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Histamine decreases myogenic tone in rat cerebral arteries by H₂-receptor-mediated K_V channel activation, independent of endothelium and cyclic AMP

Yagna P.R. Jarajapu^{a,b,*}, Charlotte Oomen^{a,c}, Victor V. Uteshev^{a,d}, Harm J. Knot^{a,b,c,e}

^a Department of Pharmacology and Therapeutics, University of Florida, Gainesville FL 32610, USA

^b Wake Forest Institute of Regenerative Medicine, Wake Forest University, Winston-Salem NC 27157, USA

^c Medical Physiology, University of Groningen, Groningen, The Netherlands

^d Department of Pharmacology MC#9629, Southern Illinois University, Springfield IL 62702, USA

^e Danish MyoTechnology, Aarhus, Denmark

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Abstract

The effect of histamine on the pressure-induced constriction was characterized in rat cerebral arteries and mechanisms were investigated. Rat cerebral arteries were pressurized to 70 mm Hg in an arteriograph and the effect of histamine on myogenic tone was studied. Histamine and amthamine, a selective histamine H₂-receptor agonist, concentration-dependently decreased myogenic tone, which was unchanged in the absence of endothelium. 2-(2-aminoethyl) pyridine, a selective histamine H₁-receptor agonist, produced concentration-dependent constriction of arteries that was significantly increased in the absence of endothelium. Imetit, a selective histamine H₃-receptor agonist, has no effect on myogenic tone. The dilation to histamine was antagonized by tiotidine, a selective antagonist of histamine H₂-receptor subtype, giving a pK_B of 7.86 that was not altered in the absence of endothelium. The histamine-mediated dilation was significantly antagonized by NF 449, a reversible inhibitor of Gs-protein activation but was not affected by ODO and SQ 22536. Dilations to histamine and amthamine were accompanied by a decrease in arterial wall calcium measured by fura-2 ratios. The dilation to histamine was significantly reduced by partial depolarization of smooth muscle by 25 mM KCl (control 91 ± 5%, 25 mM KCl 53 ± 5%, $P < 0.002$) and was not observed in the presence of strongly depolarizing 60 mM KCl. The histamine dilation was not affected by iberiotoxin, barium chloride and glibenclamide but was strongly antagonized by 4-aminopyridine (0.3 mM) and tetraethylammonium chloride (10 mM) (pEC₅₀: control: 5.6 ± 0.1, 4-aminopyridine: 4.1 ± 0.1 ($P < 0.001$); tetraethylammonium chloride: 3.2 ± 0.2 ($P < 0.0001$)). These results suggest that histamine-mediated reversal of myogenic tone in rat cerebral arteries is endothelium-independent, mediated by histamine H₂-receptor subtype with no involvement of guanylyl cyclase or adenylyl cyclase activation and most likely involves activation of K_V potassium channels.

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Keywords: Histamine; Myogenic tone; Endothelium; K_V potassium channels; Rat cerebral artery

1. Introduction

Histamine is a well studied endogenous molecule as an autacoid and neurotransmitter with multiple physiological and pathological effects in different organ systems. Histamine mediates its effects in different tissues via three different receptor subtypes, H₁-, H₂- and H₃-receptors (Hill et al., 1997).

In vasculature, histamine is considered as a general vasodilator, although in some vascular beds it produces constriction or biphasic responses. The histamine-mediated dilation in small arteries has been shown to be either endothelium-dependent (rat mesenteric (Lagaud et al., 1999; Moritoki et al., 1986) and cerebral arteries (Dacey and Bassett, 1987)) or -independent (mouse pial arteries (Rosenblum et al., 1990), human dorsal penile artery (Martinez et al., 2000)). Both dilation and constriction were observed in rabbit middle cerebral arteries (Ea et al., 1988) while no dilation was observed in rat middle cerebral arteries (Lagaud et al., 1999). Histamine receptor subtypes involved in these two different

* Corresponding author. Wake Forest Institute of Regenerative Medicine, Wake Forest University Baptist Medical Hospital, Medical Center Blvd., Winston-Salem NC 27157, USA. Tel.: +1 336 713 7293; fax: +1 336 713 7296.

E-mail address: yjarajap@wfbmc.edu (Y.P.R. Jarajapu).

responses were also shown to vary with the species and vascular bed.

Physiological effects of histamine in cerebral vascular bed and their pathophysiological significance have been documented. Tuberomammillary neurons represent the main source of brain histamine and project to a variety of brain regions including the cortex and hippocampus, with the strongest projection within the hypothalamus, basal forebrain and amygdala (Brown et al., 2001). Histamine is known to regulate sleep and wakefulness, and blood–brain barrier permeable antihistamines cause sedation. Besides being an essential brain neurotransmitter and a neuromodulator, histamine is also a major cerebral vasodilator. Histamine released from tuberomammillary nuclei, often located in the immediate vicinity of small cerebral arteries (Uteshev and Knot, 2005), may play a significant role in neurovascular control of cerebral blood flow.

