Dependency of Histamine Induced Phasic and Tonic Contractions on Intracellular and Extracellular Calcium in Guinea Pig Tracheal Smooth Muscle

Hiroyuki FUKUI, Atsushi HAYASHI, Hiroshi FUKUDA, Motohiko TAKEUMA, Atsushi YAMATODANI and Hiroshi WADA

Department of Pharmacology II, Osaka University School of Medicine, 3-57 Nakanoshima 4-chome, Kita-ku, Osaka 530, Japan

Accepted February 25, 1989

Abstract—Dependency of histamine induced phasic and tonic contractions on intracellular and extracellular calcium in guinea pig tracheal smooth muscle was investigated. In Ca**+-free Krebs-Ringer solution, the phasic contraction caused by 10^-5 M histamine was not affected, but the tonic contraction declined much faster than that in normal Krebs-Ringer solution (KR). The phasic contraction in KR containing 3 x 10^-4 M dantrolene was depressed, but reached a level similar to that in normal KR, and the tonic contraction was maintained similarly with or without dantrolene. In KR containing organic calcium channel blockers, 10^-5 M nilvadipine (FR34235), 10^-5 M verapamil or 10^-5 M diltiazem, neither phasic contraction nor tonic contraction was affected. No contraction was observed in Ca**+-free KR containing 3 x 10^-4 M dantrolene. These results suggested that the phasic contraction by histamine depended on the intracellular calcium and the tonic one, on the extracellular calcium, and the organic calcium channel blockers had no effect on both phasic and tonic contractions caused by histamine.

Guinea pig tracheal smooth muscle is contracted by histamine through the H1-receptor subtype (1). It is thought that two calcium sources, intracellular microsomal calcium storage and extracellular calcium, participate in this contraction (2-4). It is reported that the intracellular microsomal calcium is released by inositol 1,4,5-trisphosphate in tracheal smooth muscle cells (5) and other cells (6-8). It was also reported that inositol phosphates were increased in tracheal smooth muscle cells (9), ileal smooth muscle cells (10-12) and other cells (13-17) through histamine H1 receptor stimulation. The extracellular calcium is hypothesized to pass through the H1-receptor operated calcium channel (18). The histamine induced contraction of the tracheal smooth muscle can be divided into two components, the phasic and tonic contractions. We defined the parameters and analyzed the relationship between the histamine induced phasic and tonic contractions and the dependencies on the intra- and extracellular calcium sources by the parameters. In this report, we describe that the phasic contraction largely depended on the intracellular calcium and the tonic one on the extracellular calcium, when guinea pig tracheal smooth muscle was contracted by histamine.

Materials and Methods

Materials: Histamine hydrochloride was purchased from Wako Chemical Co., dantrolene sodium, nilvadipine (FR34235), verapamil and diltiazem were generously presented from Yamanouchi Pharmaceutical Co., Fujisawa Pharmaceutical Co., Eizai Pharmaceutical Co. and Tanabe Pharmaceutical Co., respectively.

Smooth muscle isolation: Male Hartley guinea pig (200-300 g) was stunned and exsanguinated and then the trachea was isolated. A five millimeter wide closed cyndrical tracheal strip was cut off and incubated at 37°C in normal Krebs-Ringer
solution (KR) (118.4 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄, 1.4 mM CaCl₂, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, 11.1 mM glucose, pH 7.4) which was constantly gassed by 95% O₂ and 5% CO₂ in a 10-ml organ bath.

