Histamine-Induced Increases in Intracellular Free Ca^{2+} Levels in Hepatoma Cells

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Abstract

The effect of histamine on intracellular free Ca^{2+} levels ($[Ca^{2+}]_i$) in HA22/VGH human hepatoma cells were evaluated using fura-2 as a fluorescent Ca^{2+} dye. Histamine (0.2-5 μM) increased $[Ca^{2+}]_i$ in a concentration-dependent manner with an EC_{50} value of about 1 μM. The $[Ca^{2+}]_i$ response comprised an initial rise, a slow decay, and a sustained phase. Extracellular Ca^{2+} removal inhibited 50% of the $[Ca^{2+}]_i$ signal. In Ca^{2+}-free medium, after cells were treated with 1 μM thapsigargin (an endoplasmic reticulum Ca^{2+} pump inhibitor), 5 μM histamine failed to increase $[Ca^{2+}]_i$. After pretreatment with 5 ⇑ M histamine in Ca^{2+}-free medium for 4 min, addition of 3 mM Ca^{2+} induced a $[Ca^{2+}]_i$ increase of a magnitude 7-fold greater than control. Histamine (5 μM)-induced intracellular Ca^{2+} release was abolished by inhibiting phospholipase C with 2 μM 1-(6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl)-1H-pyrrole-2,5-dione (U73122), and by 5 μM pyrilamine but was not altered by 50 μM cimetidine. Together, this study shows that histamine induced $[Ca^{2+}]_i$ increases in human hepatoma cells by stimulating H1, but not H2, histamine receptors. The $[Ca^{2+}]_i$ signal was caused by Ca^{2+} release from thapsigargin-sensitive endoplasmic reticulum in an inositol 1,4,5-trisphosphate-dependent manner, accompanied by Ca^{2+} entry.

Key Words: cytosolic Ca^{2+}, fura-2, HA22/VGH hepatoma cells, histamine, liver

Introduction

Histamine has been shown to exert various physiological effects by stimulating one or more of at least three types of plasmalemmal receptors (H1, H2, H3) (1-8). An increase in intracellular free Ca^{2+} levels ($[Ca^{2+}]_i$) is a key signal for many cellular processes in all cell types (9-11). Depletion of intracellular Ca^{2+} often triggers Ca^{2+} refilling from extracellular space via a process called capacitative Ca^{2+} entry (12). Histamine was shown to increase $[Ca^{2+}]_i$ in various excitable and nonexcitable cells including mesothelial cells (1), endothelial cells (2), keratinocytes (3), neuronal cells (4), human gingival fibroblasts (5), epithelial cells (6), hair cells (7), glia cells (8), etc.. The mechanism underlying histamine-induced Ca^{2+} signal may be different in different cell types. While in most cell types histamine acts by stimulating H1 receptors, it acts via H2 histamine receptors in bovine cerebral endothelial cells (2) and human keratinocytes (3), and via all three types of histamine receptors in vestibular hair cells from guinea pigs (7).

In rat hepatocytes, histamine has been shown to stimulate three of the major metabolic pathways: glycogenolysis, gluconeogenesis from lactate, and

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ureagenesis (13, 14). The effect of histamine on $[Ca^{2+}]_i$ in human hepatocytes has not been explored previously. In rat hepatocytes, it was shown that histamine stimulates glycogenolysis by stimulating H1 histamine receptors leading to an increase in inositol 1,4,5-trisphosphate levels and $[Ca^{2+}]_i$ (13). However, the sources of the $[Ca^{2+}]_i$ increase and the requirement of inositol 1,4,5-trisphosphate for the $[Ca^{2+}]_i$ increase were unclear.

This study was aimed to characterize the effect of histamine on $[Ca^{2+}]_i$ in HA22/VGH human hepatoma cells. Ca^{2+} signaling in HA22/VGH cells has not been examined. In the present study $[Ca^{2+}]_i$ changes in populations of HA22/VGH cells were measured by using the fluorescent Ca^{2+} dye fura-2. It was found that histamine induced significant $[Ca^{2+}]_i$ increases. The concentration-response relationships both in the presence and absence of extracellular Ca^{2+}, the receptors involved, the Ca^{2+} sources, and the participation of inositol 1,4,5-trisphosphate were evaluated.

