Manipulation of chloride flux affects histamine-induced contraction in rabbit basilar artery

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Dai, Yun, and John H. Zhang. Manipulation of chloride flux affects histamine-induced contraction in rabbit basilar artery. Am J Physiol Heart Circ Physiol 282: H1427–H1436, 2002.—Cl⁻ efflux induces depolarization and contraction of smooth muscle cells. This study was undertaken to explore the role of Cl⁻ flux in histamine-induced contraction in the rabbit basilar artery. Male New Zealand White rabbits (n = 16) weighing 1.8–2.5 kg were euthanized by an overdose of pentobarbital sodium. The basilar arteries were removed for isometric tension recording. Histamine produced a concentration-dependent contraction that was attenuated by the H₂ receptor antagonist chlorpheniramine (10⁻⁶ M) but not by the H₁ receptor antagonist cimetidine (3 × 10⁻⁶ M) in normal Cl⁻ Krebs-Henseleit bicarbonate solution (123 mM Cl⁻). The histamine-induced contraction was reduced by the following manipulations: 1) inhibition of Na⁺-K⁺-2Cl⁻ cotransporter and H⁺-ATPase activity by bumetanide (3 × 10⁻⁴ and 10⁻⁴ M), 2) bicarbonate-free HEPES solution to disable Cl⁻/HCO₃⁻ exchanger, and 3) blockade of Cl⁻ channels with the use of niflumic acid, 5-nitro-2-(3-phenylpropylamino) benzoic acid, and indoleacetic acid 94 R(+)-methylindazole. In addition, substitution of extracellular Cl⁻ (10 mM) with methanesulfonate acid (113 mM) transiently enhanced histamine-induced contraction. Manipulation of Cl⁻ flux affects histamine-induced contraction in the rabbit basilar artery.

Cl⁻ channels; Na⁺-K⁺-2Cl⁻ cotransporter; Cl⁻/HCO₃⁻ exchanger

Histamine, which acts as a neurotransmitter of central neurons, can be released from mast cells distributed throughout the whole brain, predominantly in the perivascular regions (40). Neurons containing histamine have been identified in the posterior hypothalamus (43), and histaminergic fibers have been shown to innervate cerebral blood vessels (37). Thus histamine might play a role in the regulation of cerebrovascular tone under either physiological or pathophysiological conditions (1, 4, 6).

The physiological response to histamine is mediated through four distinct subtype receptors (H₁–H₄) (6, 19). In cerebral arteries, activation of H₁ receptors induces contraction, whereas activation of H₂ receptors leads to relaxation. The vasomotor effects of histamine are highly dependent on species and vascular region being investigated (17, 21, 22, 40, 42, 43). In cerebral smooth muscle cells, histamine induces inositol phospholipid hydrolysis, mobilizes Ca²⁺ from intracellular store, or depolarizes membrane, and promotes Ca²⁺ entry from voltage-dependent Ca²⁺ channels (14, 15, 21, 22). Elevation of intracellular Ca²⁺ not only produces contraction, but also activates Ca²⁺-dependent K⁺ channels, Ca²⁺-dependent Cl⁻ channels, or nonselective cation channels (15, 14, 42).

Elevation of intracellular Ca²⁺ is the major source for the activation of Ca²⁺-dependent Cl⁻ channels (14, 15). Histamine increases intracellular Ca²⁺, activates Ca²⁺-dependent Cl⁻ channels in the rabbit basilar arteries and produces contraction in the rabbit middle cerebral arteries (14, 15, 23). However, the role of Na⁺-K⁺-2Cl⁻ cotransporter and HCO₃⁻/Cl⁻ exchanger in the accumulation of intracellular Cl⁻, and the functional evidence of Cl⁻ channels in histamine-induced contraction in cerebral arteries, remains to be determined. Thus Cl⁻ flux was manipulated by inhibition of Na⁺-K⁺-2Cl⁻ cotransporter and HCO₃⁻/Cl⁻ exchanger, substitute of extracellular Cl⁻ concentration (from 123 to 10 mM), and blockade of Cl⁻ channels in the rabbit basilar artery exposed to histamine.