Histamine has been shown to dilate meningeal and intracerebral arteries and increase cerebral blood flow (Heatley et al., 1982; Schmetterer et al., 1997). Histamine-induced headaches, preferentially in migraineurs, have been attributed to histamine-mediated release of nitric oxide in cerebral vasculature suggesting predominant actions of histamine on vascular endothelium (Lassen et al., 1995). Despite these lines of evidence for its physiological effects, limited information is available regarding the signaling mechanisms involved in histamine-mediated dilation in cerebral arterioles or resistance arteries. Resistance arteries have the ability to constrict in response to pressure, myogenic tone, which is the basis of pressure-mediated autoregulation of blood flow to different organs (Johnson, 1986). By virtue of myogenic tone, resistance arteries exist in partially contracted state in normal physiological conditions, modulation of which by endogenous constrictors and dilators regulates cerebral blood flow. The pressure-induced constriction involves smooth muscle membrane depolarization and influx of calcium resulting in smooth muscle contraction (Hill et al., 2001). Very few studies were focused on the effects of histamine in myogenic response in resistance arteries *in vitro* and these were aimed at characterizing receptors involved in arterial constriction and relaxation (Dacey and Bassett, 1987; Rosenblum et al., 1990).

Therefore, the present study is aimed at 1) characterizing the effect of histamine on myogenic tone, 2) characterizing the histamine receptor pharmacology and 3) elucidating mechanisms involved in the histamine-mediated dilation in rat posterior cerebral arteries.

2. Methods

Animal procedures have been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of University of Florida.

2.1. Preparation of cerebral arteries and measurement of diameter

Male Sprague–Dawley (SD) rats (250–300 g) were anesthetized by an intraperitoneal injection of pentobarbital sodium (160 mg/kg) and killed by decapitation. The brain was removed

and placed in an ice-cold oxygenated physiological saline solution (PSS, see below for composition). Posterior cerebral or cerebellar arteries were isolated and cannulated with glass pipettes mounted in an arteriograph. Arteriograph was placed on the stage of an inverted microscope and arteries were visualized by a CCD camera for the measurement of artery diameter, which was continuously monitored and the data was acquired by Myoview software ((Danish MyoTechnology (DMT), Aarhus, Denmark)). Cannulated arteries were slowly pressurized to 70 mm Hg under no flow conditions using pressure myograph (DMT, Aarhus, Denmark). PSS in the arteriograph was warmed to 37 °C while being continuously bubbled with 21% O₂, 5% CO₂, 74% N₂ (pH 7.3–7.4 in the bath).

2.2. Experimental protocol

After an equilibration period of ~20 min, arteries showed stable myogenic tone at 70 mm Hg. Concentration response curves to different agonists were generated in half log order of concentrations that were added cumulatively. Arteries were exposed to different concentrations for at least 3 min or until the observed effect reached steady state. Effects of different pharmacological tools on dilatory responses to histamine were evaluated by preincubating arterial segments for 20 min before concentration response curves to histamine were obtained and compared with the control curves obtained before treatment. In some experiments, endothelium was denuded by exposing lumen to air bubble for 1 min, followed by perfusing with PSS. Presence of functional endothelium was tested by applying 1 μM bradykinin. Endothelium-intact arteries showed almost complete relaxation (loss of myogenic tone), whereas in endothelium-denuded arteries no relaxation was observed. Obtaining the maximum possible diameter or passive diameter in calcium-free PSS (see below for composition) concluded all experiments. The myogenic tone was calculated according to the following equation:

$$\text{Myogenic tone} = (D_p - D_a)/D_p \times 100 \quad (1)$$

where D_a is the internal diameter of the arterial segment with active myogenic tone in the presence of PSS at a particular intraluminal pressure and D_p is the passive diameter obtained at the same pressure in the presence of calcium-free PSS.

2.3. Measurement of arterial wall calcium using fura-2

Arteries with stable myogenic tone were loaded with the calcium sensitive dye fura-2 using the following protocol: arteries were allowed to return to room temperature for 15 min. PSS was removed from the bath and replaced with that containing 5 μM fura-2 (acetoxymethyl ester) in dimethylsulfoxide (DMSO) with equal volume of 10% w/v pluronic F-127 (to solubilize fura-2 in aqueous solution). After 30 min, the fura-2 was washed out at room temperature and then the temperature was raised back to 37 °C. All arteries were allowed to recover myogenic tone completely before different pharmacological treatments.