**Determination of muscle contraction:** The muscle tension was isometrically determined using the strain gauge (Type UL-10GR, Minebea Co., Ltd.), DC strain amplifier (6M 92; NEC-San-ei Co., Ltd.) and 8-channel rectigraph recorder (NEC-San-ei Co., Ltd.). The tracheal strip was loaded at the resting tension of 1.0 g and washed every fifteen minutes with fresh KR gassed with 95% O₂ and 5% CO₂ warmed at 37°C. The tracheal strip was contracted by histamine in a dose-dependent manner. The full contraction was observed at higher than 10⁻⁴ M histamine. In the control experiments, 10⁻⁵ M histamine which produced 80% of the full contraction was used. In order to clearly express the degree of the tracheal smooth muscle contraction by 10⁻⁵ M histamine, the following parameters were used: Tm, time required from the drug administration to the maximal contraction; T½, time from the drug administration to 50% of the maximal contraction; and C15, percentage of the contraction at 15 min after the maximal contraction by 10⁻⁵ M histamine. Tm and T½ were used as the indicators of the speed of the phasic contraction development and C15, for the maintenance of the tonic contraction. Statistical evaluation of significant differences was performed with Student’s t-test.

In the Ca²⁺-free experiment, the tracheal strip was pre-incubated for 5 min in KR without CaCl₂. Calcium channel blockers and dantrolene were added 5 min and 15 min prior to the administration of histamine, respectively.

### Results

**Effect of extracellular Ca²⁺ depletion on histamine induced tracheal smooth muscle contraction:** Figure 1 shows typical contraction profiles of the tracheal strip in response to 10⁻⁵ M histamine in normal (a) and Ca²⁺-free (b) KRs. The tracheal strips were pre-incubated for 5 min in Ca²⁺-free KR, and histamine was administered at the time indicated by the arrow.

Similar phasic contractions were observed both in normal and Ca²⁺-free KR. After reaching the maximum level, the tonic contraction in Ca²⁺-free KR declined much faster than that in normal KR. Table 1 shows the values of the parameters of the tracheal strip contraction. No significant differences were observed in the values of (T½) and (Tm) between normal and Ca²⁺-free KRs but the value of (C15) in Ca²⁺-free KR was much smaller (34%) than that in normal KR.

**Effect of dantrolene on histamine induced tracheal smooth muscle contraction:** The tracheal strips was pre-incubated with 10⁻⁵–3×10⁻⁴ M dantrolene for 15 min prior to the administration of histamine. The tension of the strip was not changed by dantrolene.

**Table 1.** Effect of the extracellular Ca²⁺ on values of parameters of the tracheal strip contraction by histamine

|               | T½ (min) | Tm (min) | C15 (%) |
|---------------|----------|----------|---------|
| Control       | 1.0±0.1  | 6.7±0.5  | 82.6±4.1|
| Ca²⁺ free     | 1.3±0.2  | 5.4±0.4  | 28.1±6.0|

Data represent means±S.E. (n=16). T½: time required for half maximal contraction. Tm: time required for maximal contraction. C15: percentage of the contraction at 15 min after the maximal contraction. The maximal contraction by 10⁻⁵ M histamine was expressed as 100%. *P<0.001.

![Fig. 1. Contraction profiles of the tracheal strips by 10⁻⁵ M histamine in normal KR (a) and Ca²⁺-free KR (b). The administration of histamine is indicated by an arrowhead.](image)
itself at the concentration described above. Figure 2 shows the typical contraction profiles of the tracheal strip produced by $10^{-5}$ M histamine without (b) or with (a) $3 \times 10^{-4}$ M dantrolene. Dantrolene was added at the first arrow; and after 15 min pre-incubation period, histamine was added at the second arrow. When dantrolene was present, the phasic contraction was depressed but reached a level similar to that of the control. The tonic contractions were well-maintained in both conditions with and without dantrolene. Figure 3A shows the values of Tm at various concentrations of dantrolene. The value of Tm increased to about 4-fold by the increasing doses of dantrolene. Figure 3B shows the effect of dantrolene on the value of C15. When the maximal contraction was expressed as 100%, the value percentage of C15 with or without $10^{-5}$-$4 \times 10^{-4}$ M dantrolene ranged from 80–90%, and the deviation was not significant at each concentration ($P>0.05$).

