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Role of endothelium and nitric oxide in histamine-induced responses in human cranial arteries and detection of mRNA encoding H_1- and H_2-receptors by RT-PCR

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1 Histamine induces relaxation of human cranial arteries. Studies have revealed that the relaxant histamine H_1-receptor predominates in human cerebral and the H_2-receptor in temporal arteries, while H_1- and H_2-receptors are of equal importance in the middle meningeal artery. The purpose of the present study was to examine the role of the endothelium and nitric oxide in histamine-induced responses and to show the presence of mRNA encoding H_1- and H_2-receptors in human cranial arteries.

2 Electrophoresis of polymerase chain reaction (PCR) products from human cerebral, middle meningeal and temporal arteries, demonstrated products corresponding to mRNA encoding both H_1- and H_2-receptors in arteries with and without endothelium. The amplified PCR products were sequenced and showed 100% homology with the published sequences of these histamine receptors.

3 A sensitive *in vitro* system was used to study vasomotor responses to histamine. In precontracted cerebral, middle meningeal and temporal arteries with and without endothelium, histamine caused a concentration-dependent relaxation with I_{max} values between 87% and 81% and pIC_{50} values between 8.14 and 7.15. In arteries without endothelium the histamine-induced relaxation was significantly less potent (I_{max} values between 87% and 66% and pIC_{50} values between 7.01 and 6.67) than in cranial arteries with an intact endothelium.

4 The addition of histamine to arteries without endothelium and pretreated with the histamine H_2-antagonist, cimetidine (10^{-5} M), caused a concentration-dependent contraction of the cranial arteries with E_{max} values between 86% and 29% and pEC_{50} values between 7.53 and 6.77. This contraction was blocked by the histamine H_1-receptor antagonist, mepyramine (10^{-7} M), and even turned into a relaxation with I_{max} values between 84% and 14% and pIC_{50} values between 7.42 and 5.86.

5 The nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 3×10^{-5} M) significantly inhibited the relaxant response to histamine in cerebral and temporal arteries (pIC_{50} values between 7.43 and 7.13). The combined treatment with L-NAME (3×10^{-5} M) and cimetidine (10^{-5} M) caused a further displacement of the concentration-response curve (pIC_{50} values between 7.14 and 6.57) and decreased the maximum relaxant responses in all three cranial arteries (I_{max} values between 62% and 39%).

6 In conclusion, this is the first study which show mRNA encoding histamine H_1- and H_2-receptors in human cranial arteries. The results indicate that histamine-induced relaxation of human cranial arteries is partially mediated via an endothelial H_1-receptor coupled to the production of nitric oxide and partially via a H_2-receptor associated with the smooth muscle cells. In addition, there is evidence for a contractile H_1-receptor in the smooth muscle cells in these arteries.

Keywords: Histamine receptors; human cranial arteries; vasomotor responses; mRNA; RT-PCR; endothelium; nitric oxide; L-NAME; vascular smooth muscle

Introduction

Histamine is considered a regulator of the microcirculation by controlling vascular resistance through local production and release. The physiological response to histamine is mediated through distinct receptors of which three subtypes (H_1, H_2 and H_3) have now been identified functionally (Leurs *et al*., 1995). The vasomotor effects of histamine, dilatation or constriction, are highly dependent on species and vascular region being investigated. The type of response further depends on factors like the histamine concentration, the route of administration, the vessel calibre, pre-existing tone, and the relative distribution of the receptor subtypes in the vasculature. Thus, observations made in certain vascular beds or animals do not necessarily apply to the human cranial circulation.

We have in previous studies demonstrated that histamine acts as a vasodilator in the human cerebral circulation. This effect is mediated by dilator H_1- and H_2-receptors in temporal, meningeal and cerebral arteries (Ottosson *et al*., 1988; 1989; 1991). There are studies suggesting the presence of a contractile H_1-receptor on cerebral arteries, and that the action of the dilator H_1-receptor is endothelium-dependent (Toda, 1990). Receptors coupled to the endothelium often stimulate nitric oxide synthase (NOS) to produce nitric oxide (NO) and L-citrulline from L-arginine (Palmer *et al*., 1988; Moncada *et al*., 1991). The release of NO onto the smooth muscle cell results in the formation of guanosine 3′:5′-cyclic monophosphate (cyclic GMP) through guanylyl cyclase, mediating vasodilatation via a decrease in the intracellular Ca^{2+}-concentration and/or reduction of Ca^{2+}-sensitivity of the contractile proteins (Karaki,

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1989). Dilatory histamine H_2-receptors that are not endothelium-dependent are generally considered to be coupled to the adenylyl cyclase system resulting in increased levels of cyclic AMP (Johnson, 1992).