Fura-2 ratio images were obtained using a computer controlled monochromatic excitation light source (TILL Polychrome

II, TILL-photonics, Martinsried, Germany) and a cooled CCD camera with exposure control (SensiCam, TILL-Photonics). During excitation at 340 nm or 380 nm, emission at 510 nm was collected by the camera for 300 ms. A 340/380 ratio image was generated following background subtraction. In order to minimize photobleaching and photodamage calcium images were only obtained when arterial diameter was at steady state following exposure to each concentration of agonists. The decrease in fura-2 ratio by histamine and amthamine was expressed as percent of that produced by 16 mM KCl which causes maximum decrease in the arterial wall calcium due to hyperpolarization secondary to the activation of inward-rectifier potassium channels (Knot et al., 1996; McCarron and Halpern, 1990).

2.4. Data analysis and statistics

Results are expressed as mean $\pm$ S.E.M; n indicates the number of independent experiments, which equals the number of animals used. Results were compared by Students 't'-test using Prism (GraphPad software, San Diego, CA) and a 'P' value <0.05 was considered statistically significant.

The efficacy of dilatory agonists was expressed as the maximum response (maximum percent decrease in myogenic tone) produced. The potency was expressed as pEC_{50} (negative logarithm of the concentration of the agonist that produces 50% of the maximum effect). pEC_{50} values were calculated by analyzing concentration response curves using Prism (GraphPad software, San Diego, CA) that fits the data to a four-parameter logistic equation given below:

$$Y = \text{Minimum} + \frac{(\text{Maximum} - \text{Minimum})}{1 + 10^{(\log EC_{50} - X)P}} \quad (2)$$

where minimum and maximum indicate the smallest and the highest responses produced by an agonist, X is the logarithm of the molar concentration of an agonist, Y is the response at a concentration of X and P is the Hill slope.

Effect of an antagonist was evaluated by incubating arterial segment with antagonist for 30 min. Concentration response curves to histamine were obtained before and after incubation with the antagonist. The antagonist affinity was expressed as

pK_B value, which was calculated using the following equation (Schild, 1949):

$$pK_B = -\log\{[B]/(r - 1)\} \quad (3)$$

where K_B is the dissociation constant, $[B]$ is the molar concentration of the antagonist, and r is the ratio of EC_{50} of the agonist in the presence of the antagonist to that in the absence.

2.5. Drugs, chemicals and solutions

Most of the drugs used in this study were obtained from Sigma (St. Louis, MO). Amthamine dihydrobromide, and tiotidine were obtained from Tocris (MO, USA). Stock solutions (10 mM) of histamine, amthamine (2-amino-4-methyl-5-thiazoleethanamine), 2-(2-aminoethyl) pyridine (PEA), imetit (S-[2-(imidazolo-4-yl)ethyl]isothiourea dihydrobromide), bradykinin, NF 449 (4,4',4'',4'''-[carbonyl-bis[imino-5,1,3-benzenetriyl bis-(carbonylimino)]tetrakis-(benzene-1,3-disulfonic acid, 8 Na), barium chloride and dihydroouabin were prepared in distilled water. Tiotidine, ODQ (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one), SQ 22536 (9-(tetrahydro-2'-furyl)adenine), glibenclamide and forskolin were prepared in dimethylsulfoxide. Iberiotoxin (0.1 mM) was dissolved in Tris buffered saline with 0.1% bovine serum albumin at pH 7.5 as recommended by the supplier. Tetraethylammonium chloride and 4-aminopyridine were prepared in PSS. Adjustment of pH to 7.3–7.4 was needed to make clear solutions of 4-aminopyridine. Stock solution (10 mM) of fura-2 AM ester (Molecular probes, Eugene, OR, USA) was prepared in DMSO.

The composition of PSS (mmol/L): NaCl (120), KCl (3), $NaHCO_3$ (24), $NaH_2PO_4 \cdot H_2O$ (1.2), $CaCl_2$ (2.5), $MgSO_4 \cdot 7H_2O$ (1.2) and glucose (4). PSS with 16 and 60 mM KCl was prepared by replacing NaCl with an equimolar quantity of KCl. Calcium free PSS was prepared by replacing $CaCl_2$ with 2 mmol/L EGTA.

3. Results

Cerebral arteries used in the present study showed spontaneous constriction in less than 30 min after pressurization to 70 mm Hg. Passive diameter (inner) of arteries used in the present study

Fig. 1. Representative tracing of inner diameter of rat posterior cerebral artery pressurized at 70 mm Hg showing development of spontaneous constriction in response to intraluminal pressure, dilation to 16 mM extracellular KCl, concentration response curve (CRC) to histamine and passive dilation in the presence of calcium-free PSS. Dots represent application of different concentrations of histamine.

