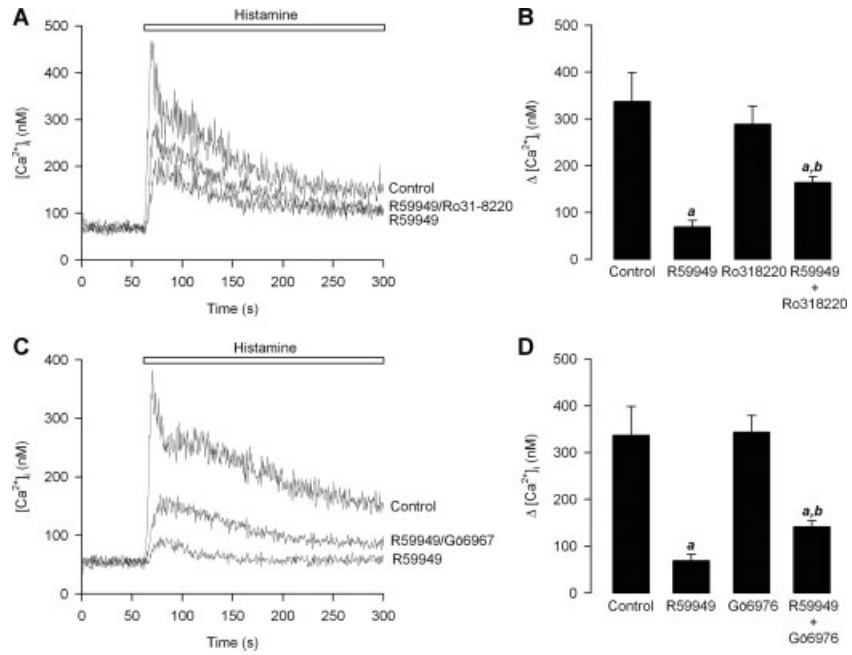

Fig. 5. PKC activation reduces histamine-induced Ca^{2+} responses. **A:** Representative traces of the Ca^{2+} response evoked by histamine (30 μM) application in the absence (control) and presence of the DAG kinase inhibitor R59949 (1 μM) alone or in combination with the general PKC inhibitor (Ro 31-8220, 200 nM). For the sake of clarity the trace for histamine and Ro 31-8220 is not shown. **B:** Analysis of 3–11 separate determinations as in (A). Bars represent mean ± SEM. (a) $P < 0.001$ when compared with control value, (b) $P < 0.05$ when compared with histamine plus R59949, ANOVA and Student-Newman-Keuls post hoc test. **C:** Recordings from fura 2–loaded U373 MG cells incubated with histamine in the absence (control**:** and presence of R59949 alone or in combination with the PKC inhibitor Gö 6967. For the sake of clarity, trace for histamine and Gö 6967 is not shown. **D:** Analysis of 3–11 determinations as in (C). (a) $P < 0.001$ when compared with control value. (b) $P < 0.05$ when compared with histamine plus R59949.