Values of the parameters for the tracheal strips contraction caused by histamine with or without dantrolene are shown in Table 2. The values of (T1/2) and (Tm) in the presence of dantrolene were 4.6-fold and 3.2-fold of the control values, respectively; and the value of C15 was similar to the control value and there was no statistically significant difference ($P>0.05$).

Effect of organic calcium channel blockers on histamine induced tracheal smooth muscle: The effect of three calcium channel blockers at $10^{-6}$ M nilvadipine, $10^{-6}$ M verapamil and $10^{-6}$ M diltiazem on histamine induced tracheal smooth muscle were examined. The values of the parameters in the presence of calcium channel blockers did not differ significantly from the control values (data not shown).

Effect of dantrolene on histamine induced tracheal smooth muscle contraction in Ca$^{++}$-free KR: Figure 4 shows the histamine-
Table 2. Effect of dantrolene on values of the parameters of tracheal strip contraction by histamine

|               | T1/2 (min) | Tm (min) | C15 (%) | n  |
|---------------|------------|----------|---------|----|
| Control       | 1.1 ±0.2   | 6.7 ±1.3 | 89.2±6.1| 20 |
| Dantrolene (3×10^{-4}) | 5.1±1.0    | 21.6±1.3 | 92.3±1.4| 15 |

Data represent means±S.E. T1/2: time required for half maximal contraction. Tm: time required for maximal contraction. C15: percentage of the contraction at 15 min after the maximal contraction. The maximal contraction by 10^{-5} M histamine was expressed as 100%. *P<0.001.

Discussion

The histamine-induced contraction profiles and the values of the parameters of guinea pig tracheal strip in Ca^{2+}-free KR clearly showed that the extracellular calcium participated in the histamine induced tonic contraction (Fig. 1 and Table 1). On the other hand, the contraction in KR containing dantrolene depressed the development of the phasic contraction (Figs. 2 and 3 and Table 2). Dantrolene was reported to be an inhibitor of the calcium release from the intracellular microsomal storage (19). This suggested the microsomal calcium participation in the histamine-induced phasic contraction. A depressed phasic contraction appeared in response to histamine in Ca^{2+}-free KR containing 10^{-4} M dantrolene, and the tonic contraction declined to the basal level which was probably due to no supply of extracellular Ca^{2+}, and no contraction was observed in Ca^{2+}-free KR containing 3×10^{-4} M dantrolene (Fig. 4). These data show that two calcium sources, the microsomal and extracellular calcium sources, are major calcium supplying sources for the tracheal smooth muscle contraction induced by histamine in guinea pigs.

Ito and Ito (20) reported that acetylcholine induced phasic contraction of tracheal smooth muscle was unaffected in Ca^{2+}-free EGTA-containing solution. Kojima et al. (21) reported that the phasic and tonic increase of aldosterone production in the adrenal cortex by angiotensin II depended on the dantrolene-sensitive microsomal and extracellular calcium sources, respectively. Joseph et al. (22) reported that the entry of the extracellular Ca^{2+} into hepatocytes is required in order to obtain a sustained phosphorylase activity by the stimulation of vasopressin and is responsible for the maintenance of a small steady state elevation of the intracellular calcium concentration. Park and Rasmussen (23) reported that extracellular Ca^{2+} entry in combination with C-kinase activation was important in the sustained contraction of tracheal smooth muscle.

It is not clear why the release of calcium
from the microsomal storage occurs first and the influx of the extracellular calcium follows this. It is becoming clear that the mechanism of the calcium release from the microsomal storage by inositol 1,4,5-trisphosphate, which is formed by phospholipase C activation, is mediated through histamine H1-receptor stimulation in tracheal smooth muscle (5, 9). Irvine and Moor suggested that inositol 1,3,4,5-tetrakisphosphate is formed by the phosphorylation of inositol 1,4,5-trisphosphate, and it opens the calcium channel for the tonic contraction (24).