Fig. 2. (A) Concentration response curves to histamine and subtype-selective agonists in rat cerebral arteries in the presence (E+) and absence (E-) of endothelium. (B) Antagonism of concentration response curves to histamine by 0.1 μ M tiotidine in rat cerebral arteries in the presence (E+, $n=8$) and absence (E-, $n=5$) of endothelium.

was 176 ± 5 μ m with an active diameter of 110 ± 4 μ m giving myogenic tone of $38 \pm 1\%$ ($n=33$) at the intraluminal pressure of 70 mm Hg. Exposure to 60 mM KCl produced a decrease of 105 ± 6 μ m (or $62 \pm 1\%$, $n=15$) in the passive diameter and 16 mM KCl resulted in complete reversal of myogenic tone or maximum dilation ($n=21$). Fig. 1 shows a representative tracing of diameter recording, depicting a typical experimental protocol with spontaneous development of myogenic tone at an intraluminal pressure of 70 mm Hg followed by dilation to 16 mM KCl, concentration response curve to histamine and conclusion of the experiment by exposing the artery to calcium-free PSS that resulted in passive relaxation.

3.1. Effects of agonists and antagonists of histamine receptors on myogenic tone

Effects of different agonists were evaluated in cerebral arteries in the presence and absence of endothelium. Histamine and amthamine, histamine H_2 -receptor selective agonist (Eriks et al., 1992), produced concentration-dependent dilation of cerebral arteries resulting in almost complete reversal of myogenic tone with pEC_{50} of 5.63 ± 0.04 ($n=14$) and 6.34 ± 0.03 ($n=12$), respectively (Fig. 2A). In the absence of endothelium, histamine- and amthamine-mediated dilations were unaltered (Fig. 2A). 2-(2-aminoethyl) pyridine (PEA), selective histamine H_1 -receptor agonist (Donaldson and Hill, 1986) produced concentration-dependent constriction of arteries with efficacy of 150 ± 6 (% myogenic tone, $n=11$) and potency (pEC_{50}) of $4.54 \pm$

0.09 (Fig. 2A). Efficacy of PEA was significantly increased in the absence of endothelium (191 ± 10 , $n=7$, $P<0.0001$) with the potency unchanged (4.79 ± 0.10) (Fig. 2A). Imetit, selective histamine H_3 -receptor agonist (Garbarg et al., 1992), had no effect on myogenic tone in the presence ($n=3$) and absence of endothelium ($n=3$) (Fig. 2A).

Results from experiments using agonists suggested a predominant role of histamine H_2 -receptors in histamine-mediated dilation in these arteries. This was further confirmed by using selective antagonist of histamine H_2 -receptor, tiotidine (Yellin et al., 1979). Concentration-dependent dilatory effects of histamine were antagonised by 0.1 μ M tiotidine with no change in the maximum response, resulting in parallel rightward shift of concentration response curves to histamine (Fig. 2B). Potency (pEC_{50}) of histamine (5.64 ± 0.04) was decreased by 8-fold (pEC_{50} 4.77 ± 0.02) giving a pK_B value of 7.86 ± 0.14 ($n=8$). Experiments with 0.1 μ M tiotidine in arteries without endothelium showed similar results, giving a similar pK_B value of 7.44 ± 0.20 ($n=5$) (Fig. 2B). Incubation of arterial segments with tiotidine for 30 min did not alter basal myogenic tone. Consistent with observations with PEA, histamine-mediated dilation was not affected by selective H_1 -receptor antagonist (Hill et al., 1981), pyrilamine, in the presence and absence of endothelium (data not shown).

3.2. The effect of NF 449 on the histamine-mediated dilation

The histamine-mediated dilation in cerebral arteries was evaluated in the presence of 10 μ M NF 449, a reversible inhibitor of Gs-protein activation (Hohenegger et al., 1998). Incubation with NF 449 for 30 min did not alter basal myogenic tone. Pre-incubation of arterial segments with this compound for 30 min produced significant rightward shift in concentration response curves to histamine with no change in the maximum dilation (Fig. 3). The potency of histamine was significantly decreased by 76-fold (pEC_{50} : control 6.02 ± 0.06 ; NF 449 4.14 ± 0.04 , $n=5$, $P<0.001$).

3.3. Effects of ODQ and SQ 22536 on the histamine-mediated dilation

Guanylyl cyclase and adenylyl cyclase inhibitors, ODQ (10 μ M) and SQ 22536 (200 μ M) (Fabbri et al., 1991; Garthwaite

Fig. 3. The antagonism of histamine-mediated dilatory responses by NF 449 in rat posterior cerebral arteries. Potency (pEC_{50}) of histamine was significantly decreased by NF 449 ($P<0.001$, $n=5$).

