Histamine Increases Vascular Tone and Intracellular Calcium Level Using Both Intracellular and Extracellular Calcium in Porcine Coronary Arteries

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Abstract—Effects of histamine on the tone and intracellular calcium level (Ca\(^{2+}\)) in porcine coronary arteries were simultaneously investigated by use of the fura-2 microscopic fluorometric method. Histamine (10\(^{-6}\)–10\(^{-4}\) M) induced concentration-dependent increases in tone and Ca\(^{2+}\), but these responses were not sustained. Histamine induced a larger contraction than did KCl with a similar increase in Ca\(^{2+}\). Depletion of the caffeine-sensitive Ca\(^{2+}\) store with ryanodine (3 \times 10\(^{-5}\) M) and repetitive applications of caffeine (2.5 \times 10\(^{-2}\) M) scarcely affected contractile and Ca\(^{2+}\) responses to histamine. In Ca\(^{2+}\)-free medium or in the presence of verapamil (10\(^{-6}\) M), histamine produced a briefer increase in Ca\(^{2+}\) and a smaller contraction than in normal medium. When histamine or caffeine was repetitively applied in Ca\(^{2+}\)-free medium, the first application produced an increase in Ca\(^{2+}\) but the second application produced no increase. Although caffeine increased Ca\(^{2+}\), after repetitive histamine applications, histamine failed to increase Ca\(^{2+}\) after repetitive caffeine applications in Ca\(^{2+}\)-free medium. These results indicate that vascular contraction induced by histamine may involve the following mechanisms: an increase in Ca\(^{2+}\) influx through Ca\(^{2+}\) channels, release of Ca\(^{2+}\) from the intracellular Ca\(^{2+}\) store which has an interaction with the caffeine-sensitive Ca\(^{2+}\) store, and sensitization of contractile proteins to Ca\(^{2+}\).

Intracellular free calcium level (Ca\(^{2+}\)) is one of the major regulatory factors of vascular contraction (1). In such a molecular mechanism, it is generally accepted that an increase in Ca\(^{2+}\) promotes the binding of Ca\(^{2+}\) to calmodulin, and myosin is phosphorylated by the Ca\(^{2+}\)-calmodulin complex-dependent myosin light chain kinase to interact with actin and eventually to produce contraction (2, 3). Ca\(^{2+}\) is elevated by Ca\(^{2+}\) influx across the plasma membrane and/or release from the intracellular Ca\(^{2+}\) store. The increased Ca\(^{2+}\) is reduced by efflux of Ca\(^{2+}\) via the Ca\(^{2+}\) pump and/or the uptake of Ca\(^{2+}\) into the intracellular store.

When an agonist binds to its receptors in vascular smooth muscle, second messengers which are produced via the receptor-coupled mechanisms induce contraction, which seems to depend on both Ca\(^{2+}\) influx and Ca\(^{2+}\) release from the intracellular Ca\(^{2+}\) store (4). On the other hand, application of excess KCl depolarizes the plasma membrane and produces contraction that mainly depends on the influxed Ca\(^{2+}\) through voltage-dependent Ca\(^{2+}\) channels (5). Some investigators have suggested that agonist-induced Ca\(^{2+}\) influx occurs through receptor-operated Ca\(^{2+}\) channels, which are thought to be different from voltage-dependent Ca\(^{2+}\) channels (6). Histamine is one of the spasmogenic substances in coronary arteries of pigs and humans (7–9), and its contractile effect is mediated by H\(_1\)-receptor activation (8, 9). It has been already suggested that the vascular contraction induced by histamine utilizes both intracellular and extracellular Ca\(^{2+}\) sources (4, 10). However, this suggestion has been put forward based on the results obtained by studies in which only contractile responses to histamine were measured, and no Ca\(^{2+}\) has been measured (4, 10). There-
fore, in the present study, we investigated the effects of histamine on force and Ca\(^{2+}\) in the porcine coronary artery to clarify further the relationship between vascular contraction and changes in Ca\(^{2+}\) induced by histamine.