MATERIALS AND METHODS

Tissue Preparation

Male New Zealand White rabbits (n = 16) weighing 2–2.5 kg were anesthetized by intravenous injection of acepromazine (5 mg), ketamine (50 mg), and xylazine (25 mg) and euthanized by an overdose of pentobarbital sodium (250 mg). The brain was removed and the basilar arteries were cut into 2-mm-thick rings and placed in an ice-cold modified Krebs-Henseleit bicarbonate solution containing (in mM) 120 NaCl, 4.5 KCl, 1 MgSO₄, 27 NaHCO₃, 1.2 KH₂PO₄, 2.5 CaCl₂, and 10 dextrose and bubbled with 95% O₂-5% CO₂.

Isometric Tension

The isometric tension study procedures were described previously (7, 23). Briefly, the rings were suspended at 400 g resting tension (Radnoti Transducer, Radnoti Glass) between stainless steel hooks in 10-ml water-jacketed tissue baths (Radnoti Glass) in modified Krebs-Henseleit bicarbonate solution with 95% O₂-5% CO₂ at 37°C. The rings were
incubated for 90 min until a stable rest tension was achieved. The solution was changed every 20 min to remove metabolites. The tissues were challenged with KCl (60 mM) twice at 30-min intervals before the experiment. Isometric force transducers were connected to arterial rings and the contraction was recorded with an eight-channel MacLab 8E and stored on a Power Macintosh computer. The low Cl⁻ solution was prepared by replacing 120 mM NaCl with 120 mM NaOH and by titrating the pH of the buffer to 7.4 with the use of methanesulfonate acid (MS⁻).

In all experiments, whereas the extracellular Cl⁻ concentration was being changed, the low-level Cl⁻ solution was prewarmed and preoxygenated in a 37°C water bath, mixed with the agonists at the designated concentration, and injected slowly into the water bath. The control group with normal Cl⁻ solution was done in the same way. The HEPES buffer was made by replacing 27 mM NaHCO₃ with 20 mM HEPES and titrating the pH of the solution to 7.4 using 1 M NaOH solution. The tension developed by histamine application was shown as a ratio to 60 mM KCl (except the low Cl⁻-solution-induced contraction was calculated as the ratio to 90 mM KCl). The measured osmolarity of the normal Cl⁻ buffer and the low concentration Cl⁻ buffer was 284 and 288 mosmol/kg, respectively.

**Chemicals**

Histamine, d(-)+-chlorpheniramine (H₁ receptor antagonist), cimetidine (H₂ receptor antagonist), bumetanide (Na⁺-K⁺-2Cl⁻ cotransporter inhibitor), and nifuramic acid (Ca²⁺-dependent Cl⁻ channel blocker) were purchased from Sigma Aldrich. 5-Nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB), a nonselective Cl⁻ channel blocker, and indocyanine acid 94, R(+)-methylindazone (IAA-94), a nonselective Cl⁻ channel blocker, were purchased from Tocris and Alexis, respectively.

**Statistics**

All data are shown as means ± SE, and n is the number of arterial rings in each group. One-way ANOVA and Student's t-test were used as analysis methods. A value of *P* < 0.05 was considered to be significantly different.

**RESULTS**

**Histamine Induces Contraction via H₁ Receptor**

Histamine (10⁻⁸–10⁻⁵ M) produced dose-dependent contraction in the rabbit basilar artery. H₁ receptor antagonist d(-)+-chlorpheniramine (10⁻⁸ M) shifted the concentration-dependent response curve to the right and reduced significantly (*P* < 0.05, ANOVA) the maximum contraction to histamine (Fig. 1). The 50% effective concentration (EC₅₀) and the maximum normalized response in the absence of H₁ receptor antagonist d(-)+-chlorpheniramine are 1.70 ± 0.10 μM and 124.84 ± 2.16%, respectively. The maximum normalized response in the presence of H₁ receptor antagonist d(-)+-chlorpheniramine is 60.28 ± 4.86%. EC₅₀ value in the presence of d(-)+-chlorpheniramine was not calculated due to the lack of maximum contraction.