Materials and Methods

Cell Culture

HA22/VGH cells were obtained from American Type Culture Collection, and were cultured in Dulbecco's modified Eagle medium plus 10% fetal bovine serum. The medium was supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were kept at 37°C in 5% CO_2-containing humidified air.

Solutions

Ca^{2+} medium (pH 7.4) contained (in mM): NaCl 140; KCl 5; $MgCl_2$ 1; $CaCl_2$ 2; Hepes 10; glucose 5. Ca^{2+}-free medium contained no Ca^{2+} plus 1 mM EGTA.

Optical measurements of $[Ca^{2+}]_i$

Trypsinized cells (10^6/ml) were loaded with 2 μM 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2'-amino-5'-methylphenoxy)-ethane-N,N,N,N-tetraacetic acid pentaacetoxymethyl ester (fura-2/AM) for 30 min at 25°C in culture medium. Fura-2 fluorecence measurements were performed in a water-jacketed cuvette (25°C) with continuous stirring; the cuvette contained 1 ml of medium and 0.5 million cells. Fluorescence was monitored with a Shimadzu RF-5301PC spectrofluorophotometer (Shimadzu Corporation, Kyoto, Japan) by recording excitation signals at 340 and 380 nm and emission signal at 510 nm at 1-s intervals. Maximum and minimum fluorescence values were obtained by adding 0.1% Triton X-100 and 20 mM EGTA sequentially at the end of each experiment. $[Ca^{2+}]_i$ was calculated as previously described (15).

Chemical Reagents

The reagents for cell culture were from Gibco (Gaithersburg, MD, USA). Fura-2/AM was from Molecular Probes (Eugene, OR, USA). Histamine, U73122 (1-(6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl)-1H-pyrrole-2,5-dione) and U73343 (1-(6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl)-2,5-pyrrolidine-dione) were from Biomol (Plymouth Meeting, PA, USA). The other reagents were from Sigma (St. Louis, MO, USA).

Statistical Analysis

The traces were typical of 4-5 similar responses. All values were reported as means±SEM of 4-5 experiments. Because the data from each experiment were the average of responses from 0.5 million cells, the variation among experiments was small. This means that the mean±SEM of 4-5 experiments can reveal significant results. Statistical comparisons were determined by using Student's t test, and significance was accepted when $P<0.05$.

Results

Effect of Histamine on $[Ca^{2+}]_i$ in HA22/VGH Cells

At concentrations between 0.2-5 μM histamine increased $[Ca^{2+}]_i$ in the presence of extracellular Ca^{2+}. Figure 1A shows typical recordings of 5 μM (trace a) and 1 μM (trace b) histamine-induced $[Ca^{2+}]_i$ increases. At a concentration of 0.1 μM histamine had no effect (trace c). The basal $[Ca^{2+}]_i$ was 99±5 nM (n=5). The $[Ca^{2+}]_i$ signal comprised an initial rise, a slow decay and a plateau. The response saturated at 5 μM histamine because 50 μM histamine did not induce a greater response. At a concentration of 5 μM (trace a) histamine induced a $[Ca^{2+}]_i$ increase which reached a net maximum value of 221±4 nM (baseline subtracted; n=4). The $[Ca^{2+}]_i$ signal gradually decayed and reached a plateau of a net value of 41±3 nM. The concentration-dependent plot is shown in Figure 1C (solid circles) which indicates an EC_{50} value of about 1.2 μM, calculated by fitting the Hill equation to the data.