**Materials and Methods**

Porcine coronary arteries were used as material. Fresh porcine hearts were transported on ice from a local slaughter house to the laboratory. Small coronary arteries (about 1 mm in outer diameter) were dissected, and adhering fat and connective tissues were removed in oxygenated physiological salt solution (PSS) of the following composition: 140 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl\(_2\), 2.5 mM MgCl\(_2\), 11.1 mM glucose and 3 mM 4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid (HEPES; Dojin Chemicals, Kumamoto, Japan), pH 7.4. The isolated coronary arteries were dissected into rings about 1-mm long and reversed endothelial side out. Force and Ca\(^{2+}\) of the coronary ring preparations were measured simultaneously by the previously described method (11). Briefly, for Ca\(^{2+}\) measurement, coronary ring preparations loaded with fura-2 by incubation in the PSS containing 10 /\(\mu\)M fura-2 acetoxy methyl ester (Dojin Chemicals) and 0.01–0.1% Pluronic F-127 (BASF, Parsippany, NJ, U.S.A.) at 37°C for 2–3 hr. The coronary ring was mounted on two tungsten hooks, one of which was glued to the arm of a force displacement transducer, Aker element (SensoNor, Horten, Norway). The other hook was glued to a glass rod. Endothelial cells were removed by gentle rubbing with forceps. Resting tension was adjusted so that it was 5 to 10 mN during the measurement. The whole apparatus was placed horizontally in a perfusion bath (400 ml, 37°C), which was mounted on the stage of an inverted microscope (TND-8, Nikon, Tokyo, Japan). The microscope, equipped with a fluorometer (CAM-200, Japan Spectroscopic, Tokyo, Japan), was focused on the smooth muscle layers. Tissue was illuminated for 2 sec every 4 or 10 sec with excitation light of alternating 340- and 380-nm wavelength chopped at a frequency of 100 Hz, and fluorescence signals of 500-nm wavelength to the excitation light were measured with a photomultiplier. Force, fluorescence signals induced by excitation at 340 nm (F\(_{340}\)) and 380 nm (F\(_{380}\)) and their ratio (F\(_{340}/F_{380}\)) were simultaneously recorded on a thermal pen recorder (Recti-Horiz-8K, NEC-San-ei, Tokyo, Japan). The tissue was superfused at rate of 4 ml/min by use of a roller pump (Taiyo, Tokyo, Japan). The ratio of the fluorescence signals was used as an index of Ca\(^{2+}\). Although absolute values of Ca\(^{2+}\) could be calculated based on the fura-2 fluorescence ratio and the dissociation constant of fura-2 for Ca\(^{2+}\) binding (12), recent works have suggested that the dissociation constant of fura-2 for Ca\(^{2+}\) in the cytosol is different from that measured in the absence of protein (13). Thus, changes in force and Ca\(^{2+}\) in response to various intervention were expressed as a % of each response to that elicited by 10-min exposure to 90 mM KCl, which was performed at the beginning of each experiment in the present study. The fura-2 fluorescence ratio was corrected by subtraction of autofluorescence, which was determined by quenching fura-2 fluorescence signals with MnCl\(_2\) after the cell membrane had been lysed with the detergent Triton X-100 (Wako). The intensity of autofluorescence was 25–50% of the measured fluorescence. Ca\(^{2+}\)-free PSS was made by the removal of CaCl\(_2\) from normal PSS and addition of 1 mM ethylene glycol-bis (\(\beta\)-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA, Dojin Chemicals); and high KCl-PSS was made by equimolar replacement of NaCl with KCl. Histamine dihydrochloride (Wako, Osaka, Japan), verapamil hydrochloride (Eisai, Tokyo, Japan) and ryanodine (Penick, Lyndhurst, NJ, U.S.A.) were dissolved in distilled water at concentrations of 2x10\(^{-6}\) and 6x10\(^{-3}\) M, respectively, and diluted to the desired concentrations with PSS. Caffeine (Wako) was dissolved in PSS at a concentration of 2.5x10\(^{-2}\) M. These agents did not affect fura-2 fluorescence.

**Results**

In normal Ca\(^{2+}\) medium: Histamine at concentrations of 10\(^{-6}\)–10\(^{-4}\) M induced contraction and an increase in Ca\(^{2+}\) in a concentration-dependent manner (Figs. 1 and 2A). The maximum contraction produced by his-
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**Fig. 1.** Typical recordings of the effects of KCl and histamine on force and fluorescence ratio (F\(_{340}/F_{380}\)), which is an index of intracellular calcium level (Ca\(^{2+}\)), in a porcine coronary arterial ring loaded with fura-2. The upper panel and lower panel were sequentially recorded, and 20 min were allowed between the end of 60 mM KCl application and the start of 10\(^{-4}\) M histamine application.