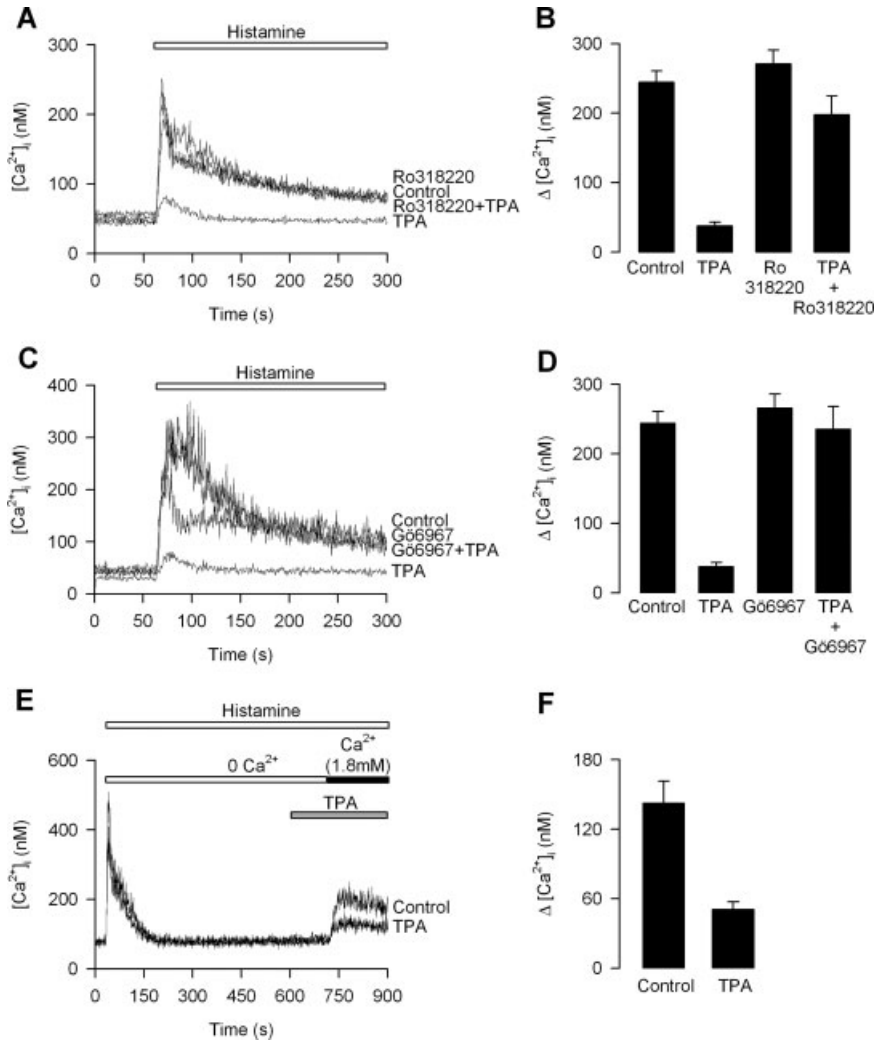

Fig. 6. PKC activation reduces the histamine-induced Ca^{2+} response. **A:** Representative recordings of the Ca^{2+} response evoked by histamine (30 μM) in fura 2–loaded U373 MG cells in absence (control) and presence of the PKC inhibitor Ro 31-8220 alone or in combination with the PKC activator TPA. For the sake of clarity trace for histamine and Ro 31-8220 is not shown. **B:** Analysis of 5–26 separate determinations as in (A). Bars represent mean ± SEM. The response in the presence of TPA was statistically different from the control value ($P < 0.001$, ANOVA and Student-Newman-Keuls *post hoc* test). **C:** Recordings from fura 2–loaded U373 MG cells that were incubated with histamine in the absence (control) and presence of the PKC antagonist Gö 6967 alone or in combination with TPA. **D:** Analysis of 14–26 determinations as in (C). The response in the presence of TPA was statistically different from the control value ($P < 0.05$). **E:** Representative traces of $[Ca^{2+}]_i$ measured by using the Ca^{2+} add-back protocol. Histamine application induces a second raise of $[Ca^{2+}]_i$ after addition of extracellular Ca^{2+} that is blocked by TPA application. **F:** Analysis of 5–6 experiments as in (E). The response in the presence of TPA was statistically different from the control value ($P < 0.05$, Student's *t*-test).

Together, these data indicate that PKC activation negatively regulates histamine-evoked Ca^{2+} entry in U373 MG cells and may help explain the effects of DAG on the Ca^{2+} response triggered by H_1 receptor activation. Given that the H_1 receptor is rapidly desensitized by PKC phosphorylation in a DAG-dependent manner (Bristow and Zamani, 1993; Warashina, 1997; Montero et al., 2003), our data imply that histamine-induced Ca^{2+} entry in U373 MG cells does not involve a mechanism dependent on DAG-activated channels. It has also been reported that PKC can directly modulate DAG-activated channels (Venkatachalam et al., 2003). To test this possibility, TPA was applied before OAG-induced Ca^{2+} mobilization and no significant effect was observed (not shown), suggesting that DAG-activated channels present in U373 MG cells are not regulated by PKC.

Human Astrocytoma U373 MG Cells Express Genes Related to Capacitative Ca^{2+} Entry

Although the molecular identity of the store-operated Ca^{2+} channels is not yet fully resolved, a growing body of evidence suggests that members of the transient receptor potential (TRP) channel superfamily are part of

the CCE pathway in many cell types (Montell et al., 2002; Vazquez et al., 2004). Particular focus has concentrated on the canonical TRPs (TRPCs), and a considerable amount of experimental evidence supports the notion that TRPC1 and/or TRPC4 may form or function as components of SOC (Smyth et al., 2006; Trebak et al., 2007). Likewise, through the use of RNAi technology, two other proteins (STIM1 and Orai1) have recently been involved in the store operated Ca^{2+} entry mechanism. STIM1 is a single spanning membrane protein with a Ca^{2+} binding EF-hand, which appears to function as the sensor of endoplasmic reticulum luminal Ca^{2+}, then transducing information to Orai1, a tetraspanning plasma membrane protein that functions as the Ca^{2+} permeable channel (Smyth et al., 2006; Hewavitharana et al., 2007).