Cimetidine (3 × 10⁻⁶ M), a specific H₂ receptor antagonist, was used in another series of studies (Fig. 1). The concentration-dependent response curve to histamine remained the same either in the presence or in the absence of cimetidine. The maximum contraction to histamine was slightly increased in the presence of cimetidine (*P* < 0.05, ANOVA). The EC₅₀ and the maximum normalized response in the presence of cimetidine was 1.73 ± 1.42 μM and 165.42 ± 20.09% compared with 1.70 ± 0.10 μM and 124.84 ± 2.16% (in the absence of cimetidine), respectively.

**Effect of Bumetanide on Histamine-Induced Contraction**

Rings were pretreated with 3 × 10⁻⁵ M (n = 7) and 10⁻⁴ M (n = 7) bumetanide (Na⁺-K⁺-2Cl⁻ cotransporter inhibitor) for 30 min before application of histamine (Fig. 2, A–C) from 10⁻⁸ to 10⁻⁵ M. Bumetanide significantly suppressed the contraction in a concentration-dependent and reversible manner. The EC₅₀ and the maximum normalized response to histamine from 10⁻⁸ to 10⁻⁵ M was 0.75 ± 0.14 μM and 84.24 ± 3.39% for bumetanide (30 μM); 0.73 ± 0.04 μM and 62.04 ± 0.50% for bumetanide (100 μM); and 1.70 ± 0.10 μM and 124.84 ± 2.16% for rings without bumetanide, respectively (Fig. 2D). Bumetanide reduced significantly the histamine-induced contraction (*P* < 0.05, ANOVA).

**Effect of HEPES on Histamine-Induced Contraction**

Rings were pretreated in HEPES solution (2 × 10⁻² M, HCO₃⁻ free, n = 8) for 30 min before application of histamine at 10⁻⁶ M. The HEPES solution significantly (*P* < 0.05, Student’s t-test) suppressed the contraction induced by histamine (Fig. 3, A and B).
Effect of HEPES on KCl- and K-Methanesulfonate-Induced Contraction

To test whether HEPES solution itself has an effect on contractile proteins by changing intracellular pH, we pretreated arterial rings with 20 mM HEPES (2 × 10⁻² M, HCO₃⁻ free, n = 6) for 30 min (in Fig. 4 the time between the two contractions was mostly omitted that did not show HEPES was pretreated with the rings for 30 min) and then applied KCl at the dosage of 60 mM. HEPES solution did not change the contraction induced by 60 mM KCl (P > 0.05, Student’s t-test) (Fig. 4, A and B). Similarly, HEPES failed to reduce K-methanesulfonate-induced contraction, which indicates that the contractile function of the arterial rings was intact. K-methanesulfonate produced similar contraction in the absence (1.7 ± 0.2 g) or presence (1.55 ± 0.18 g) of HEPES.

Effect of Low Cl⁻ Concentration on Histamine-Induced Contraction

Direct application. Prewarmed and preaerated low concentration Cl⁻ (10 mM Cl⁻) buffer, having already mixed with histamine at 10⁻⁶ M, was used to compare with the effect of histamine at 10⁻⁶ M in normal Cl⁻ buffer. The contractile response to histamine dissolved at 10⁻⁶ M was enhanced transiently in low-Cl⁻ (10 mM Cl⁻) buffer for 5 to 8 min, followed by a prolonged relaxation toward the rest tension level (Fig. 5A). Thus low-Cl⁻ (10 mM Cl⁻) buffer enhanced the initial contraction but reduced plateau contraction induced by histamine (P < 0.05, Student’s t-test, Fig. 5B).