**Fig. 2.** Effects of histamine on force and Ca\(^{2+}\) in porcine coronary arteries. Changes in force and Ca\(^{2+}\) in response to histamine are expressed in % of their changes induced by 10 min exposure to 90 mM KCl medium which was carried out at the beginning of each experiment. A: Histamine was applied in normal PSS (control) (n=8). B: Histamine was applied after depletion of the caffeine-sensitive Ca\(^{2+}\) store by sequential application of ryanodine (3\(\times\)10\(^{-5}\) M) and caffeine (2.5\(\times\)10\(^{-2}\) M), two times, as shown in Fig. 3 (n=8). C: Histamine was applied after 5 min perfusion with 0 mM Ca\(^{2+}\) and 1 mM EGTA containing (Ca\(^{2+}\)-free) medium (n=7). Each value represents the mean±S.E.M.

tamine (10\(^{-4}\) M) was about 80% of that induced by 90 mM KCl medium, but the maximum increase in Ca\(^{2+}\), was about 40% of the 90 mM KCl-induced one. The histamine-induced contractions reached a peak at about 3 min after application and then gradually wore off even in the presence of histamine. The peak increase in Ca\(^{2+}\), was obtained at about 1 min after application, and the increase in Ca\(^{2+}\), lasted for a shorter time than the increase in tension.

**After depletion of the caffeine-sensitive Ca\(^{2+}\) store:** Since it is known that caffeine mobilizes intracellularly stored Ca\(^{2+}\) (14, 15) and ryanodine keeps the caffeine-sensitive Ca\(^{2+}\) release channel open (16), a combination of these two agents was used for depletion of the intracellular Ca\(^{2+}\) store. Before depletion of the Ca\(^{2+}\) store, caffeine (2.5\(\times\)10\(^{-2}\) M) produced a transient large increase in Ca\(^{2+}\), which was comparable with that induced by 90 mM KCl medium, but only a transient small contraction that was about one tenth of the 90 mM KCl medium-induced one.
Fig. 3. Typical recordings of the effects of histamine on force and fluorescence ratio \((F_{340}/F_{380})\) in a fura-2 loaded porcine coronary artery after depletion of the caffeine-sensitive \(Ca^{2+}\) store by treatment with ryanodine and caffeine. The upper panel and lower panel were sequentially recorded, and 10 min were allowed between the end of the second application of caffeine and the start of the application of \(10^{-6}\) M histamine.

After washout of caffeine, \(Ca^{2+}\) was decreased slightly below the resting level (Fig. 3). Repetitive applications of caffeine produced similar contractile and \(Ca^{2+}\) responses (data not shown). After a 5 min exposure to ryanodine \((3\times10^{-5}\) M\), which affected neither vascular tone nor \(Ca^{2+}\), the first application of caffeine produced contractile and \(Ca^{2+}\) responses similar to those before ryanodine, but \(Ca^{2+}\) remained slightly higher than the resting level after washout of caffeine; and the second application of caffeine produced no responses (Fig. 3, upper panel). Disappearance of the second caffeine responses after exposure to ryanodine indicates depletion of the caffeine-sensitive \(Ca^{2+}\) store.

After depletion of the caffeine-sensitive \(Ca^{2+}\) store with ryanodine and repetitive caffeine applications, histamine at concentrations of \(10^{-6}-10^{-4}\) M induced essentially the same contractile and \(Ca^{2+}\) responses as those observed under normal conditions (cf. Fig. 2, A and B). In some cases, the rate of increase in \(Ca^{2+}\), however, was slower after depletion of the caffeine-sensitive \(Ca^{2+}\) store than in normal PSS (cf. Fig. 1, lower panel and Fig. 3, lower panel). Under these conditions, caffeine failed to increase \(Ca^{2+}\) after histamine application (data not shown). Therefore, the effect of ryanodine was thought to be sustained even after histamine application.

In \(Ca^{2+}\)-free medium: When extracellular \(Ca^{2+}\) was removed with 1 mM EGTA (\(Ca^{2+}\)-free), \(Ca^{2+}\) decreased but vascular tone did not change (Fig. 4). As 90 mM KCl responses were abolished after 5 min exposure of the tissues to \(Ca^{2+}\)-free medium, we left them for 5 min in \(Ca^{2+}\)-free medium for removal of extracellular \(Ca^{2+}\). In \(Ca^{2+}\)-free medium, the contractile response to \(10^{-6}-10^{-4}\) M histamine were greatly reduced in height and duration, and the contractions produced by \(10^{-4}\) M histamine was only about 25% of the contraction observed in normal medium (cf. Fig. 2, A and C). In contrast, the increases in \(Ca^{2+}\) induced by histamine were similar to those observed in normal medium, and only the duration of the increase became shorter (Figs. 4 and 2C).
In the presence of verapamil: Verapamil at a concentration of 10^{-6} M tended to slightly decrease Ca^{2+}, but did not change vascular tone. In the presence of verapamil, contractions induced by 20–60 mM KCl media were nearly abolished and increases in Ca^{2+} induced by KCl were strongly inhibited (Fig. 5). However, under these conditions, 10^{-6}–10^{-4} M histamine produced contractions and increases in Ca^{2+} in a concentration-dependent manner, although the contractions were smaller and the increases in Ca^{2+} were briefer (Fig. 5) than those observed in normal medium (Fig. 1). Responses induced by histamine in the presence of verapamil were similar to those observed in Ca^{2+}-free medium except that the decrease in resting level of Ca^{2+} is small.