Having shown that the Ca^{2+} influx triggered by histamine in the U373 MG cells is related to the opening of SOCs, we next sought to determine which channels involved in the capacitative Ca^{2+} entry were present in the U373 MG cells. Table I shows the sets of oligonucleotides that were designed to amplify in RT-PCR experiments the different human *Trpc* genes, as well as human STIM1 and Orai1 from the U373 MG cells. As shown in Figure 7, three of the six pairs of primers tested amplified products corresponding to the expected size (h*Trpc*1, -4, and -6), whereas no mRNA transcripts of the h*Trpc*3, -5, and -7 genes were detected. Amplicons were purified and sequenced to confirm their identity by database homology searches. Similarly, RT-PCR experiments in the U373 MG cells that used specific primers for human STIM1 and Orai1 revealed the presence of two fragments of 316 and 309 bp, respectively, providing experimental evidence for the presence of the mRNA coding these capacitative Ca^{2+} channel subunits. It is important to point out that although the pair of primers designed for STIM1 is able to detect also STIM2, the identity of the amplicon determined by automatic sequencing corresponded to STIM1 (data not shown). These results indicate that multiple genes coding proteins involved in CCE are expressed in the human astrocytoma U373 MG cell line.

Expression of TRPC proteins was also detected by confocal immunofluorescence microscopy. In agreement with the results from RT-PCR experiments, specific staining for TRPC1, -4, and -6 was observed (Fig. 8). All of the expressed TRPC proteins displayed ubiquitous subcellular distribution, showing predominant intracellular localization with a punctate pattern presumably corresponding to clusters of channels. In addition, and unexpectedly, by using a polyclonal antibody that recognizes TRPC5, we found that this protein is also expressed in U373 MG cells. As shown in Figure 7, the results of experiments in which RT-PCR was performed and specific primers were used to probe for TRPC mRNAs showed that only bands corresponding to TRPC1, -4, and -6 were amplified from these cells. Therefore, the expression of TRPC5 protein was a surprising result, and in consequence we used three different sets of

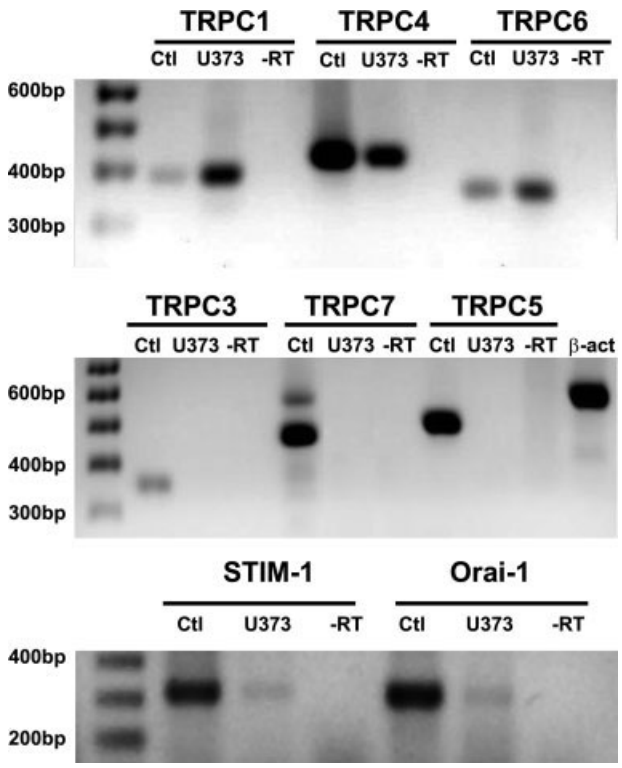

Fig. 7. Selective expression of SOCs in U373 MG cells as determined by RT-PCR. RNA isolated from U373 MG cell preparations was probed with selective primers for TRPC1, -4, and -6 (top), TRPC3, -5, and -7 (middle) as well as for STIM1 and Orai1 (bottom). Bands shown by ethidium bromide staining were confirmed by dideoxynucleotide sequencing. Rat brain-derived cDNA was used as a positive control (Ctl) for the various TRPC primers to test their ability to produce the correct amplicon, while human brain–derived cDNA was used as positive control (Ctl) for STIM1 and Orai1 expression. (−)RT denotes negative controls without retrotranscriptase.

TRPC5 oligonucleotides in a new series of RT-PCR experiments to provide evidence for the expression of the mRNA coding for this channel. Although the use of these primers showed the presence of bands with sizes that correctly matched the expected lengths of the amplified fragments of TRPC5 in rat brain (positive controls), bands representing TRPC5 mRNA were not detected in the total RNA sample from U373 MG cells. The reason for this discrepancy is unknown, but perhaps it lies within the presence of a novel splice variant not revealed in our RT-PCR experiments. If this was the case, our data suggest that a deletion might occur in the cytoplasmic region or regions coded by exons 5 and/or 7, because a PCR product was undetectable in the U373 MG cells by using the set of primers shown in Table I (nucleotides 1435–1930, corresponding to a PCR product of 495 bp) for TRPC5, while a product was detected in the positive controls (data not shown). It is also possible that the anti-TRPC5 antibody detected a variant form of TRPC5 in the U373 MG cells.

