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Short communication

Mechanisms of histamine-induced intracellular Ca^{2+} release and extracellular Ca^{2+} entry in MG63 human osteosarcoma cells

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Abstract

The effect of histamine on intracellular free Ca^{2+} levels ($[Ca^{2+}]_i$) in MG63 human osteosarcoma cells was explored using fura-2 as a Ca^{2+} dye. Histamine increased $[Ca^{2+}]_i$ in a concentration-dependent fashion with an EC_{50} value of 0.5 μM. Extracellular Ca^{2+} removal inhibited the $[Ca^{2+}]_i$ signals. Histamine failed to increase $[Ca^{2+}]_i$ in Ca^{2+}-free medium after cells were pretreated with thapsigargin (an endoplasmic reticulum Ca^{2+} pump inhibitor). Addition of Ca^{2+} induced concentration-dependent $[Ca^{2+}]_i$ increases after preincubation with histamine in Ca^{2+}-free medium. Histamine-induced intracellular Ca^{2+} release was abolished by inhibiting phospholipase C with 1-(6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl)-1*H*-pyrrole-2,5-dione (U73122). The $[Ca^{2+}]_i$ increase induced by histamine in Ca^{2+} medium was abolished by cimetidine, but was not altered by pyrilamine, nifedipine, verapamil, and La^{3+}. Together, this study shows that histamine increased in $[Ca^{2+}]_i$ in osteosarcoma cells by stimulating H2 histamine receptors. The Ca^{2+} signal was caused by Ca^{2+} release from the endoplasmic reticulum in a phospholipase C-dependent manner. The Ca^{2+} release was accompanied by Ca^{2+} influx.

Keywords: Bone; Ca^{2+} signaling; Fura-2; MG63 cells; Osteosarcoma

1. Introduction

Changes in cytosolic free Ca^{2+} levels ($[Ca^{2+}]_i$) regulate diverse cellular events, ranging from secretion and contraction to modulation of cell growth and proliferation [1–4]. In non-excitable cells, receptor-evoked $[Ca^{2+}]_i$ increases usually are initiated by a rapid IP_3-dependent release of Ca^{2+} from the endoplasmic reticulum [3,4], followed by an extracellular Ca^{2+} influx termed capacitative Ca^{2+} entry [5,6].

Histamine is a biogenic amine found in large quantities in most tissues, especially in mast cells [7]. Three types of histamine receptors have been characterized, coded as the H1, H2, and H3 receptors based on their sensitivity to different agonists and antagonists [8,9]. Histamine was shown to increase $[Ca^{2+}]_i$ in excitable and non-excitable cells [10–12]. The aim of this study was to investigate the effect of histamine on $[Ca^{2+}]_i$ in osteosarcoma cells. Both extracellular Ca^{2+} levels and intracellular Ca^{2+} signaling play important roles in the pathophysiology of osteoblasts [13–15]. The effect of histamine on Ca^{2+} signaling in bone cells is unclear. The MG63 human osteosarcoma cell line is a useful model in bone cell research. It was shown that the calmodulin inhibitor W-7 increases $[Ca^{2+}]_i$ in MG63 cells [16], however, the effects of other agonists are unknown.

It was found that histamine increased $[Ca^{2+}]_i$ in MG63 cells when fura-2 was used as a Ca^{2+} dye. The concentration–response relationships and the mechanisms underlying the $[Ca^{2+}]_i$ increase, such as the receptors involved and the Ca^{2+} sources, were investigated.

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Abbreviations: $[Ca^{2+}]_i$; Cytosolic free Ca^{2+} concentration; and IP_3, inositol 1,4,5-trisphosphate.