Change Cl⁻ concentration during sustained contraction. The rings were contracted first in a normal 123 mM Cl⁻ solution by histamine at 10⁻⁶ M for at least 20 min. After the tension attained stable and plateau phase, the normal Cl⁻ buffer was quickly drained and exchanged for either “fresh” normal Cl⁻ buffer (123 mM Cl⁻) or low-Cl⁻ (MS⁻, 10 mM Cl⁻) buffer containing the same concentration of histamine. No marked change in tension was observed when normal Cl⁻ solution was changed to fresh normal Cl⁻ solution (Fig. 6A). In contrast, tension was markedly and transiently enhanced to its maximum within 5–8 min after the low-Cl⁻ buffer was applied, followed by a rapid relaxation toward the rest tension level (Fig. 6B). The low-Cl⁻ buffer potentiated histamine-induced contraction (P < 0.05, Student’s t-test) but reduced sustained contraction (P < 0.05, Student’s t-test) of histamine (Fig. 6C).

Change low-Cl⁻ solution into normal Cl⁻ buffer. To investigate the nature of the transient enhancement by low-Cl⁻ buffer further, low-Cl⁻ solution with histamine (10⁻⁶ M) was applied first and then followed by histamine (10⁻⁶ M) in normal Cl⁻ solution. In this different sequence, histamine in low-Cl⁻ solution produced a transient contraction, followed by relaxation (Fig. 7A). Restoring normal Cl⁻ produced a slow-developing contraction (Fig. 7A). These studies showed that although low-Cl⁻ solution enhanced transiently of histamine-induced initial peak contraction, extracellular Cl⁻ is necessary for the sustained contractile phase (Fig. 7B).
Effect of depletion of intracellular Cl⁻ on histamine-induced contraction. This experiment tested the effect of histamine on contraction when intracellular Cl⁻ was depleted. The arterial rings were incubated in low-Cl⁻ solution (10 mM Cl⁻) for 20 min before histamine (10⁻⁶ M) was applied. The rings were then incubated in normal Cl⁻ solution (123 mM Cl⁻) for at least 60 min before applying histamine at the same concentration. Histamine (10⁻⁶ M) produced significantly higher tension in normal Cl⁻ solution than in low-Cl⁻ solution (Fig. 8).

Effect of low-Cl⁻ solution on K-methanesulfonate-induced contraction. To test whether low-Cl⁻ solution has an effect on intracellular pH or contractile proteins without adding back Cl⁻ to the buffer solution, K-methanesulfonate was used instead of KCl. K-methanesulfonate was applied first in normal Cl⁻ solution and then washed and applied in low-Cl⁻ solution. There was no significant difference in K-methanesulfonate (60 mM)-induced contraction either in low-Cl⁻ or normal Cl⁻ solutions (Fig. 9).

DISCUSSION

The present study supports the hypothesis that manipulation of Cl⁻ flux affects histamine-induced con-
traction in the rabbit basilar arteries. The Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter inhibitor bumetanide, the HCO\(_3\)\(^-\) free solution (HEPES), and the Cl\(^-\) channel blockers NPPB, niflumic acid, and IAA-94 all depressed histamine-induced contraction. Low extracellular Cl\(^-\) solution transiently enhanced the initial components of the contraction induced by histamine. However, normal Cl\(^-\) concentration seems critical in the maintaining of the plateau phase of the contraction induced by histamine.

Role of Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter in histamine-induced contraction. The Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter, also known as the bumetanide-sensitive cotransporter, is an electrically neutral pathway for the coupled movement of Na\(^+\), K\(^+\), and Cl\(^-\) across the plasma membrane (3, 33). Transport is bidirectional and is driven by the transmembrane gradients of all three ions. The Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter is one of the three mechanisms responsible for the accumulation of

Fig. 5. Low-Cl\(^-\) buffer [113 methanesulfonate acid (MS\(^-\)) and 10 mM Cl\(^-\)] on histamine-induced contraction. A: original tracings of histamine-induced contraction (10\(^{-6}\) M) in the normal (123 mM Cl\(^-\)) or low-Cl\(^-\) (10 mM Cl\(^-\)) solutions. B: summary of the effects of low-Cl\(^-\) solution on the initial and plateau phases of histamine-induced contraction. *P < 0.05, peak vs. peak control and plateau vs. plateau control, Student’s t-test.