Three groups of organic calcium channel blockers, dinydropyridine, papaverine and benzothiazepine groups, were developed for the investigation of calcium channels and the therapy of cardiovascular diseases (25–27). These calcium channel blockers clearly block the L-type voltage dependent calcium channel (28). However, it is not clear whether receptor operated calcium channels are blocked by these compounds. The present results clearly showed that histamine induced contraction was insensitive to these three calcium channel blockers.

Acknowledgments: We thank Prof. S. Shibata (Dept. of Pharmacology, University of Hawaii, U.S.A.) for valuable discussions and Mrs. K. Tsuji for the secretarial assistance. We also thank Yamanouchi Pharmaceutical Co., Fujisawa Pharmaceutical Co., Eizai Pharmaceutical Co. and Tanabe Pharmaceutical Co. for their kind gifts of dantrolene, nilvadipine, verapamil and diltiazem, respectively. This work is supported by grants from the Japanese Ministry of Education, Science and Culture.

References
1 Douglas, W.W.: Histamine, pharmacological effects: H1 and H2 receptors. In The Pharmacological Basis of Therapeutics. Seventh Edition, Edited by Gilman, A.G., Goodman, L.S., Rall, T.W. and Murad, F., p. 607–611, McMillan, New York (1985)
2 Kirkpatrick, C.: Excitation and contraction in bovine tracheal smooth muscle. J. Physiol. (Lond.) 244, 263–281 (1975)
3 Creese, B.R. and Denborough, M.A.: Sources of calcium contraction of guinea-pig isolated tracheal smooth muscle. Clin. Exp. Pharmacol. Physiol. 8, 175–182 (1981)
4 Mitchell, H.W.: Electrochemical effects of teatraethylammonium and K+ on histamine-induced contraction in pig isolated tracheal smooth muscle. Lung 165, 129–142 (1987)
5 Hashimoto, T., Hirata, M. and Ito, Y.: A role for inositol 1,4,5-trisphosphate in the initiation of agonist-induced contractions of dog tracheal smooth muscle. Br. J. Pharmacol. 86, 191–199 (1985)
6 Streb, H., Irvin, R., Berridge, M.J. and Schulz, I.: Release of Ca++ from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. Nature 306, 67–69 (1983)
7 Burgess, G.M., Godfrey, P.P., McKinney, J.S., Berridge, M.J., Irvine, R.F. and Putney, J.W., Jr.: The second messenger linking receptor activation to internal Ca release in liver. Nature 309, 63–66 (1984)
8 Prentkin, M., Biffen, T.J., Janjic, D., Irvine, R.F., Berridge, M.J. and Wells, C.B.: Rapid mobilization of Ca++ from rat insulinoma microsomes by inositol-1,4,5-trisphosphate. Nature 309, 562–564 (1984)
9 Grandordy, B.M., Cuss, F.M. and Barnes, P.J.: Breakdown of phosphoinositides in airway smooth muscle: lack of influence of anti-asthmatic drugs. Life Sci. 41, 1621–1627 (1987)
10 Donaldson, J. and Hill, S.J.: Histamine-induced inositol phospholipid breakdown in the longitudinal smooth muscle of guinea-pig ileum. Br. J. Pharmacol. 85, 499–512 (1985)
11 Best, L., Brooks, K.J. and Bolton, T.B.: Relationship between stimulated inositol lipid hydrolysis and contractility in guinea pig visceral longitudinal smooth muscle. Biochem. Pharmacol. 34, 2297–2301 (1985)
12 Bielkiewicz-Vollrath, B., Carpenter, J.R., Schulz, R. and Cook, D.A.: Early production of 1,4,5-inositol trisphosphate and 1,3,4,5-inositol tetrakisphosphate by histamine and carbachol in ileal smooth muscle. J. Pharmacol. Exp. Ther. 31, 513–522 (1987)