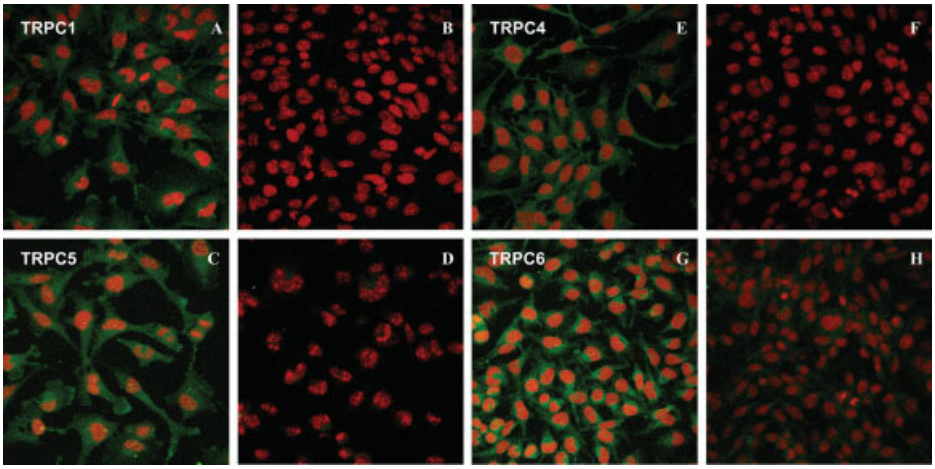

Fig. 8. U373 MG cells express different types of TRPC channels. Confocal indirect immunofluorescence images obtained from U373 MG cells labeled with specific antibodies against TRPC1 (**A**), TRPC4 (**E**), TRPC5 (**C**) and TRPC6 (**G**). Green and red signals are derived from fluorescein and nuclear staining with propidium iodide, respectively. Negative controls consisted in incubation of the samples with primary antibodies preincubated with their corresponding antigen peptides (**B,D,F,H**).

Although splice variants for human TRPC5 have not been definitely reported, the presence of a splice variant form of this protein has been suggested in human epidermoid carcinoma A431 cells (Yoshida et al., 2005). Likewise, it is worth mentioning that TRPC5 is intimately related to TRPC4, a protein for which 10 different splice variants have been so far reported (Cavalie, 2007). On the other hand, immunocytochemistry data gave a true picture of the TRPC expression (including TRPC5) because using the specific primary antibodies blocked with the corresponding peptide antigen showed negligible fluorescence staining (Fig. 8). Last, in addition to TRPC proteins, expression of STIM1 and Orai1 subunits was also detected by confocal immunofluorescence microscopy. Both expressed proteins displayed ubiquitous subcellular distribution, showing predominant intracellular localization with a punctate pattern (Fig. 9).

Together, the above mentioned results indicate that the U373 MG cells express TRPC1, -4, and -5, which are channels operated by store depletion, as well as TRPC6, a DAG-dependent channel that in under some circumstances may act also as a SOC (Liao et al., 2007). The finding of transcripts for STIM1 and Orai1 opens the interesting possibility that histamine might induce Ca^{2+} entry through more than one species of SOCs in the U373 MG cells.

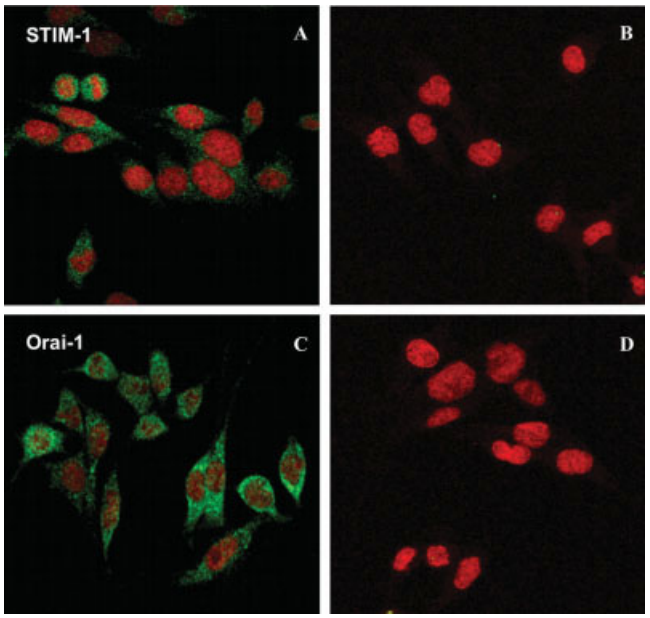

Fig. 9. U373 MG cells express STIM1 and Orai1 channel subunits. Confocal indirect immunofluorescence images obtained from cells labeled with specific antibodies against STIM1 (**A**) and Orai1 (**C**). Green and red signals are derived from fluorescein and nuclear staining with propidium iodide, respectively. Negative controls consisted in incubation of the samples with primary antibodies preincubated with their corresponding antigen peptides (**B,D**).