Fig. 4. Representative fura-2 ratio images of rat cerebral arteries pressurized at 70 mm Hg with myogenic tone (control) and with different treatments, exposure to 60 and 16 mM KCl and different concentrations of histamine were shown. Fura-2 ratios and diameter (microns) were provided for each image shown. Summary of the effects of histamine ($n=6$) and amthamine ($n=5$) on arterial wall calcium expressed as per cent response (decrease) to 16 mM KCl was graphically represented in the top right (see text for details).

et al., 1995), respectively, were used to evaluate the possible involvement of cGMP and cAMP, in cellular signaling involved in histamine-mediated dilation in cerebral arteries. ODQ increased basal myogenic tone by $11 \pm 3\%$ ($n=4$) while SQ 22536 ($n=4$) had no effect at the concentration used. Pre-incubation with either of these two compounds for 30 min did not alter histamine-mediated dilation. As positive control experiments, effects of ODQ and SQ 22536 were evaluated on dilatory responses to bradykinin and forskolin, respectively, in these arteries under the same conditions. ODQ (10 μ M) produced $78 \pm 8\%$ ($n=4$) decrease in dilation to bradykinin (3 μ M) and SQ 22536 (200 μ M) produced $89 \pm 8\%$ ($n=4$) decrease in dilation to forskolin (3 μ M).

3.4. Effects of histamine and amthamine on the arterial wall calcium

Dilatory effects of histamine and amthamine were evaluated for simultaneous changes in arterial wall calcium in fura-2 loaded arterial segments. Representative fura-2 images of an arterial segment with myogenic tone and with different treatments were shown in Fig. 4. Arteries showed fura-2 ratios of 0.76 ± 0.02 and 1.29 ± 0.03 ($n=15$) before and after the development of myogenic tone at the intraluminal pressure of 70 mm Hg. Fura-2 ratio was increased and decreased by exposure to 60 (1.93 ± 0.04 , $n=15$) and 16 mM KCl (0.79 ± 0.02 , $n=15$) as depicted in Fig. 4. Changes in fura-2 ratios produced by agonists were expressed as percent of the effect produced by 16 mM KCl that dilates the arteries by hyperpolarization. Both agonists decreased arterial wall calcium in a concentration-dependent manner with a potency (pEC_{50}) of 5.44 ± 0.2 (histamine, $n=6$) and 6.20 ± 0.3 (amthamine, $n=4$)

that paralleled their potency in reducing the myogenic tone in this group of experiments (pEC_{50} : histamine: 5.81 ± 0.2 ; amthamine: 6.44 ± 0.3) (Fig. 4). These observations suggested that histamine-mediated dilation involves either hyperpolarization of smooth muscle membrane potential or antagonism of voltage-gated calcium channels. Following experiments were performed to investigate these possible mechanisms.

3.5. The effect of high potassium-induced depolarization on the histamine-mediated dilation

The histamine-mediated dilation was evaluated in the arteries with myogenic tone that were further constricted with 25 mM KCl, which partially depolarizes smooth muscle membrane and 60 mM KCl that produces higher degree of depolarization resulting in almost maximum calcium influx in these arteries

Fig. 5. The histamine-mediated dilation in cerebral arteries pre-constricted with either 25 or 60 mM KCl. The histamine-mediated dilation was significantly decreased in the presence of 25 mM KCl ($P<0.002$) and was completely blocked in the presence of 60 mM KCl.

(Knot and Nelson, 1998). Treatment with 25 and 60 mM KCl increased basal myogenic tone by $39 \pm 2\%$ ($n=5$) and $59 \pm 1\%$ ($n=7$), respectively. Histamine-mediated dilation was significantly decreased in the presence of 25 mM KCl (control $91 \pm 5\%$, 25 mM KCl 53 ± 8 , $n=6$, $P<0.002$, Fig. 5) and was not observed in the presence of 60 mM KCl ($n=6$, Fig. 5).