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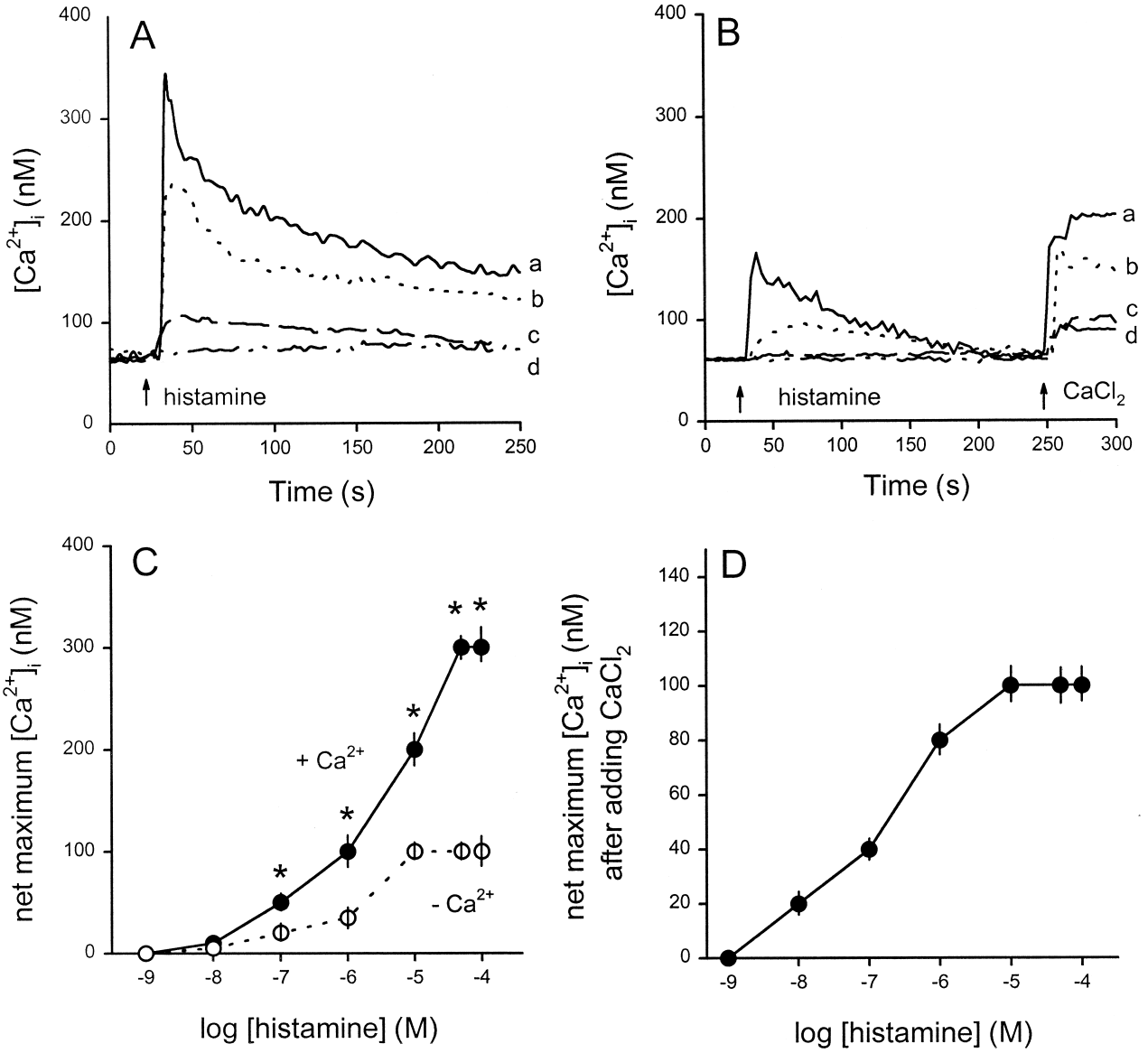

Fig. 1. (A) Concentration-dependent effects of histamine on $[Ca^{2+}]_i$ in MG63 cells. The concentration of histamine was 10 μM in *trace a*, 1 μM in *trace b*, 0.1 μM in *trace c*, and 10 nM in *trace d*. The experiments were performed in Ca^{2+} medium. (B) Effect of external Ca^{2+} removal on histamine-induced $[Ca^{2+}]_i$ increases and the effect of readmission of Ca^{2+}. Histamine (10 nM–100 μM) was added at 30 sec in Ca^{2+}-free medium followed by addition of 3 mM $CaCl_2$ at 250 sec. The concentration of histamine was 100 μM, 1 μM, 10 nM, and zero in *traces a, b, c*, and *d*, respectively. (C) Concentration–response plots of histamine-induced Ca^{2+} signals both in Ca^{2+} medium (filled circles) and Ca^{2+}-free medium (open circles). The y axis is the net maximum $[Ca^{2+}]_i$ of the response with baseline subtracted. The data were means ± SEM of 4–5 experiments. *$2P < 0.05$. (D) A concentration–response plot of histamine-induced capacitative Ca^{2+} entry. The y axis is the net maximum $[Ca^{2+}]_i$ obtained after adding 3 mM $CaCl_2$. The data were means ± SEM of 4–5 experiments.

2. Materials and methods

2.1. Cell culture

MG63 cells obtained from the American Type Culture Collection were cultured in modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin at 37° in 5% CO_2-containing humidified air.

2.2. 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.