DISCUSSION

Human astrocytoma U373 MG cells respond to exogenously applied histamine with a large transient increase in $[Ca^{2+}]_i$ followed by a phase of sustained Ca^{2+} entry across the cell membrane. This action is mediated by the activation of H_1 receptors, expressed in relatively high density in the plasma membrane of U373 MG cells (Arias-Montaño et al., 1994) because MEP, a selective antagonist at H_1 receptors, abolished the increase in intracellular Ca^{2+} induced by histamine application (Fig. 2).

Our data with 1.8 mM external Ca^{2+} and a Ca^{2+}-free external solution (Fig. 3) corroborate that the histamine-mediated increase in intracellular Ca^{2+} occurs by both intracellular release and Ca^{2+} entry. The initial fast transient increase in intracellular Ca^{2+} appears to be primarily due to intracellular Ca^{2+} release whereas the sustained plateau level of increased Ca^{2+} reflects Ca^{2+} influx. This same pattern of Ca^{2+} release and entry has previously been reported for different cell types including endothelial and adrenal cells as well as astrocytes after histamine application (Zerbes et al., 1998; Jow and Numann, 2000; Jung et al., 2000; Mauban et al., 2006).

It is well established that Ca^{2+} influx can be mediated by various channel proteins located on the plasma membrane, such as receptor-operated or second messenger-activated channels (Hille, 2001). Likewise, store-operated Ca^{2+} infux is controlled by the filling state of intracellular Ca^{2+} pools located in the endoplasmic reticulum. Interestingly, TRPC proteins currently appear as the most attractive candidates for channels that mediate CCE (Montell et al., 2002; Vazquez et al., 2004; Putney, 2005; Smyth et al., 2006; Albert et al., 2007; Trebak et al., 2007). The notion that TRPC1 and -4 may form or be part of SOCs is supported mainly by two different lines of experimental evidence. First, ectopic expression of TRPC1 or -4 in several cell lines increases endogenous store-operated entry, and second, the use of antisense constructs directed against TRPC1 or -4, as well as genetic disruption of the corresponding genes, have efficiently reduced store-operated entry in a variety of cell types. In contrast, the role of TRPC3 and -5 in CCE is controversial (Smyth et al., 2006; Trebak et al., 2007). Last, when members of the TRPC3/6/7 subfamily are heterologously expressed in mammalian cell lines, all of them have been shown to be activated by membrane-permeant DAG analogs such as OAG (Hofmann et al., 1999; Vazquez et al., 2001; Smyth et al., 2006; Trebak et al., 2007).

To gain insight into the molecular mechanisms involved in the histamine-induced Ca^{2+} influx, U373 MG cells were first challenged with OAG. The results obtained show that these cells possess an endogenous cation influx pathway that can be activated by DAG. This pathway appears to be nonspecific in that it allows not only the influx of Ca^{2+} but also, and to a comparable extent, the influx of the surrogate cations Ba^{2+} and Sr^{2+} (Table III). According to what is mentioned in the preceding paragraph, members of the TRPC3/6/7 subfamily of channels may mediate the cation influx activated by DAG. Consistent with this possibility, both the mRNA transcript and evidence for the expression of the TRPC6 protein were found in the U373 MG cells. However, our data suggested that the DAG-activated channels were different from those responsible for histamine-evoked CCE. First, activation of Ca^{2+} influx by depleting endoplasmic reticulum Ca^{2+} stores with TG resulted in highly selective influx of Ca^{2+}, but little influx of Ba^{2+} and Sr^{2+} (Table III). Second, a variety of inhibitors blocked TG-induced CCE but not OAG-induced cation influx. Third, OAG actually causes a decrease in Ca^{2+} influx in the presence of histamine. Taken together, these findings indicate that the U373 MG cells possess a cation-influx pathway activated by DAG, which is unrelated to CCE evoked by H_1 receptor activation, and correlated with the presence of endogenous TRPC6 proteins in the plasma membrane.

Interestingly, H_1 receptor-mediated Ca^{2+} responses in U373 MG cells decreased after OAG application or increased endogenous DAG resulting from DAG kinase inhibition by R59949 (Figs. 4 and 5). It is known that H_1 receptor stimulation activates PLC to promote phosphoinositide hydrolysis in different cell types, including the U373 MG cell line (Bristow and Zamani, 1993; Hishinuma and Ogura, 2000; Mauban et al., 2006). Because PLC produces the endogenous PKC activator DAG, the possibility exists that the Ca^{2+} responses evoked by the activation of H_1 receptors may be autoregulated via PKC by a desensitization process. In line with this, PKC antagonists (Ro 31-8220 and Gö 6967) prevented in a partial but significant manner the inhibition caused by R59949. Furthermore, direct activation of PKC with TPA caused a decrease of the H_1 receptor-mediated Ca^{2+} response that was reversed by the PKC inhibitors. Taken as a whole, these results suggest the involvement of PKC in the regulation of the histamine-induced Ca^{2+} increase and point to the existence of a feedback mechanism that uses PKC to limit the increase in $[Ca^{2+}]_i$.