Fig. 6. Low-Cl\(^-\) buffer (113 mM MS\(^-\), 10 mM Cl\(^-\)) on histamine-induced sustained contraction. A: original tracing of histamine (10\(^{-6}\) M) induced contraction in the normal Cl\(^-\) buffer (123 mM Cl\(^-\)). Replacement of “fresh” normal Cl\(^-\) buffer did not change the contractility. B: original tracing of histamine (10\(^{-6}\) M)-induced contraction in the normal Cl\(^-\) buffer (123 mM Cl\(^-\)). Replacement of low-Cl\(^-\) buffer enhanced transiently histamine-induced contraction, followed by relaxation toward the resting level. C: summary of low Cl\(^-\) on the sustained contraction induced by histamine at 10\(^{-6}\) M. *P < 0.05, peak vs. peak control and plateau vs. plateau control, Student’s t-test.
intracellular Cl\(^-\) in smooth muscle cells (2, 3, 16, 26–28, 33). The Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter in intact smooth muscle cells was demonstrated first by Deth et al. (12) in rat and rabbit aortas. In rat femoral arterial smooth muscle cells, norepinephrine (NE) activates the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter to increase intracellular Cl\(^-\) accumulation and to depolarize membrane potential (11). Other agonists, such as angiotension II, phenylephrine, endothelin (ET), and KCl, all acutely enhance the activity of Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter measured by the flux of \(^{86}\)Rb\(^+\) in smooth muscle cells. On the contrary, vasodilators such as nitric oxide and nitroprusside inhibited the basal Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter-1 activity (2, 3). The Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter is more active functionally in arterial smooth muscles in the deoxycorticosterone acetate-salt model of hypertension than in normotensive control rats (5, 8–11). Consequently, the level of intracellular Cl\(^-\) is higher, and the extracellular Cl\(^-\) is more positive in hypertension, thus contributing to the hypertensive depolarization and maintenance. Those studies indicate that the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter plays an im-

![Fig. 7](http://ajpheart.physiology.org/)

**Fig. 7.** Low-Cl\(^-\) buffer (113 mM MS\(^-\), 10 mM Cl\(^-\)) on histamine-induced contraction. A: original tracing of histamine-induced contraction (10 M) in the presence of low-Cl\(^-\) buffer. A transient contraction was followed by a relaxation to the resting level. Replacing low Cl\(^-\) with normal Cl\(^-\) solution (123 mM Cl\(^-\)) containing histamine restored contraction. B: summary of low-Cl\(^-\) buffer and normal Cl\(^-\) buffer on histamine-induced contraction. \(*P < 0.05, \text{normal Cl}\(^-\) vs. low-Cl\(^-\) solutions, ANOVA."

![Fig. 8](http://ajpheart.physiology.org/)

**Fig. 8.** Histamine (10 M)-induced contraction after depletion of intracellular Cl\(^-\). A: original trace of an arterial ring in response to histamine (10 M) after incubation of the arterial ring in low-Cl\(^-\) (10 mM Cl\(^-\)) solution for 20 min. Slanted bars, Discontinuity. B: summary of histamine-induced response in low-Cl\(^-\) (10 mM Cl\(^-\)) and normal Cl\(^-\) solution (123 mM Cl\(^-\)). \(*P < 0.05, \text{normal Cl}\(^-\) vs. low-Cl\(^-\) solutions, ANOVA."

