Vol. 135, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 26, 1986 Pages 172-177

# HISTAMINE ACTIVATES $H_1$-RECEPTORS TO INDUCE CYTOSOLIC FREE CALCIUM TRANSIENTS IN CULTURED VASCULAR SMOOTH MUSCLE CELLS FROM RAT AORTA

Takahiro Matsumoto, Hideo Kanaide, Junji Nishimura,
Yoshito Shogakiuchi, Sei Kobayashi,
and Motoomi Nakamura

Research Institute of Angiocardiology and Cardiovascular Clinic,
Faculty of Medicine, Kyushu University, Fukuoka 812, Japan

Received January 13, 1986

SUMMARY: Using an intracellularly trapped dye, quin 2, the effects of histamine on cytosolic free calcium concentrations in rat aortic vascular smooth muscle cells in primary culture were recorded, microfluorometrically. When the cells were exposed to histamine, both in the presence and the absence of extracellular $Ca^{2+}$, there was a rapid, transient and dose-dependent elevation of cytosolic $Ca^{2+}$ concentrations, with a similar time course. This elevation of cytosolic $Ca^{2+}$ was dose-dependently inhibited by mepyramine , but not by cimetidine. Thus, histamine activates $H_1$- but not $H_2$- receptors to mediate a release of $Ca^{2+}$ from the store sites, and there is a rapid and transient elevation of cytosolic $Ca^{2+}$. 

Histamine is present in significant concentrations in the blood, in the vessel wall (1) and in sympathetic nerves (2). The effect of histamine on vascular smooth muscle is debatable, particularly as related to spasm of the coronary artery (3-5). We found that histamine produced spasm of the coronary artery in miniature swine with experimentally-induced atherosclerotic lesions (3). Pharmacological experiments suggested that histamine contracts vascular smooth muscles by acting on the $H_1$-receptor and relaxes these muscles via the $H_2$-receptor (6-8). There are also data that activation of vascular smooth muscle by histamine may involve both the influx of $Ca^{2+}$ and the release of intracellular $Ca^{2+}$ (9).

ABBREVIATIONS

$[Ca^{2+}]_i$: intracellular free calcium concentration
EGTA: ethylene glycol-bis (β-aminoethylether) N, N'-tetraacetic acid
HEPES: N-2-Hydroxyethyl-piperazine-N'-2-ethane sulfonic acid
PSS: physiological saline solution
VSMC: vascular smooth muscle cell

0006-291X/86 $1.50

Using quin 2-microfluorometry (10,11), we investigated the effect of histamine on cytosolic free calcium concentrations ($[Ca^{2+}]_i$) of primary cultured vascular smooth muscle cells (VSMCs) from the rat aorta. We obtained evidence that histamine activates the $H_1$-receptors to induce $[Ca^{2+}]_i$ transients in VSMCs, irrespective of the presence or the absence of extracellular $Ca^{2+}$.

## MATERIALS AND METHODS

Cell culture and loading cells with quin 2: rat aortic medial smooth muscle cells were cultured as described (12). On days 5 to 6, just before reaching confluence, the cultured cells on Lux chamber slides were loaded with quin 2, as the acetoxymethyl ester (quin 2/AM) (13), as described (10,11). Unless otherwise indicated, the measurements of $[Ca^{2+}]_i$ transients were performed in physiological saline solution (PSS) at 25°C. The millimolar composition of the normal PSS (pH7.4 at 25°C) was: NaCl 135; KCl 5; $CaCl_2$ 1; $MgCl_2$ 1; glucose 5.5; HEPES 10. The composition of $Ca^{2+}$-free PSS was as in normal PSS, except that it contained 2 mM EGTA instead of 1 mM $CaCl_2$. We used primary cell cultures throughout.

Microfluorometry of quin 2: we recorded the fluorescence intensity of VSMCs, using microfluorometry. Briefly, we used a fluorescence microscope (model standard 18, Zeiss) equipped with a water immersion objective system (Plan-Neofluor 63, Zeiss) and an appropriate combination of filters, in which the cells were excited at wavelengths between 350 and 360 nm and analyzed at fluorescent wavelengths between 470 and 560 nm. Using a pinhole diaphragm (Zeiss) in the light axis, the fluorescence intensity in a spot (<1 $\mu m^2$) of the cytosol 3 $\mu m$ apart from the nucleus was measured. Each cell was exposed to the excitation light, only once, for not longer than 2 seconds in order to avoid the photobleaching effect on the dye. To read the fluorescence intensity, an input-output calculator (model 97S, Hewlett-Packard) was used.