The nature of the Ca^{2+} permeable channels involved in CCE has been largely investigated in nonexcitable cells. As mentioned earlier, although its molecular identity remains elusive, canonical TRPC family members 1 and 4, have been put forward as possible candidates in different cell types (Wu et al., 2000; Philipp et al., 2000; Freichel et al., 2001; Beech et al., 2003). Because the histamine-evoked Ca^{2+} influx in the U373 MG cells was store-dependent, we hypothesized that Ca^{2+} permeable channels underlying CCE might be constituted of TRPC proteins, especially of the TRPC1 and -4 type. By RT-PCR, we found that both TRPC1 and -4 were endogenously expressed in the U373 MG cells. In contrast, several attempts to isolate h*Trpc*5 products by amplification of different regions were unsuccessful. In addition, no products were observed for h*Trpc*2, consistent with the observation that *Trpc*2 is a pseudogene in humans (Vannier et al., 1999). Last, by confocal laser scanning microscopy, we were able to detect the expression of TRPC1 and TRPC4 as well as TRPC5. The presence of multiple TRPCs in the U373 MG cells raises the possibility that channels could be comprised of homo- and heterotetramers, each with unique properties.

Likewise, a recent advance in the understanding of the potential molecular composition of SOCs has been the discovery of two families of transmembrane proteins, STIM and Orai, which have been proposed to mediate I_{CRAC}, a highly Ca^{2+}-selective current characterized in T-lymphocytes (Albert et al., 2007; Cahalan et al., 2007). STIM is single spanning transmembrane protein

located in the endoplasmic reticulum that functions as the sensor of endoplasmic reticulum luminal Ca^{2+} and transduces information directly to the plasma membrane. Orai, a tetra-spanning protein located in the plasma membrane, is a Ca^{2+} selective channel gated by interaction with STIM (Albert et al., 2007; Cahalan et al., 2007). Interestingly, RT-PCR analysis indicated that both STIM1 and Orai1 are expressed by U373 MG cells (Fig. 6).

However, because there are vast differences between the biophysical properties of I_{CRAC} and many SOCs (Albert et al., 2007), it seems unlikely that Orai proteins alone mediate the pore-forming subunits of all SOCs in U373 MG cells and other cell types. In fact, recent studies have suggested that STIM1 and Orai1 may interact with TRPC proteins to modify their function (Huang et al., 2006; Ong et al., 2007). Likewise, overexpression of Orai proteins was shown to enable TG to activate TRPC3 and -6 through a STIM1-mediated mechanism (Liao et al., 2007). These data suggest that STIM may act as a store-operated regulator of SOCs and also that Orai may combine with TRPCs to produce functional SOCs either through acting as a pore-forming subunit or as a regulatory subunit (Huang et al., 2006; Ong et al., 2007).

Last, it is worth noting that it remains to be determined which of these proteins (TRPCs, STIM1, and Orai1) expressed in U373 MG astrocytoma cells contribute to capacitative Ca^{2+} entry in a substantive way. In the meantime, our data add support to the hypothesis that H_1 receptor activation leads to capacitative Ca^{2+} entry in addition to intracellular Ca^{2+} release in this cell line.

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REFERENCES

Albert AP, Saleh SN, Peppiatt-Wildman CM, Large WA. 2007. Multiple activation mechanisms of store-operated TRPC channels in smooth muscle cells. J Physiol 583:25–36.

Araque A, Carmignoto G, Haydon PG. 2001. Dynamic signaling between astrocytes and neurons. Annu Rev Physiol 63:795–813.

Arias-Montaño JA, Berger V, Young JM. 1994. Calcium-dependence of histamine- and carbachol-induced inositol phosphate formation in human U373 MG astrocytoma cells: comparison with HeLa cells and brain slices. Br J Pharmacol 111:598–608.

Beech DJ, Xu SZ, Mc Hugh D, Flemming R. 2003. TRPC1 store-operated cationic channel subunit. Cell Calcium 33:433–440.

Berridge MJ. 1997. Elementary and global aspects of calcium signaling. J Physiol 499:291–306.

Bird GS, Putney JW Jr, Liveremont BJ, Wedel M, Trebak G, Vazquez J Jr. 2004. Mechanisms of phospholipase C–regulated calcium entry. Curr Mol Med 4:291–301.

Breunig E, Michel K, Zeller F, Seidl S, Hann V Weyhern CW, Schemann M. 2007. Histamine excites neurones in the human submucous plexus through activation of H1, H2, H3 and H4 receptors. J Physiol 583:731–742.