13 Resink, T.J., Grigoñan, G.Y., Moldabaeva, A.K., Danilov, S.M. and Bühler, F.R.: Histamine-induced phosphoinositide metabolism in cultured human umbilical vein endothelial cells. Association with thromboxane and prostacyclin release. Biochim. Biophys. Res. Commun. 144, 438–446 (1987)
14 Lo, W.W.Y. and Fan, T.-P.D.: Histamine stimulates inositol phosphate accumulation via the H1-receptor in cultured human endothelial cells. Biochem. Biophys. Res. Commun. 148, 47–53 (1985)
15 Noble, E.P., Bommer, M., Sincini, E., Costa, T. and Herz, A.: H1-histaminergic activation stimulates inositol-1-phosphate accumulation in chro-
maffin cells. Biochem. Biophys. Res. Commun. 135, 566–573 (1986)
16 Carswell, H. and Young, J.M.: Regional variation in the characteristics of histamine H₁-agonist mediated breakdown of inositol phospholipids in guinea-pig brain. Br. J. Pharmacol. 89, 809–817 (1986)
17 Nakahata, N. and Harden, T.K.: Regulation of inositol trisphosphate accumulation by muscarinic cholinergic and H₁ histamine receptors on human astrocytoma cells. Biochem. J. 241, 337–344 (1987)
18 Karaki, M. and Weiss, G.B.: Calcium channels in smooth muscle. Gastroenterology 87, 960–970 (1984)
19 Desmedt, J.E. and Hainaut, K.: Dantrolene and A23187 ionophore: specific action on calcium channels revealed by aequorine method. Biochem. Pharmacol. 28, 957–964 (1979)
20 Ito, Y. and Ito, T.: The roles of stored calcium in contractions of cat tracheal smooth muscle produced by electrical stimulation, acetylcholine and high K⁺. Br. J. Pharmacol. 83, 667–676 (1984)
21 Kojima, I., Kojima, K., Kreutter, D. and Rasmussen, H.: The temporal integration of the aldosterone secretory response to angiotensin occurs via two intracellular pathways. J. Biol. Chem. 259, 14448–14457 (1984)
22 Joseph, S.K., Coll, K.E., Thomas, A.P., Rubin, R. and Williamson, J.R.: The role of extracellular Ca⁺⁺ in the response of the hepatocyte to Ca⁺⁺-dependent hormones. J. Biol. Chem. 260, 12508–12515 (1985)
23 Park, S. and Rasmussen, H.: Activation of tracheal smooth muscle contraction: Synergism between Ca⁺⁺ and activators of protein kinase C. Proc. Natl. Acad. Sci. U.S.A. 82, 8835–8839 (1985)
24 Irvin, R.F. and Moor, R.M.: Micro-injection of inositol 1,3,4,5-tetrakisphosphate activates sea urchin eggs by a mechanism dependent on external Ca⁺⁺. Biochem. J. 240, 917–920 (1986)
25 Grün, G. and Fleckenstein, A.: Die Elektromechanische Entkoppelung der glatten Gefäßmuskulatur als Grundprinzip der Coronardilatation durch 4-[(2'-Nitrophenyl)-2,6-dimethyl-1,4-dihydropyridin-3,5-dicarbonsäure-dimethyl-ester (Bay a 1040, Nifedipine). Arzneimittel-forschung 22, 334–344 (1972)
26 Kohlhardt, M., Bauer, B., Krause, M. and Fleckenstein, A.: Selective inhibition of Ca conductivity of mammalian cardiac fibers by use of specific inhibitors. Pflugers Arch. Ges. Physiol. 335, 309–322 (1972)
27 Nakajima, H., Hoshiyama, M., Yamashita, K. and Kiyomoto, K.: Effect of diltiazem on electrical and mechanical activity of isolated cardiac ventricular muscle of guinea pig. Japan. J. Pharmacol. 25, 383–392 (1975)
28 Nowycky, M.C., Fox, A.P. and Tsien, R.W.: Three types of neuronal calcium channel with different agonist sensitivity. Nature 316, 440–443 (1985)