Cell viability: A high viability (>95%) of the cells was maintained during the course of each experimental procedure, as assessed by the trypan blue exclusion test (12).

Quin 2/AM was purchased from DOTITE (Japan), histamine dihydrochloride was purchased from WAKO (Japan), and mepyramine maleate and cimetidine were purchased from Sigma Chemical Co.

## RESULT AND DISCUSSION

When the VSMCs were incubated with quin 2/AM, the fluorescence of the quin 2-$Ca^{2+}$ complex was observed almost exclusively in the cytosol; the nucleus and myofilaments were negatively stained, as reported (10, 11). During the course of all experiments, morphological changes were nil, as assessed in a phase contrast microscope at x400.

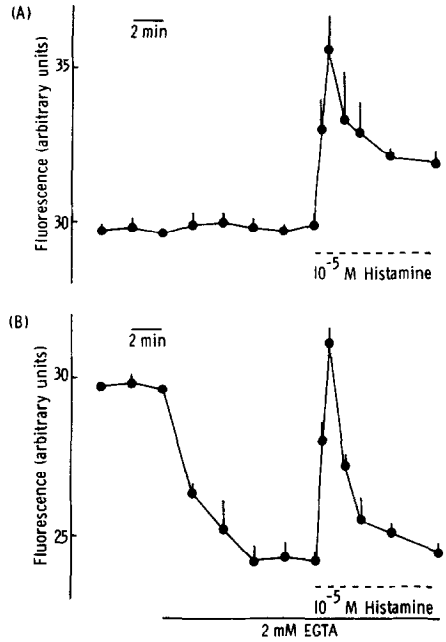


Figure 1. Time courses of the response of fluorescence change in the cytosol induced by $10^{-5}$ M histamine (broken line) in normal PSS (1 mM $Ca^{2+}$) (A) and in $Ca^{2+}$-free PSS containing 2 mM EGTA (solid line) (B).

When VSMCs were incubated in $Ca^{2+}$-free PSS containing 2 mM EGTA, the $[Ca^{2+}]_i$ decreased gradually and reached a steady state, within 6 minutes. This steady state remained unchanged for at least 60 minutes, as previously noted (10, 11). Data are mean ± SD of 4 experiments, and the number of cells counted in each plot was 8.

Figure 1 shows that histamine induced a transient elevation of fluorescence levels in the cytosol, both in the presence (1.0 mM $Ca^{2+}$) and absence (0 mM $Ca^{2+}$, 2 mM EGTA) of extracellular $Ca^{2+}$. In the presence of extracellular $Ca^{2+}$ (Fig. 1A), fluorescence reached a peak level at 1 minute (the first component) and then rapidly declined to a fairly steady state (the second component), within 10 minutes, and remained at this level as long as VSMCs were exposed to histamine. When VSMCs were exposed to $Ca^{2+}$-free media containing 2 mM EGTA, $[Ca^{2+}]_i$ decreased gradually to the steady state level, within 6 minutes (Fig. 1B). In the absence of extracellular $Ca^{2+}$, histamine induced a $[Ca^{2+}]_i$ transient, which reached a peak level at 1 minute to make the first component, however, then, rapidly declined to the pre-exposure level in 8 minutes, and there was no second component. The extent of the

transient $[Ca^{2+}]_i$ elevation of the first component induced by histamine was concentration-dependent, the range being between $10^{-6}$ and $10^{-5}$ M ($P<0.05$ by analysis of variance), both in the presence and the absence of extracellular $Ca^{2+}$ (Fig. 2A, B). The finding that histamine induces the first component of a transient and dose-dependent $[Ca^{2+}]_i$ elevation, even in the absence of extracellular $Ca^{2+}$, indicates that this component was produced by a release of $Ca^{2+}$ from cellular store sites. This proposal is compatible with findings that histamine induced a contraction of vascular tissues in $Ca^{2+}$-free solution, thereby indicating that histamine mediates a release of intracellular $Ca^{2+}$ (9). Because of similarities between the time courses of the first component observed in the presence and the absence of extracellular $Ca^{2+}$ and the lack of the second component in the absence of extracellular $Ca^{2+}$, it is conceivable that in the presence of extracellular $Ca^{2+}$, the first transient component may reflect a release of cellular $Ca^{2+}$, while the second steady component may be due to an influx of extracellular $Ca^{2+}$.