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

Trypsinized cells (10^6/mL) were loaded with 2 μM fura-2/AM for 30 min at 25° in modified Eagle's medium. Fura-2

fluorecence measurements were performed in a water-jacketed cuvette (25°) with continuous stirring; the cuvette contained 1 mL of medium and 0.5 million cells. Fluorescence was monitored with a Shimadzu RF-5301PC spectrofluorophotometer by recording excitation signals at 340 and 380 nm and the emission signal at 510 nm at 1-sec intervals. Maximum and minimum fluorescence values were obtained by adding 0.1% Triton X-100 and 20 mM EGTA sequentially at the end of an experiment. $[Ca^{2+}]_i$ was calculated as previously described [17–19].

2.4. Chemical reagents

The reagents for cell culture were from GIBCO. Fura-2/AM was from Molecular Probes. U73122 (1-(6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl)-1*H*-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. The other reagents were from Sigma.

2.5. Statistical analysis

The traces are 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 typically small. This means that the mean ± SEM of 4–5 experiments can reveal significant results. Statistical comparisons were determined by using Student's paired *t*-test, and significance was accepted when $P < 0.05$.

3. Results and discussion

3.1. Effect of histamine on $[Ca^{2+}]_i$ in MG63 cells

At concentrations between 10 nM–100 μM, histamine increased $[Ca^{2+}]_i$ in the presence of extracellular Ca^{2+} (Fig. 1A). The $[Ca^{2+}]_i$ signal comprised an initial rise, a slow decay, and a sustained phase within 250 sec. At a concentration of 1 nM, histamine had no effect. The response saturated at 10–100 μM histamine. The $[Ca^{2+}]_i$ signal induced by 1–100 μM histamine comprised an immediate rise, a slow decay, and a sustained phase. At a concentration of 100 μM, histamine induced a $[Ca^{2+}]_i$ increase that reached a net maximum value of 298 ± 8 nM (baseline subtracted; *trace a*; N = 4; P < 0.05) followed by a sustained phase. The $[Ca^{2+}]_i$ had a net value of 90 ± 6 nM at 250 sec. The rising speed of the Ca^{2+} signal was independent of the concentration of histamine. The concentration-dependent plot indicates an EC_{50} of 0.5 μM, calculated by fitting the Hill equation to the data. The experiments were performed in serum-free solution because serum is known to modify Ca^{2+} signals and IP_3 formation.

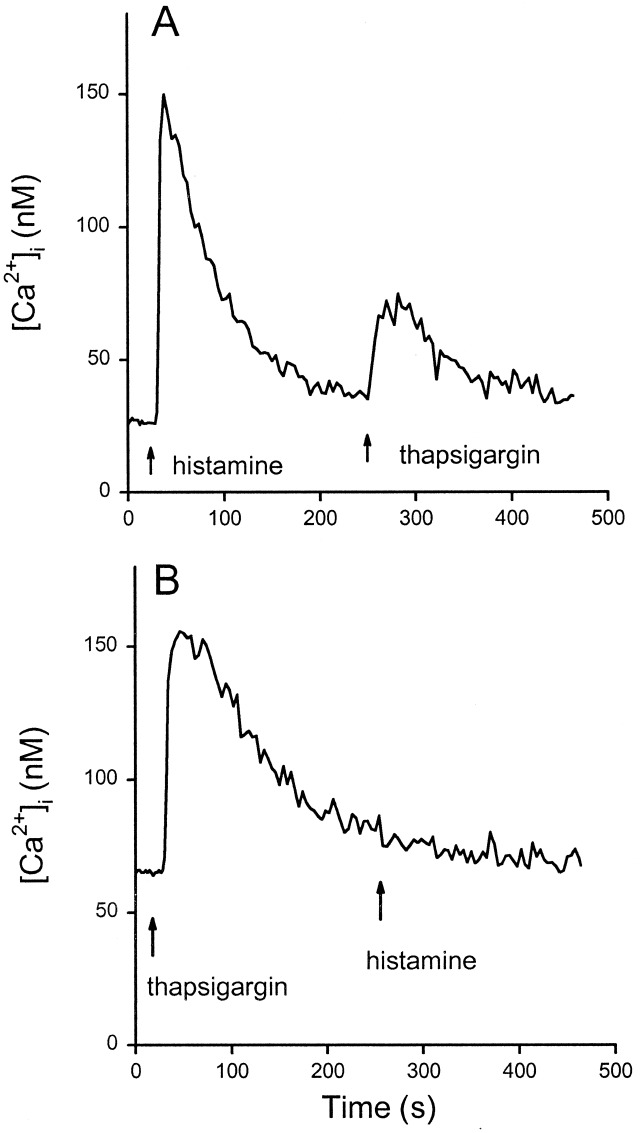

Fig. 2. Internal Ca^{2+} sources of histamine-induced $[Ca^{2+}]_i$ increases. (A) In Ca^{2+}-free medium, 100 μM histamine and 1 μM thapsigargin were added at 30 and 250 sec, respectively. (B) Similar to (A) except that the order of drug application was reversed.