**Intraction of the histamine- and the caffeine-sensitive Ca^{2+} store in Ca^{2+}-free medium:** When histamine (10^{-4} M) or caffeine (2.5\times 10^{-2} M) were applied repetitively in Ca^{2+}-free medium, the first application of these agents produced a transient increase in Ca^{2+}, which decayed more rapidly than that in normal PSS and a transient small contraction, but their second application produced neither an increase in Ca^{2+} nor contraction. The histamine- and the caffeine-sensitive Ca^{2+} store were easily depleted by the first application of the agents at such concentrations in Ca^{2+}-free medium. After depletion of the histamine-sensitive Ca^{2+} store, application of caffeine produced a transient small increase in Ca^{2+} (Figs. 6A and 7A). However, histamine was unable to increase Ca^{2+} after depletion of the caffeine-sensitive Ca^{2+} store (Figs. 6B and 7B). Since the responses to the first application of caffeine and histamine were observed even after 25 min exposure to Ca^{2+}-free medium, disappearance of histamine responses after caffeine was not due to a time-dependent effect of exposure to Ca^{2+}-free medium.

**Discussion**

In the present study, histamine produced contractions and an increase in Ca^{2+} in isolated porcine coronary arteries (Fig. 1).
The maximum contraction produced by $10^{-4}$ M histamine was about 80% of the 90 mM KCl medium-induced one, but the maximum increase in Ca$_{2+}$ was about 40% of that induced by 90 mM KCl medium. In order to compare the relationship of the histamine-induced contraction and increase in Ca$_{2+}$ with that of KCl-induced ones, Ca$_{2+}$-force relations are plotted in Fig. 8. The Ca$_{2+}$-force relations obtained with histamine were positioned to the left of that determined with KCl, indicating that the histamine-induced increase in Ca$_{2+}$ produced a larger contraction than the KCl-induced Ca$_{2+}$ increase. Similar results have been obtained in rat aorta with norepinephrine (17). Histamine and norepinephrine produce contractions in vascular tissues via stimulation of H$_1$- and $\alpha_1$-receptors, respectively (4, 10); and these receptors are known to be coupled to phosphoinositide breakdown (18), which generates two second messengers, inositol trisphosphate (IP$_3$) and diacylglycerol (DG). DG activates protein kinase C (19). We have already shown that activation of protein kinase C by phorbol ester sensitizes contractile proteins to Ca$_{2+}$ in the intact porcine coronary artery (20). The Ca$_{2+}$ sensitizing ac-

![Fig. 7.](image1)

![Fig. 8.](image2)
tion of protein kinase C activated by phorbol esters has also been demonstrated in skinned vascular preparations (21-23). Thus, it is likely that activation of protein kinase C is responsible for the Ca\(^{2+}\), sensitizing of histamine seen in the present study.