To identify the contributions of subtypes of histamine receptors ($H_1$, $H_2$) to the $[Ca^{2+}]_i$ transients in cultured VSMCs from the rat aorta, the effects of mepyramine ($H_1$ antagonist) and cimetidine ($H_2$ antagonist) on the levels of the first component of $[Ca^{2+}]_i$ elevation induced by $10^{-5}$ M histamine were studied in normal PSS (1 mM $Ca^{2+}$) and in $Ca^{2+}$-free PSS containing 2 mM EGTA. Both in the presence and the absence of extracellular $Ca^{2+}$, mepyramine reduced, dose-dependently, the peak values (at 1 minute) of transient fluorescence elevations by $10^{-5}$ M histamine, and at higher concentrations of mepyramine, the peak levels of fluorescence were neither more nor less than the level recorded in cells pre-exposed to histamine (Fig. 3A, B). Cimetidine had little or no effect on elevation of the fluorescence induced by $10^{-5}$ M histamine, irrespective of the presence of extracellular $Ca^{2+}$. Thus, it is suggested that the transient elevation of $[Ca^{2+}]_i$ by application of histamine both in the presence and absence of extracellular $Ca^{2+}$ is

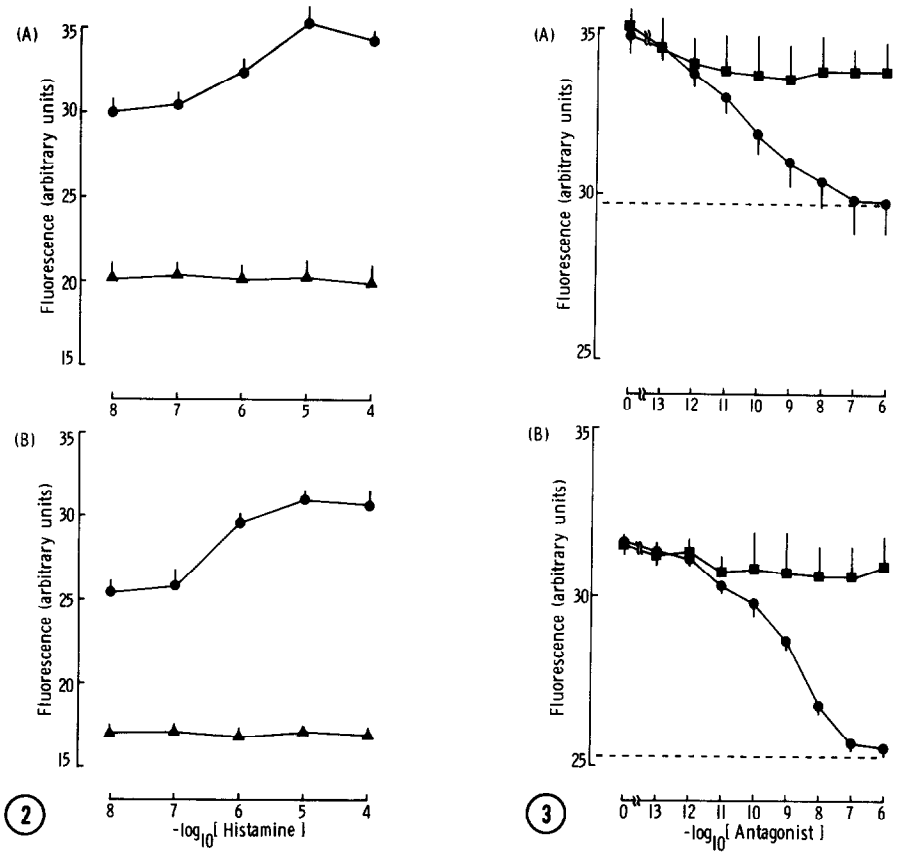


Figure 2. Dose-dependent effect of histamine on the first component of the peak elevation (1 minute) of $[Ca^{2+}]_i$ of VSMCs in normal PSS (1 mM $Ca^{2+}$) (A) and in $Ca^{2+}$-free PSS containing 2 mM EGTA (B). Microfluorometry was carried out 1 minute after application of histamine in the cytosol of quin 2-loaded cells (●) and the unloaded cells (▲). Data are mean ± SD of 4 experiments.

Figure 3. The effect of various concentrations of mepyramine (●) and cimetidine (■) on a peak levels of $[Ca^{2+}]_i$ elevations induced by $10^{-5}$ M histamine, in normal PSS (1 mM $Ca^{2+}$) (A) or in $Ca^{2+}$-free PSS containing 2 mM EGTA (B). Broken lines indicate the pre-exposure levels of fluorescence to histamine in normal PSS (A) and in $Ca^{2+}$-free PSS (B), respectively. Microfluorometry was carried out 1 minute after histamine and antagonists were simultaneously applied. Data are mean ± SD of 4 experiments.