3.2. Effect of external Ca^{2+} removal on the histamine response

Extracellular Ca^{2+} removal inhibited histamine-induced $[Ca^{2+}]_i$ increases (Fig. 1B). The concentration–response relationships of the histamine responses in the presence and absence of extracellular Ca^{2+} are depicted in Fig. 1C. Ca^{2+} removal inhibited 0.1–100 μM histamine-induced $[Ca^{2+}]_i$ increases by 50 ± 5% at the net maximum value (N = 5; P < 0.05). The data suggest that extracellular Ca^{2+} influx and intracellular Ca^{2+} release contributed equally to the peak Ca^{2+} signal. The elevated phase of the histamine response was abolished by Ca^{2+} removal, indicating that this phase could be attributed to Ca^{2+} entry.

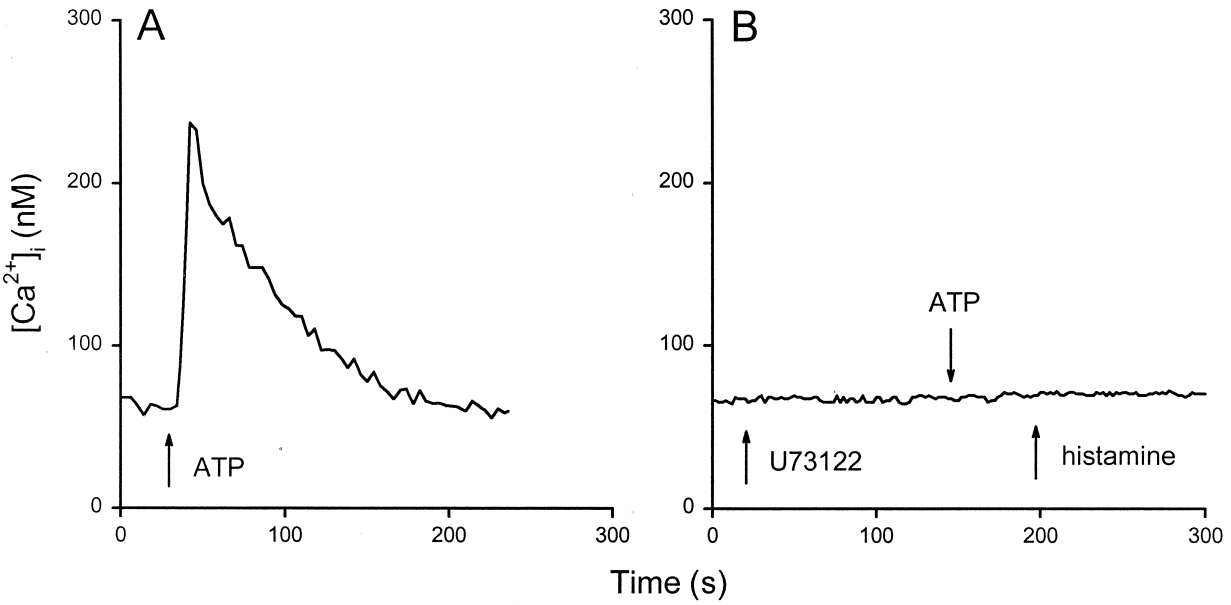

Fig. 3. Effect of U73122 on histamine-induced intracellular Ca^{2+} release. (A) In Ca^{2+}-free medium, 10 μM ATP was added at 30 sec. (B) In Ca^{2+}-free medium, 2 μM U73122, 10 μM ATP, and 100 μM histamine were added at the time indicated by arrows.

3.3. Effect of histamine on Ca^{2+} entry

Mobilization of intracellular Ca^{2+} in most cells activates capacitative Ca^{2+} entry [5,6]. Most researchers measure capacitative Ca^{2+} entry by reintroducing Ca^{2+} following Ca^{2+} store depletion with the tested agent in Ca^{2+}-free medium. Fig. 1B shows that after pretreatment with 10 nM–100 μM histamine for 220 sec, addition of 3 mM $CaCl_2$ increased $[Ca^{2+}]_i$ concentration-dependently (*traces a–c*), and these responses were all higher than control (*trace d*; $N = 4$; $P < 0.05$). Fig. 1D shows the concentration–response curve of Ca^{2+}-induced Ca^{2+} entry. These results indicate that histamine may activate Ca^{2+} influx via capacitative Ca^{2+} entry.