Generally, IP\(_3\), another product of phosphoinositide breakdown, is thought to release Ca\(^{2+}\) from the intracellular Ca\(^{2+}\) store (24); and the same molecular mechanism has also been postulated in vascular smooth muscle (25, 26). In the present study, histamine transiently increased Ca\(^{2+}\) even in Ca\(^{2+}\)-free and 1 mM EGTA-containing medium (Figs. 4, 6 and 7). This suggests that histamine releases Ca\(^{2+}\) from the intracellular Ca\(^{2+}\) store. Thus, a question comes up about whether the contraction and an increase in Ca\(^{2+}\) induced by histamine in Ca\(^{2+}\)-free medium depend on IP\(_3\). Iino et al. (27) have proposed that there are two distinguished intracellular Ca\(^{2+}\) stores: one is released by IP\(_3\) and the other by both IP\(_3\) and caffeine in the guinea pig taenia caeci, portal vein and pulmonary artery. The proportions of these two stores were different among various tissues (27). If their proposal is right, IP\(_3\) could release Ca\(^{2+}\) after depletion of the caffeine-sensitive Ca\(^{2+}\) store. In other words, agonists whose action involves generation of IP\(_3\) could increase Ca\(^{2+}\) after depletion of the caffeine-sensitive Ca\(^{2+}\) store. In the present study, however, histamine failed to release Ca\(^{2+}\) after depletion of the caffeine-sensitive Ca\(^{2+}\) store. Nevertheless, caffeine could release Ca\(^{2+}\) after depletion of the histamine-sensitive Ca\(^{2+}\) store (Figs. 6 and 7). The possibility that the disappearance of the second histamine responses in Ca\(^{2+}\)-free medium was due to the desensitization of histamine receptors was also ruled out, because histamine responses were easily restored after the Ca\(^{2+}\) store was loaded again by perfusion with Ca\(^{2+}\)-containing medium (data not shown). Saida and van Breemen (28) have obtained similar results in the rabbit mesenteric artery using norepinephrine as an agonist. These observations suggest that caffeine and these agonists can release Ca\(^{2+}\) from one common Ca\(^{2+}\) store, and the store can be depleted completely by caffeine, but only partially by agonists. However, Saida and van Breemen (28) thought that the caffeine- and the norepinephrine-sensitive store were different from each other, because the rates of depletion and recovery of these stores were different. They have further suggested that the norepinephrine-sensitive Ca\(^{2+}\) store is too small to produce contraction directly and that Ca\(^{2+}\) released from the norepinephrine-sensitive store may release Ca\(^{2+}\) from the caffeine-sensitive Ca\(^{2+}\) store by the Ca\(^{2+}\)-induced Ca\(^{2+}\) release mechanism and leads to contraction. Even if our results are also explainable in the same way, it is still unclear whether the contraction and Ca\(^{2+}\) responses to histamine in Ca\(^{2+}\)-free medium depend on IP\(_3\) or not.

As described in the results section, caffeine releases intracellularly stored Ca\(^{2+}\) (14, 15), and ryanodine keeps the Ca\(^{2+}\)-induced Ca\(^{2+}\) release channels open (16). Therefore, the caffeine-sensitive Ca\(^{2+}\) store is supposed to be depleted after sequential application of ryanodine and caffeine, two times. Although the rate of increase in Ca\(^{2+}\) induced by histamine was less steep after the depletion of the caffeine-sensitive Ca\(^{2+}\) store in some cases (Fig. 3), histamine produced contraction and an increase in Ca\(^{2+}\) almost to the same extent as observed under normal conditions (Fig. 2). These results suggest that vascular contraction induced by histamine mainly depends on Ca\(^{2+}\) influx, because the histamine-sensitive Ca\(^{2+}\) store would not function after the depletion of the caffeine-sensitive Ca\(^{2+}\) store (Figs. 6 and 7). Harder (29) has reported in an electrophysiological study that histamine increased the Ca\(^{2+}\) inward current which was blocked by manganese ions in canine coronary arterial smooth muscle cells. In the present study, verapamil, a Ca\(^{2+}\) entry blocker, inhibited vascular contraction and an increase in Ca\(^{2+}\) in response to histamine (Fig. 5). These data strongly suggest that the main portion of the increase in Ca\(^{2+}\) induced by histamine predominantly depends on Ca\(^{2+}\) influx through Ca\(^{2+}\) channels.

Furthermore, we observed that a transient increase in Ca\(^{2+}\) produced by caffeine was comparable to that induced by 90 mM KCl medium, but contraction induced by caffeine was only one tenth of that induced by 90 mM KCl medium. The contraction qualitatively reflects Ca\(^{2+}\), but not always quantitatively.
This is particularly the case with caffeine because caffeine has multiple actions other than Ca\(^{2+}\) release (30) such as phosphodiesterase inhibition.

In conclusion, contractile response induced by histamine may involve three mechanisms: 1) an increase in Ca\(^{2+}\) influx through Ca\(^{2+}\) channels, 2) release of Ca\(^{2+}\) from the intracellular store and 3) sensitization of contractile proteins to Ca\(^{2+}\), which may be due to protein kinase C activation. Recently, we demonstrated the involvement of these three mechanisms in the endothelin contraction in canine and porcine coronary arteries (31). Endothelin is a vasoconstrictor peptide discovered in the cultured medium of endothelial cells (32), and recent reports have demonstrated that phosphoinositide breakdown is associated with the contraction induced by endothelin (33, 34). Involvement of these three mechanisms may be common in the contraction induced by agonists which accompany phosphoinositide breakdown.

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