Bristow DR, Zamani MR. 1993. Desensitization of histamine H_1 receptor-mediated inositol phosphate production in HeLa cells. Brit J Pharmacol 109:353–359.

Cahalan MD, Zhang SL, Yeromin AV, Ohlsen K, Roos J, Stauderman KA. 2007. Molecular basis of the CRAC channel. Cell Calcium 42:133–144.

Cavalie A. 2007. Ionic channels formed by TRPC4. Handb Exp Pharmacol 179:93–108.

Chakrabarti R, Chakrabarti R. 2006. Calcium signaling in non-excitable cells: Ca^{2+} release and influx are independent events linked to two plasma membrane Ca^{2+} entry channels. J Cell Biochem 99:1503–1516.

Freichel M, Suh SH, Pfeifer A, Schweig U, Trost C, Weissgerber P, Biel M, Philipp S, Freise D, Droogmans G, Hofmann F, Flockerzi V, Nilius B. 2001. Lack of an endothelial store-operated Ca^{2+} current impairs agonist-dependent vasorelaxation in $TRP4^{-/-}$ mice. Nat Cell Biol 3:121–127.

Grykiewicz G, Poenie M, Tsien RY. 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 260:3440–3450.

Gschwendt M, Dieterich S, Rennecke J, Kittstein W, Mueller HJ, Johannes FJ. 1996. Inhibition of protein kinase C μ by various inhibitors. Differentiation from protein kinase C isoenzymes. FEBS Lett 392:77–80.

Hewavitharana T, Deng X, Soboloff J, Gill DL. 2007. Role of STIM and Orai proteins in the store-operated calcium signaling pathway. Cell Calcium 42:173–82.

Haas H, Panula P. 2003. The role of histamine and the tuberomamillary nucleus in the nervious system. Nat Rev Neurosci 4:121–130.

Hille B. 2001. Ion channels of excitable membranes,third ed., Sinauer Associates Inc., Sunderland, MA.

Hishinuma S, Ogura K. 2000. Ca^{2+}/calmodulin-mediated regulation of the desensitizing process in G_q protein-coupled histamine H_1 receptor-mediated Ca^{2+} responses in human U373 MG astrocytoma cells. J Neurochem 75:772–781.

Hofmann T, Obukhov AG, Schaefer M, Harteneck C, Gudermann T, Schultz G. 1999. Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. Nature 397:259–263.

Huang GN, Zeng W, Kim JY, Yuan JP, Han L, Muallem S, Worley PF. 2006. STIM1 carboxyl-terminus activates native SOC, Icrac and TRPC1 channels. Nat Cell Biol 8:1003–1010.

Inagaki N, Wada H. 1994. Histamine and prostanoid receptors on glial cells. Glia 11:102–110.

Jow F, Numann R. 2000. Histamine increases [Ca^{2+}]i and activates Ca-K and nonselective cation currents in cultured human capillary endothelial cells. J Membr Biol 173:107–116.

Jung S, Pfeiffer F, Deitmer JW. 2000. Histamine-induced calcium entry in rat cerebellar astrocytes: evidence for capacitative and non-capacitative mechanisms. J Physiol 527:549–561.

Keller HU, Niggli V. 1993. The PKC-inhibitor Ro 31-8220 selectively suppresses PMA- and diacylglycerol-induced fluid pinocytosis and actin polymerization in PMNs. Biochem Biophys Res Commun 194:1111–1116.

Liao Y, Erxleben C, Yildirim E, Abramowitz J, Armstrong DL, Birnbaumer L. 2007. Orai proteins interact with TRPC channels and confer responsiveness to store depletion. Proc Natl Acad Sci U S A 104:4682–4687.

Mauban JR, Wilkinson K, Schach C, Yuan JX. 2006. Histamine-mediated increases in cytosolic Ca^{2+} involve different mechanisms in human pulmonary artery smooth muscle and endothelial cells. Am J Physiol 290:C325–C336.

Meldolesi J, Clementi E, Fasolato C, Zacchetti D, Pozzan T. 1991. Ca influx following receptor activation. Trends Pharmacol Sci 12:289–292.

Montell C, Birnbaumer L, Flockerzi V. 2002. The TRP channels, a remarkably functional family. Cell 108:595–598.

Montero M, Lobatón CD, Gutierrez-Fernández S, Moreno A, Alvarez J. 2003. Modulation of histamine-induced Ca^{2+} release by protein kinase C. Effects on cytosolic and mitochondrial [Ca^{2+}] peaks. J Biol Chem 278:49972–49979.

Ong HL, Chen KT, Liu X, Bandyopadhyay BC, Paria BC, Soboloff J, Pani B, Gwack Y, Srikanth S, Singh BB, Gill D, Ambudkar IS. 2007. Dynamic assembly of TRPC1-STIM1-Orai1 ternary complex is involved in store-operated calcium influx. Evidence for similarities in store-operated and calcium-release-activated calcium channel components. J Biol Chem 282,9105–9116.