mediated by $H_1$-receptors and that the $H_2$-receptors probably have no role in the $[Ca^{2+}]_i$ regulation in cultured VSMCs from rat aorta. In accord with this result, histamine contracts the rat aorta and the effect was inhibited by mepyramine while cimetidine had no influence whatever (8). Thus, irrespective of the presence or absence of extracellular $Ca^{2+}$, histamine activates $H_1$-receptors to induce a rapid, transient and dose-dependent elevation of $[Ca^{2+}]_i$ by releasing $Ca^{2+}$ from cellular

store sites. This is the first report that histamine activates $H_1$-receptors to induce $[Ca^{2+}]_i$ transients in VSMCs.

## ACKNOWLEDGMENTS

We thank M. Hasegawa for assistance with the experiments. N. Hayashi and N. Misaka for secretarial services, and M. Ohara for comments on the manuscript. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 58570395 and 59440044) from the Ministry of the Education, Science and Culture, a Research Grant for Cardiovascular Disease (60 C-1) from the Ministry of Health and Welfare, Japan, a Grant from the Kanae Foundation of Research for New Medicine (1985), and a Grant from the Calcium Signal Workshop on Cardiovascular Systems.

## REFERENCES

1. Ryan, M.J. and Brody, M.J. (1972) J. Pharmacol. Exp. Ther. 181, 83-91.
2. Ryan, M.J. and Brody, M.J. (1970) J. Pharmacol. Exp. Ther. 174, 123-132.
3. Shimokawa, H., Tomoike, H., Nabeyama, S., Yamamoto, H., Araki, H., Nakamura, M., Ishii, Y. and Tanaka, K. (1983) Science 221, 560-562.
4. Kalsner, S. and Richard, R. (1984) Science 223, 1435-1437.
5. Forman, M.B., Oates, J.A., Robertson, D., Roberts, II, L.J. and Virmani, R. (1985) N. Engl. J. Med. 1138-1141.
6. Hagen, M. and Paegelow, I. (1979) Agents and Actions 9, 253-256.
7. Tenner, Jr., T.E. and McCully, J.P. (1981) Eur. J. Pharmacol. 73, 293-300.
8. Von De Voorde, J. and Leusen, I. (1983) Eur. J. Pharmacol. 87, 113-120.
9. Watkins, R.W. and Davidson, I.W.F. (1980) Experientia 36, 582-584.
10. Kobayashi, S., Kanaide, H. and Nakamura, M. (1985) Science 229, 553-556.
11. Kobayashi, S., Kanaide, H. and Nakamura, M. (1985) Biochem. Biophys. Res. Comm. 129, 877-884.
12. Yamamoto, H., Kanaide, H. and Nakamura, M. (1983) Br. J. Exp. Pathol. 64, 156-165.
13. Tsien, R.Y., Pozzan, T. and Rink, T.J. (1982) J. Cell. Biol. 94, 325-334.