3.6. Effects of different potassium channel blockers on the histamine-mediated dilation

Different potassium channel blockers were used to identify the involvement of membrane hyperpolarization evoked by potassium channel activation in the histamine dilation. Barium chloride (30 μ M), a selective blocker of inward-rectifying potassium channels (Kir channels) (Nelson and Quayle, 1995; Quayle et al., 1993) did not affect dilatory responses to histamine ($n=5$, Fig. 6). Iberitoxin (100 nM), a selective inhibitor of large conductance calcium-activated potassium channels (K_{Ca} channels) (Galvez et al., 1990), had no effect on histamine-mediated dilation ($n=4$, Fig. 6). A selective blocker of KATP channels, glibenclamide, also had no effect on histamine dilation ($n=3$, data not shown). 4-Aminopyridine, a selective blocker of voltage-dependent potassium channels (K_V channels) (Knot and Nelsom, 1995), was used at two different concentrations that produced concentration-dependent antagonistic effects on histamine dilation. Significant decrease in the potency of histamine resulting in rightward shift of concentration response curves was observed in the presence of 0.3 and 3 mM 4-aminopyridine (pEC_{50} : control 5.6 ± 0.1 ($n=15$), 4-aminopyridine (0.3 mM) 4.1 ± 0.1 (39-fold decrease, $P<0.001$, $n=4$); 4-aminopyridine (3 mM) 3.8 ± 0.1 (70-fold decrease, $P<0.0001$, $n=6$). Finally, tetraethylammonium chloride (10 mM), a non-selective potassium channel blocker at this concentration, right-shifted concentration response curves to histamine decreasing the potency by 269-fold (pEC_{50} : 3.2 ± 0.2). In the presence of 4-aminopyridine or tetraethylammonium chloride, histamine could not achieve maximum dilatory effect in the concentration range used i.e. up to 300 μ M. Pre-incubation for 30 min with barium chloride, iberitoxin, 4-aminopyridine and tetraethylammonium chloride increased basal myogenic tone by 36 ± 9 , 30 ± 5 , 40 ± 4 and $44 \pm 6\%$, respectively. In order to rule out, rightward shift in concentration response curves to histamine was

due to increased constriction to potassium channel blockers, 4-aminopyridine and tetraethylammonium chloride, histamine dilation was checked in arteries pre-constricted with 5-hydroxytryptamine (5HT) in addition to myogenic tone. 5HT (1 μ M) increased basal myogenic tone by $54 \pm 5\%$, which was higher than that produced by pretreatment with different potassium channel blockers. Arteries precontracted with 5HT were dilated to histamine in a similar fashion as control with no change in the potency (pEC_{50} : control 5.5 ± 0.1 ; 5HT 5.4 ± 0.1 , $n=4$) and efficacy (% dilation: control 97 ± 2 ; 5HT 92 ± 4) of histamine suggesting that the effects of potassium channel blockers on the histamine dilation were not due to the increased constriction but due to blockade of the potassium channel activity.

Finally, the histamine dilation was evaluated in the presence of dihydroouabain, a selective inhibitor of Na^+K^+ -ATPase (Daut, 1983). The activation of this enzyme causes smooth muscle membrane hyperpolarization and arterial dilation. Incubation of arterial segments with 10 μ M dihydroouabain resulted in $34 \pm 4\%$ increase in basal myogenic tone ($n=6$). In the presence of 10 μ M dihydroouabain, the histamine produced similar amount of dilation with no change in the potency (pEC_{50} : control 5.8 ± 0.1 ; dihydroouabain 5.6 ± 0.1 , $n=6$).

4. Discussion

The present study suggests that the histamine-mediated dilation in rat posterior cerebral arteries is endothelium-independent and mediated via Gs-protein activation by histamine H_2 -receptor subtype in smooth muscle. It does not involve production of cAMP or cGMP in smooth muscle. This is the first study to evaluate histamine pharmacology on pressure-induced constriction, myogenic tone, and to show that the dilation to histamine H_2 -receptor stimulation was accompanied by decreased arterial wall calcium and most likely involves activation of K_V channels.

Studies with agonists in arterial segments in the presence and absence of endothelium showed the histamine dilation is exclusively mediated by receptors on smooth muscle. This was further supported by studies with selective histamine H_2 -receptor antagonist, tiotidine that showed a pK_B of 7.86 and 7.44 in the presence and absence of endothelium, respectively that are in agreement with published affinity values of this antagonist to the histamine H_2 -receptor subtype (Yellin et al., 1979; Hill et al., 1997). PEA increased constriction of pressurised arteries suggesting a constrictor role for histamine in these arteries that was mediated by histamine H_1 -receptors. PEA-mediated constriction was increased in the absence of endothelium, which could be due to the absence of basal release of endothelial dilators that would otherwise antagonise constriction to some extent. However, responses to histamine did not show constriction throughout the concentration range used, suggesting a predominant dilatory effect of histamine that would mask the histamine H_1 -receptor-mediated constriction. On the other hand, selective agonist of histamine H_3 -receptor subtype has no effect on the basal myogenic constriction ruling out a role for histamine H_3 -receptors in histamine-mediated responses in these arteries. These findings are in agreement with earlier reports showing histamine dilation by histamine H_2 -receptors in smooth muscle of resistance arteries

Fig. 6. Effects of different potassium channel blockers, barium chloride ($n=5$), iberitoxin ($n=4$), 0.3 mM ($n=5$) and 3 mM ($n=6$) 4-aminopyridine and tetraethylammonium chloride (TEA, $n=4$) on histamine-mediated dilation. The histamine dilation was significantly antagonized by 4-aminopyridine and TEA (see text for details).