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Role of extracellular Cl\(^-\) concentration in histamine-induced contraction. In this study, low-Cl\(^-\) solution potentiated transiently but significantly histamine-induced contraction, no matter if low-Cl\(^-\) solution was used initially or was added on the top of histamine-induced sustained contraction. Regardless of whether low-Cl\(^-\) solution was used initially or was added on the top of histamine-induced contraction, low-Cl\(^-\) solution consistently produced, after a transient contraction, a relaxant response to a level slightly above the resting tension. After the extracellular Cl\(^-\) was restored to normal concentration, the rings gradually developed a sustained contraction to histamine to a level significantly higher than that induced by histamine in low-Cl\(^-\) solution. These results obtained in the present study are somehow different from one of our previous studies. Low-Cl\(^-\) solution enhanced both the initial and the sustained components of ET-1-induced contraction in the rabbit basilar artery (7). Low-Cl\(^-\) solution did not cause relaxation in the presence of ET-1 (7). Even though the mechanism for low-Cl\(^-\) solution-induced relaxation in the presence of histamine is not clear, it seems a unique phenomenon for histamine-induced contraction in the rabbit basilar artery. Low-Cl\(^-\) solution did not change KCl or K-methanesulfonate-induced contraction (Fig. 9), potentiated both the initial and sustained contractions as well. Consistently, pretrat-
Fig. 10. Effects of Cl⁻ channel inhibitors on histamine-induced contraction. 
A–C: original tracings of histamine (10⁻⁶ M) induced contraction relaxed by the application of 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), indolacetic acid 94, R-(+)-methylidazole (IAA-94), and niflumic acid (10⁻⁶ - 10⁻⁴ M). 
D: summary of the effects of NPPB, IAA-94, and niflumic acid on histamine-induced contraction. *P < 0.05 vs. control as 100% ANOVA.

Fig. 11. Illustration of proposed mechanism for histamine-induced contraction in the rabbit basilar arteries. Histamine activates H₁ receptor (R) and generates D-myo-inositol 3-phosphate [Ins(3)P] that releases Ca²⁺ from internal stores through Ins(3)P. Elevation of internal Ca²⁺ may trigger Ca²⁺ release from ryanodine receptor (RyR) as Ca²⁺-induced Ca²⁺ release. Opening of Cl⁻ channels depolarizes membrane potential and activates voltage-dependent Ca²⁺ channels (VDCC) and results in Ca²⁺ entry. Elevation of internal Ca²⁺ opens Ca²⁺-activated or other types of Cl⁻ channels that may be blocked by IAA-94, NPPB, and niflumic acid. The Na⁺/K⁺-2Cl⁻ cotransporter and HCO₃⁻/Cl⁻ exchanger are involved in smooth muscle contraction and can be inhibited by bumetanide and HEPES. Inhibition of Na⁺/K⁺-2Cl⁻ cotransporter and HCO₃⁻/Cl⁻ exchanger will reduce Cl⁻ accumulation and hyperpolarizes membrane potential.

Smooth muscle
sustained component of histamine-induced contraction. The apparent discrepancy between these two studies might be related to the different experimental conditions. First, in our study, the contractile apparatus was exposed to low-Cl\(^-\) solution and histamine simultaneously, whereas in the other study (14), the tissues were treated with low-Cl\(^-\) solution for 15–30 min before histamine was applied. In our study, the depletion of intracellular Cl\(^-\) was avoided and thus a transient enhancement of the histamine-induced contraction occurred at the initial phase of the contraction. This hypothesis is supported by our experiment that depletion of intracellular Cl\(^-\) by incubating the arterial rings in low-Cl\(^-\) solution (10 mM Cl\(^-\)) for 20 min reduced histamine-induced contraction compared with the contraction by histamine in normal Cl\(^-\) solution (Fig. 8). Thus long-term depletion of intracellular Cl\(^-\) might affect contractility. Indeed, low-Cl\(^-\) solution also produced a relaxant response in histamine-induced contraction as discussed above. A normal level of extracellular Cl\(^-\) might be necessary for the sustained contraction induced by histamine. Second, methanesulfonate ion and glutamate ion, respectively, were used in these two studies. Methanesulfonate ion, which is an impermeant Cl\(^-\) channel anion, was used in our study. Because of its negligible permeability and apparent lack of interaction with Cl\(^-\), it is used as the preferred impermeant Cl\(^-\) substitute in substitution studies of membrane conductance (26, 27, 34). The low-Cl\(^-\) buffer replaced with MS\(^-\) potentiated NE-induced contraction in the rat aorta (26, 27) and ET-1-induced contraction in the rabbit basilar artery (7).