Parekh AB, Penner R. 1997. Store depletion and calcium influx. Physiol Rev 77:901–930.

Philipp S, Trost C, Warnat J, Rautmann J, Himmerkus N, Schroth G, Kretz O, Nastainczyk W, Cavalie A, Hoth M, Flockerzi V. 2000. TRP4 (CCE1) protein is part of native calcium release-activated Ca^{2+}-like channels in adrenal cells. J Biol Chem 275:23965–23972.

Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. 2006. Orai1 is an essential pore subunit of the CRAC channel. Nature 443:230–233.

Putney JW Jr. 1986. A model for receptor-regulated calcium entry. Cell Calcium 7:1–12.

Putney JW Jr. 2001. Pharmacology of capacitative calcium entry. Mol Interv 1:84–94.

Putney JW Jr. 2005. Capacitative calcium entry: sensing the calcium stores. J Cell Biol 169:381–382.

Putney JW Jr. 2007. Recent breakthroughs in the molecular mechanism of capacitative calcium entry (with thoughts on how we got here). Cell Calcium. 42:103–110.

Smyth JT, Dehaven WI, Jones BF, Mercer JC, Trebak M, Vazquez G, Putney JW Jr. 2006. Emerging perspectives in store-operated Ca^{2+} entry: roles of Orai, Stim and TRP. Biochim Biophys Acta 1763:1147–1160.

Streb H, Irvine RF, Berridge MJ, Schulz I. 1983. Release of Ca from nonmitochondrial intracellular store in pacnreatic acinar cells by inositol-(1,4,5)-trisphosphate. Nature 306:67–69.

Suh BC, Hille B. 2006. Does diacylglycerol regulate KCNQ channels? Pflugers Arch 453:293–301.

Takemura H, Putney JW Jr. 1989. Capacitative calcium entry in parotid acinar cells. Biochem J 258:409–412.

Trebak M, Vazquez G, Gary SJB, Putney JW Jr. 2003. The TRPC3/6/7 subfamily of cation channels. Cell Calcium 33:451–461.

Trebak M, Lemonnier L, Smyth JT, Vazquez G, Putney JW Jr. 2007. Phospholipase C-coupled receptors and activation of TRPC channels. Handb Exp Pharmacol 179:593–614.

Vannier B, Peyton M, Boulay G, Brown D, Qin N, Jiang M, Zhu X, Birnbaumer L. 1999. Mouse trp2, the homologue of the human trpc2 pseudogene, encodes mTrp2, a store depletion-activated capacitative Ca^{2+} channel. Proc Natl Acad Sci U S A 96:2060–2064.

Vazquez G, Lièvremont JP, Bird GS, Putney JW Jr. 2001. Human Trp3 forms both inositol trisphosphate receptor-dependent and receptor independent store-opareted cation channels in DT40 avian B-lymphocytes. Proc Natl Acad Sci U S A 98:11777–11782.

Vazquez G, Wedel BJ, Aziz O, Trebak M, Putney JW Jr. 2004. The mammalian TRPC cation channels. Biochim Biophys Acta 1742:21–36.

Venkatachalam K, Zheng F, Gill DL. 2003. Regulation of canonical transient receptor potential (TRPC) channel function by diacylglycerol and protein kinase C. J Biol Chem 278:29031–29040.

Warashina A. 1997. Involvement of protein kinase C in homologous desensitization of histamine-evoked secretory responses in rat chromaffin cells. Brain Res 762:40–46.

Way KJ, Chou E, King GL. 2000. Identification of PKC-isoform-specific biological actions using pharmacological approaches. Trends Pharmacol Sci 21:181–187.

Weiger T, Stevens DR, Wunder L, Haas HL. 1997. Histamine H1 receptors in C6 glial cells are coupled to calcium-dependent potassium channels via release of calcium from internal stores. Naunyn Schmiedeberg's Arch Pharmacol 355:559–565.

Wu X, Babnigg G, Villereal ML. 2000. Functional significance of human trp1 and trp3 in store-operated Ca^{2+} entry in HEK-293 cells. Am J Physiol 278:C526–C536.

Yeromin AV, Zhang SL, Jiang W, Yu Y, Safrina O, Cahalan MD. 2006. Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. Nature 443:226–229.

Yoshida J, Ishibashia T, Imaizumib N, Takegamic T, Nishioa M. 2005. Capacitative Ca^{2+} entries and mRNA expression for TRPC1 and TRPC5 channels in human epidermoid carcinoma A431 cells. Eur J Pharmacol 510:217–222.

Zerbes M, Bunn SJ, Powis DA. 1998. Histamine causes Ca^{2+} entry via both a store-operated and a store-independent pathway in bovine adrenal chromaffin cells. Cell Calcium 23:379–386.