Effect of Extracellular Ca^{2+} Removal on the Histamine Response

Figure 1B shows 5 μM histamine-induced response in Ca^{2+}-free medium (trace a; time points

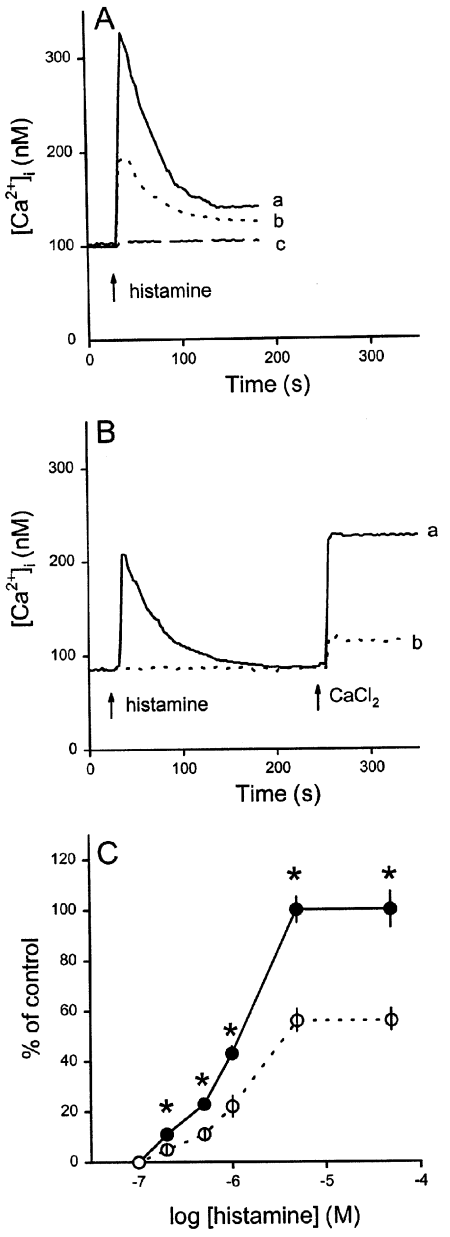

Fig. 1. Effect of histamine on $[Ca^{2+}]_i$. (A) Concentration-dependent effects of histamine on $[Ca^{2+}]_i$. The concentration of histamine was 5 μM in trace a, 1 μM in trace b, and 0.1 μM in trace c. Experiments were performed in Ca^{2+} medium. (B) Effect of extracellular Ca^{2+} removal on histamine-induced $[Ca^{2+}]_i$ increases and the effect of reintroduction of Ca^{2+}. Trace a: Histamine (5 μM) was added at 30 s in Ca^{2+}-free medium followed by addition of 3 mM $CaCl_2$ at 260 s. Trace b: control effect of $CaCl_2$ without histamine preincubation. (C) Concentration-response plots of histamine-induced Ca^{2+} signals both in Ca^{2+} medium (solid circles) and Ca^{2+}-free medium (open circles). The y axis is the percentage of control which was defined as the net (baseline subtracted) peak $[Ca^{2+}]_i$ of 5 μM histamine-induced $[Ca^{2+}]_i$ signal. *P<0.05. Traces were typical of 4-5 similar experiments.

between 30-250 s). The basal $[Ca^{2+}]_i$ was 81±5 nM (n=4). The response had a net maximum value of 111±5 nM. The sustained phase of histamine-induced $[Ca^{2+}]_i$ increases in Ca^{2+} medium was abolished. The concentration-response relationship of histamine-induced $[Ca^{2+}]_i$ increases in Ca^{2+}-free medium is shown in Figure 1C (open circles). The two curves in Figure 1C suggest that Ca^{2+} removal inhibited 0.2-50 μM histamine-induced $[Ca^{2+}]_i$ increases by 49±2% in the net maximum value (n=4-5; P<0.05).