Role of Ca\(^{2+}\)-dependent Cl\(^-\) channels in histamine-induced contraction. Histamine activates H\(_1\) receptors in the rabbit pulmonary and the guinea pig tracheal myocytes, releases Ca\(^{2+}\) from the intracellular caffeine-sensitive Ca\(^{2+}\) store, and increases membrane conductance to Cl\(^-\) and K\(^+\) ion (13, 17, 41, 42). In the rabbit middle cerebral artery, histamine induces a transient contraction by releasing Ca\(^{2+}\) from intracellular store, in the presence of Co\(^{2+}\) (1 mM), which prevents Ca\(^{2+}\) influx through voltage-dependent Ca\(^{2+}\) channels and nonselective cation channels (14, 15). Histamine activates an inward current close to the equilibrated potential for Cl\(^-\) in the rat basilar artery and Cl\(^-\) channel blocker niflumic acid (100 \(\mu\)M) reversed completely the inward current (23). Thus histamine releases intracellular Ca\(^{2+}\), activates Cl\(^-\) channels, and produces contraction in cerebral arteries.

The histamine-induced contraction can be reduced or abolished by Cl\(^-\) channel blockers, as shown in the present study as well as reported previously (15, 23). Three Cl\(^-\) channel blockers were used in the present study and all achieved a relaxant effect, although IAA-94 seems more potent than those of NPPB or niflumic acid. Niflumic acid is the only specific inhibitor for the Ca\(^{2+}\)-dependent Cl\(^-\) channels and it blocks the Ca\(^{2+}\)-dependent Cl\(^-\) channels in smooth muscle, including cerebral vascular smooth muscle cells (15, 32). Niflumic acid, at the same concentration as used in this study, reduced histamine-induced inward current in tracheal myocytes and attenuated ET-1-induced contraction in the pulmonary artery in the rat (18, 24). Niflumic acid reduced the amplitude of depolarization and contraction by histamine in the rabbit middle cerebral artery and the rabbit basilar artery (7, 15, 23). However, due to the poor selectivity of these Cl\(^-\) channel blockers and their other effects on K\(^+\) channels (13, 25, 38) or voltage-dependent Ca\(^{2+}\) channels (13, 24, 25), both channels are important to the normal function of cerebral vascular function (31, 35), the exact type of Cl\(^-\) channels involved and their possible gating mechanism in the rabbit basilar artery cannot be determined by the current study.

In conclusion, this is the first investigation to our knowledge of the effect of Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter and HCO\(_3\)/Cl\(^-\) exchanger in histamine-induced contraction in cerebral arteries. Various approaches, as shown in Fig. 11, were used to demonstrate that modulation of Cl\(^-\) flux alters histamine-induced contraction in the rabbit basilar arteries.

The mechanisms that involve Cl\(^-\) flux in histamine-induced contraction are presumed that histamine activates H\(_1\) receptor, which results in an elevation of intracellular Ca\(^{2+}\), either by releasing Ca\(^{2+}\) from internal stores or by promoting Ca\(^{2+}\) entry from extracellular space. Elevation of intracellular Ca\(^{2+}\) induces contraction and in the mean time activates Cl\(^-\) channels, especially the Ca\(^{2+}\)-activated Cl\(^-\) channels. The Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter and the HCO\(_3\)/Cl\(^-\) exchanger are involved either in the initial or in the plateau phases of histamine-induced contraction. Disabling the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter or the HCO\(_3\)/Cl\(^-\) exchanger abolishes the contractile response of the rabbit basilar artery to histamine. Although a low extracellular Cl\(^-\) concentration enhances the initial contraction to histamine, a normal level of Cl\(^-\) is needed to maintain a sustained contraction.

This study revealed a unique phenomenon: normal level of Cl\(^-\) is necessary to some extent for a sustained contraction to histamine in the rabbit basilar artery. The mechanism underneath this phenomenon remains unclear although relaxants released from endothelial cells might not be involved. A proper understanding of Cl\(^-\) flux in histamine-induced contraction in cerebral arteries might be importance because maintaining a prolonged contraction is essential for hypertension and cerebral vasospasm for which we currently need more therapies (20, 30, 39).

REFERENCES