(rat middle cerebral arteries (Benedito et al., 1991), intracerebral arterioles (Dacey and Bassett, 1987), meningeal arteries (Dux et al., 2002), mesenteric arteries (Adeagbo and Oriowo, 1998) and mouse cerebral arteries (Rosenblum et al., 1990)). However, these studies showed additional involvement of histamine H_1 -receptors in endothelium suggesting a significant role of endothelium in histamine dilation (Adeagbo and Oriowo, 1998; Rosenblum et al., 1990). These results together with the published information on histamine receptor pharmacology reveals that the distribution of histamine receptor subtypes in endothelium and smooth muscle and the resultant effect of their stimulation by histamine in intact arteries varies with the vascular bed and species being studied. It is evident from this study that the histamine-mediated regulation of cerebral blood flow by its opposing effects on myogenic tone is independent of endothelial derived dilating factors.

Mechanisms of the histamine-mediated dilation, which was exclusively by its effect on arterial smooth muscle, were dissected out by using different pharmacological tools. Initially, antagonism of histamine dilation by NF 449 was evaluated to confirm the involvement of Gs-protein coupling of histamine H_2 -receptor (Hill, 1990). This compound does not affect the interaction between Gs-protein and the activated receptor instead it competitively inhibits association of $G\alpha$ subunit with GTP (Hohenegger et al., 1998). NF 449, confirming the involvement of Gs-protein activation in this response, competitively antagonised histamine-mediated dilation resulting in 76-fold decrease in the potency of histamine.

Then, we evaluated whether the histamine H_2 -receptor–Gs-protein activation causes activation of guanylyl cyclase and adenylyl cyclase. Activation of these cyclases results in the production of nucleotide second messengers cGMP and cAMP, respectively, which in turn causes smooth muscle relaxation by different mechanisms, mainly involving decreased sensitivity of myosin light chain kinase to calcium (McDaniel et al., 1994). In some preparations, activation of different potassium channels that predominantly contribute to the agonist-mediated smooth muscle relaxation or vascular dilation was shown to be mediated by cGMP- and cAMP-activated kinases (Cole et al., 1996; Aiello et al., 1998; Porter et al., 1998; Schubert and Nelson, 2001). However, pharmacological inhibitors of guanylyl cyclase and adenylyl cyclase, ODC and SQ 22536, did not affect histamine dilation, ruling out the contribution of cGMP and cAMP in Gs-protein-mediated histamine dilation therefore involvement of guanylyl cyclase and adenylyl cyclase activation.

Then, we evaluated the effect of the histamine H_2 -receptor activation in the regulation of arterial wall calcium, depletion of which causes dilation. In rat cerebral arteries myogenic tone was shown to accompany calcium influx predominantly via voltage-gated calcium channels (Knot and Nelson, 1998). Fura-2 ratio imaging studies clearly showed concentration-dependent decrease in myogenic tone by histamine and amthamine accompanied decrease arterial wall calcium. Therefore, histamine dilation involves alterations in the ionic regulation of calcium influx. This could be secondary to one of the several mechanisms i.e. by blocking voltage-gated calcium channels, decreasing the membrane excitability (membrane hyperpolarization) by activating Na^+K^+ -ATPase or potassium channels that would in turn cause

closure of voltage-gated calcium channels. These possibilities were further explored as described below.

The histamine dilation was evaluated in the presence of elevated extracellular K^+ to 25 and 60 mM that would increase membrane potentials to -47 and -23 mV respectively, as calculated by the Nernst equation, assuming intracellular $[K^+]$ of 145 mM at 37°C . The membrane potential of pressurized posterior cerebral arteries is close to the theoretical (Nernst) K^+ 'equilibrium potential' (E_K) when extracellular K^+ is elevated above 16 mM concentration, as shown by Knot and Nelson (1998). The membrane depolarization enhances opening of voltage-gated calcium channels resulting in calcium influx causing arterial smooth muscle contraction (Nelson et al., 1990). Depolarization by 25 mM K^+ decreased histamine dilation while depolarization by 60 mM K^+ abolished histamine dilation. If histamine dilation involves blockade of calcium channels, similar or higher dilation would be expected when arteries were depolarized by either 25 or 60 mM K^+ and therefore, calcium channel blockade is unlikely to be involved in the dilatory effect of histamine. The alternative possibility for the histamine-mediated decrease in arterial wall calcium and dilation could be the membrane hyperpolarization by the activation of Na^+K^+ -ATPase or potassium channels. The activation of Na^+K^+ -ATPase was ruled out by the lack of inhibitory effect of dihydroouabain on the histamine dilation. Therefore, the role of different potassium channels was evaluated as described below, that would also explain the decrease in dilatory responses to histamine in the presence of 30 mM extracellular K^+ .