Mechanism of Histamine-Induced Extracellular Ca^{2+} Entry

Mobilization of intracellular Ca^{2+} in most cells activates extracellular Ca^{2+} entry via capacitative Ca^{2+} entry (12). Capacitative Ca^{2+} entry is often evaluated by reintroducing extracellular Ca^{2+} following depleting intracellular Ca^{2+} stores with the tested agent in Ca^{2+}-free medium. Figure 1B shows that after cells were pretreated with 5 μM histamine for 220 s (trace a), addition of 3 mM Ca^{2+} induced capacitative Ca^{2+} entry with a net maximum value of 142±5 nM (n=5) which was 7-fold greater than control (trace b, without histamine treatment; n=5, P<0.05).

Effect of Cimetidine and Pyrilamine on Histamine-Induced $[Ca^{2+}]_i$ Increases

Figure 2A shows that 5 μM histamine-induced $[Ca^{2+}]_i$ increases in Ca^{2+} medium were not altered by pretreatment with 50 μM cimetidine (a H2 histamine receptor antagonist) (n=4). Figure 2B shows that the histamine was abolished by pretreatment with 5 μM pyrilamine (a H1 histamine receptor antagonist) (n=5). Cimetidine and pyrilamine did not affect basal $[Ca^{2+}]_i$.

Intracellular Sources of Histamine-Induced $[Ca^{2+}]_i$ Increases

Figure 3 shows that in Ca^{2+}-free medium, addition of thapsigargin (an endoplasmic reticulum Ca^{2+} pump inhibitor (16)) induced a $[Ca^{2+}]_i$ increase with a net maximum value of 32±3 nM (n=4). Addition of 5 μM histamine 4 min after thapsigrgin treatment failed to increase $[Ca^{2+}]_i$.

Involvement of Inositol 1,4,5-Trisphosphate in Histamine-Induced Ca^{2+} Release

Figure 4 shows that incubation with the phospholipase C inhibitor U73122 (2 μM) (17) for 170 s in Ca^{2+}-free medium to suppress inositol 1,4,5-trisphosphate formation did not elevate basal $[Ca^{2+}]_i$ but abolished 5 μM histamine-induced $[Ca^{2+}]_i$ increases (n=4). In contrast, U73343 (10 μM), an inactive U73122 analogue, had no effect (not shown).

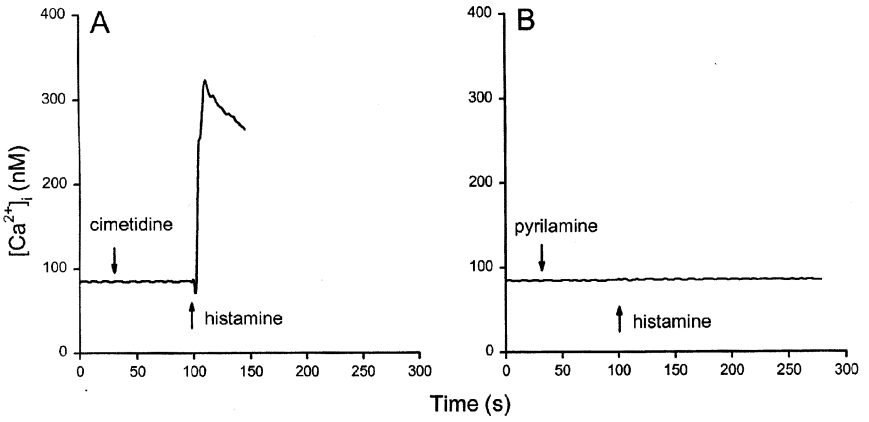

Fig. 2. Effect of H1 and H2 histamine receptor antagonists on histamine-induced $[Ca^{2+}]_i$ increases. Experiments were performed in Ca^{2+}-free medium. (A) 50 μM cimetidine was added at 20 s followed by 5 μM histamine added at 100 s. (B) 5 μM pyrilamine was added at 20 s followed by 5 μM histamine added at 100 s. Traces were typical of 4-5 similar experiments.