3.4. The intracellular sources of histamine-induced $[Ca^{2+}]_i$ increases

Experiments were performed to explore the role of the endoplasmic reticulum Ca^{2+} store in histamine-induced Ca^{2+} release. Fig. 2A shows that in Ca^{2+}-free medium, after 100 μM histamine-induced $[Ca^{2+}]_i$ responses had subsided to baseline, 1 μM thapsigargin, an endoplasmic reticulum Ca^{2+} pump inhibitor [20], evoked a $[Ca^{2+}]_i$ increase with a net maximum value of 35 ± 4 nM ($N = 4$). Conversely, Fig. 2B shows that 1 μM thapsigargin induced a $[Ca^{2+}]_i$ increase with a net maximum value of 91 ± 5 nM ($N = 4$; $P < 0.05$). After thapsigargin incubation for 230 sec, 100 μM histamine failed to increase $[Ca^{2+}]_i$ ($N = 4$). Thus, the Ca^{2+} store for the histamine response appears to be the thapsigargin-sensitive endoplasmic reticular compartment.

3.5. Mechanism of histamine-induced Ca^{2+} release

The role of IP_3 in the histamine response was examined. Fig. 3A shows that the IP_3-dependent Ca^{2+} mobilizer ATP (10 μM) increased $[Ca^{2+}]_i$ in Ca^{2+}-free medium, suggestive of the existence of IP_3-coupled Ca^{2+}-releasing pathways. The effect of inhibiting phospholipase C on the histamine response was investigated. Fig. 3B shows that incubation with the phospholipase C inhibitor U73122 (2 μM) [21] for 120 sec did not elevate basal $[Ca^{2+}]_i$, but rather abolished 10 μM ATP-induced $[Ca^{2+}]_i$ increases ($N = 4$; $P < 0.05$). U73343 (10 μM), an inactive U73122 analogue, did not affect basal $[Ca^{2+}]_i$ or the ATP response ($N = 4$; not shown). This suggests that U73122 effectively inhibited phospholipase C-coupled IP_3 formation. After U73122 and ATP pretreatment, 100 μM histamine failed to increase $[Ca^{2+}]_i$ ($N = 4$). The data suggest that histamine increases $[Ca^{2+}]_i$ by activation of phospholipase C.

3.6. Effect of histamine receptor antagonists on histamine-induced $[Ca^{2+}]_i$ increases

In Ca^{2+} medium, pretreatment with 10 μM cimetidine (an H2 histamine receptor antagonist) for 30 min abolished 50 μM histamine-induced $[Ca^{2+}]_i$ increases. In contrast, 50 μM pyrilamine (an H1 histamine receptor antagonist) had no effect ($N = 5$; not shown). Histamine appears to increase $[Ca^{2+}]_i$ via either H1 or H2 receptors depending on cell types [10–12]. Our data indicate that in MG63 cells, H2, but not H1, histamine receptors are responsible for histamine-induced $[Ca^{2+}]_i$ increases.

3.7. Effect of Ca^{2+} entry blockers on histamine-induced $[Ca^{2+}]_i$ increases

Pretreatment with 10 μM La^{3+}, nifedipine, verapamil, or diltiazem for 30 sec did not alter 50 μM histamine-induced $[Ca^{2+}]_i$ increases (N = 4–5; not shown).

3.8. Conclusion

In summary, this study shows that histamine increased $[Ca^{2+}]_i$ in MG63 cells concentration-dependently by activating H2 receptors. The signal was contributed by phospholipase C-coupled Ca^{2+} release from thapsigargin-sensitive stores, and by Ca^{2+} influx. The only drug that was found to increase $[Ca^{2+}]_i$ in MG63 cells is the calmodulin inhibitor W-7 [16]. The response induced by histamine and W-7 differs in that the W-7-induced $[Ca^{2+}]_i$ increase did not decay, and U73122 did not inhibit the W-7 response. Thus, these two agents appear to employ different Ca^{2+}-signaling pathways. Because Ca^{2+} signals interact intricately with other transduction messengers such as cyclases, protein kinases, and phospholipases to regulate cell function [22, 23], the *in vivo* significance of histamine-induced $[Ca^{2+}]_i$ signals in bone cell physiology remains to be investigated.

Acknowledgments

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