Different potassium channels that fine-tune the smooth muscle membrane potential control tone and dilation in cerebral arteries (Nelson et al., 1990; Faraci and Heistad, 1998). Different studies showed evidence for the presence of functional Kir (McCarron and Halpern, 1990), K_{Ca} (Brayden and Nelson, 1992) and K_V (Bonnet et al., 1991; Knot and Nelsom, 1995) and K_{ATP} (Zimmerman et al., 1997) potassium channels in cerebral arteries. We investigated for the possible involvement of potassium channel activation in histamine-mediated dilation in rat cerebral arteries. Studies with different potassium channel blockers showed that dilation to histamine was resistant to the blockade of Kir, BK and K_{ATP} channels but was strongly antagonized by K_V channel blocker 4-aminopyridine at different concentrations used that were selective for K_V channels (Knot and Nelsom, 1995). Tetraethylammonium chloride, at a concentration of non-selectivity, also strongly antagonized histamine dilation. These results suggest that histamine H_2 -receptor–Gs-protein activation by histamine causes K_V channel activation resulting in membrane hyperpolarization, which in turn reduces or inhibits calcium influx by causing closure of voltage-dependent calcium channels resulting in smooth muscle relaxation.

These observations also explain histamine dilation in the presence of 25 mM but not 60 mM extracellular K^+ . Membrane potential activates K_V channels resulting in outward potassium currents at membrane potential positive to E_K (-85 mV). The activation and inactivation of K_V channel currents are membrane potential-dependent. At the membrane potential that was clamped at -47 mV by 25 mM K^+ , K_V channels exist in an activated state but further increase in the clamped membrane potential to -23 mV by 60 mM K^+ results in inactivation of K_V

channel-mediated outward potassium currents (Robertson and Nelson, 1994). Partial dilation by histamine with 25 mM of extracellular K^+ was possible due to the presence of active/open K_V channels at this membrane potential that could be activated by histamine, which was not likely the case in the presence of 60 mM extracellular K^+ . However, the present study was purely functional pharmacological study and a systematic electrophysiological study has to be carried out for better understanding of histamine-mediated alterations in the smooth muscle membrane ionic currents.

Earlier studies in rat mesenteric vascular bed showed evidence for the involvement of potassium channels in the histamine dilation that was blocked by dequalinium, a selective blocker of small conductance calcium-activated potassium channels but the role of cAMP was not evaluated (Adeagbo and Oriowo, 1998). However, the activation of potassium channels by receptor-coupled G-protein activation independent of cAMP and cGMP was reported with different dilators in different experimental models. β_3 -adrenoceptor-mediated relaxation in guinea pig gastrointestinal smooth muscles by Gs-protein-coupled K_V channel activation (Horinouchi et al., 2003), constriction of cerebral arteries to melatonin by Gi/o-coupled inhibition of BK channels (Geary et al., 1997), A1-adenosine receptor-mediated relaxation of rat ventricular myocytes by $G_{i\alpha}$ coupled K_{ATP} channel activation (Kirsch et al., 1990), coupling of somatostatin receptors to K_{ATP} and SK channels by G_{i2} -proteins in rat pancreatic α cells (Gromada et al., 2001) and coupling of Kir channels to M_2 and M_4 -muscarinic receptors by Gs-proteins (rat sympathetic neurons (Fernandez-Fernandez et al., 1999)) and to VIP (vasoactive intestinal polypeptide) receptors by $G?$ -protein (bovine pulmonary endothelial cells (Pasyk et al., 1996)).

Present study suggesting functional coupling of histamine H_2 -receptors to K_V channels provides a new insight into the physiological significance of these ion channels in the histamine-mediated dilation in cerebral resistance arteries. Pressure-induced constriction involves membrane depolarization that results in the activation of K_V and K_{Ca} channels but not K_{ATP} or Kir channels (Brayden and Nelson, 1992; Knot and Nelsom, 1995), which would limit pressure-induced depolarization and constriction. It is interesting to find out from the present study that histamine utilizes some of those pressure-activated ionic mechanisms to dilate cerebral arteries and regulate cerebral blood flow. Further studies are required to understand cellular signaling events that activate K_V channels by the histamine H_2 -receptor-coupled Gs-protein activation.

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