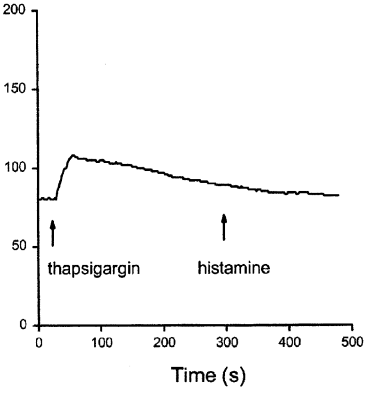

Fig. 3. Intracellular Ca^{2+} stores of histamine-induced $[Ca^{2+}]_i$ increases. In Ca^{2+}-free medium, 1 μM thapsigargin was added at 20 s followed by 5 μM histamine added at 300 s. Traces were typical of 4-5 similar experiments.

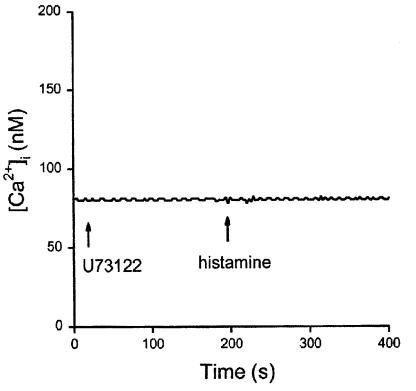

Fig. 4. Effect of inhibiting inositol 1,4,5-trisphosphate formation on histamine-induced intracellular Ca^{2+} release. In Ca^{2+}-free medium, 2 μM U73122 was added at 20 s followed by 5 histamine added at 200 s. Traces were typical of 4-5 similar experiments.

Discussion

The present study is the first to examine the effect of histmine in human hepatocytes. The data suggest that histamine induced a concentration-dependent $[Ca^{2+}]_i$ increase between 0.2-5 μM with an EC_{50} of about 1 μM in hepatoma cells. This Ca^{2+} signal is equally contributed by extracellular Ca^{2+} entry and intracellular Ca^{2+} release because it was inhibited by 50% by extracellular Ca^{2+} removal. Specifically, the sustained phase of the response in Ca^{2+} medium was abolished by Ca^{2+} removal, suggesting that this phase was attributed to extracellular Ca^{2+} influx.

The data indicate that H1 histamine receptors but not H2 histamine receptors mediate histamine-induced $[Ca^{2+}]_i$ increases because the signal was abolished by pyrilamine but was not affected by cimetidine. Histamine appears to release intracellular Ca^{2+} from the thapsigargin-sensitive endoplasmic reticular store because in Ca^{2+}-free medium histamine failed to increase $[Ca^{2+}]_i$ after thapsigargin pretreatment for several min. Further, the data suggest that histamine employs inositol 1,4,5-trisphosphate to release the stored Ca^{2+} because the histamine-induced Ca^{2+} release was abolished after inositol 1,4,5-trisphosphate formation was suppressed by inhibiting phospholipase C with U73122. This is consistent with previous findings that histamine utilizes inositol 1,4,5-trisphosphate as a second messenger to mobilize intracellular Ca^{2+} in other cell types (1-8).

Another question was how histamine induces Ca^{2+} influx. Histamine may cause Ca^{2+} influx via capacitative Ca^{2+} entry because reintroduction of 3 mM Ca^{2+} to cells that were depleted of intracellular Ca^{2+} by 5 μM histamine induced a $[Ca^{2+}]_i$ increase which was substantially greater than control.

However, the data do not exclude the possibility that histamine may cause Ca^{2+} entry through other types of channels such as receptor-operated channels and second messenger-operated channels, in a manner independent of Ca^{2+} store depletion.

Together, this study shows that histamine induces significant increases in $[Ca^{2+}]_i$ in human hepatoma cells. Histamine increases $[Ca^{2+}]_i$ in a concentration-dependent manner with an EC_{50} of 1 μM by activating H1 histamine receptors. The signal is caused by inositol 1,4,5-trisphosphate-induced intracellular Ca^{2+} release from thapsigargin-sensitive endoplasmic reticulum stores, and also by extracellular Ca^{2+} entry.

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