Recently, the human H_1- and H_2-receptor genes have been cloned (Gantz *et al*., 1991; De Backer *et al*., 1993; Fukui *et al*., 1994). This has provided a powerful instrument to demonstrate the presence and distribution of histamine receptor subtypes in different tissues. The aim of the present investigation was two fold: firstly, to demonstrate the role of the endothelium and NO in histamine-induced responses and the localization of the histamine receptors in human cranial arteries by vasomotor experiments *in vitro*. Secondly, to show the presence of mRNA encoding H_1- and H_2-receptors in human cerebral, meningeal and temporal arteries with and without endothelium by the use of reverse transcription (RT) of total mRNA and subsequent polymerase chain reaction (PCR).

Methods

For the vasomotor experiments human cerebral, meningeal and temporal arteries were obtained either in conjunction with neurosurgical operations or at autopsy within 2 to 6 h *post-mortem*. For RNA-experiments, tissue was collected from 2 persons at autopsy. Specimens were collected in accordance with Swedish legislation (The transplants act, § 1), and approved by the local ethics committee.

Vasomotor responses

Immediately after removal the vessel segments were placed in a buffer solution containing (mM): NaCl 119, $NaHCO_3$ 15, KCl 4.6, $CaCl_2$ 1.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2 and glucose 11. Circular vessel segments with an inner diameter of 0.3–0.6 mm (cerebral arteries) or 0.5–1 mm (meningeal and temporal arteries), and a length of 2–4 mm were mounted in a temperature-controlled tissue bath (37°C) containing buffer solution continuously bubbled with a mixture of 95% O_2 and 5% CO_2 giving a pH of about 7.4 (Högestätt *et al*., 1983). The presence of intact endothelium was assessed by a relaxant response to ACh and light microscopy of *en face* preparations stained with $AgNO_3$ (0.25%) for 2 min. Some experiments were performed in the absence of endothelium. These vessels were treated before mounting with a 10 s perfusion of 0.1% Triton-X 100. Removal of the endothelium was confirmed by loss of relaxant response to acetylcholine (ACh). The vessel segments were suspended between 2 L-shaped metal holders (0.1 or 0.2 mm in diameter). The distance between the holders could be varied by changing the position of one of the holders through a movable unit, thereby allowing adjustment of the resting tension of the segments. The other holder was connected to a Grass FT-03 transducer for registration of alterations in vascular tone. The signals were amplified, digitalized by a digital converter and recorded by a computer. The vessel segments were given a tension of 2–8 mN depending on the vessel size (usually 4 mN) and were allowed to accommodate for 1–1.5 h until the tension had stabilized at the desired level. Vessel reactivity was tested by exposure to a buffer solution containing 60 mM KCl, obtained by an equimolar substitution of NaCl for KCl. Only vessels with a reproducible potassium-induced contraction after washout with the sodium buffer solution, were used for further investigation. The potassium-induced contraction amounted to 1.94 ± 0.34 mN ($n=27$) and 4.58 ± 1.66 mN ($n=7$) in cerebral arteries, 6.64 ± 1.55 mN ($n=25$) and 6.60 ± 2.20 mN ($n=18$) in meningeal arteries and 11.32 ± 1.21 mN ($n=18$) and 20.37 ± 2.88 mN ($n=16$) in temporal arteries with and without endothelium, respectively. For the study of relaxant responses, the vessel segments were precontracted by addition of noradrenaline (NA) at a concentration of 3×10^{-6} M. At the point where a relaxant agent was added to the tissue baths (the stable level of tension) the precontraction amounted to 1.32 ± 0.24 mN ($n=36$) and 4.85 ± 1.67 mN ($n=6$) in cerebral arteries, 3.95 ± 0.80 mN ($n=33$) and 5.34 ± 1.96 mN ($n=11$) in meningeal arteries and 3.96 ± 0.81 mN ($n=24$) and 5.29 ± 1.61 mN ($n=5$) in temporal arteries with and without endothelium, respectively.

Concentration-response data were obtained by cumulative addition of histamine to the tissue bath. Antagonists were added to the tissue baths 20 min before the responses to histamine were tested. The values for relaxation are expressed as percentage of the level of contraction due to NA (3×10^{-6} M). The responses were characterized in terms of E_{max} (maximum contractile effect obtained with an agonist), I_{max} (maximum relaxant effect obtained with an agonist), EC_{50} (the concentration eliciting half-maximum contractile effect), IC_{50} (the concentration eliciting half-maximum relaxant effect) and pEC_{50} or pIC_{50} (negative logarithm of EC_{50} or IC_{50}) values. When testing constrictor responses, the E_{max} value represent agonist-induced contraction measured as percentage of contraction induced by 60 mM potassium in the same vessel segment. Values are given as mean ± s.e.mean with median values within parentheses. Kruskal-Wallis non-parametric ANOVA followed by Mann-Whitney U-test was used to determine statistical significance with respect to differences in E_{max}, I_{max}, pIC_{50} and pEC_{50} values. Statistical significance was assumed when $P<0.05$.

Drugs

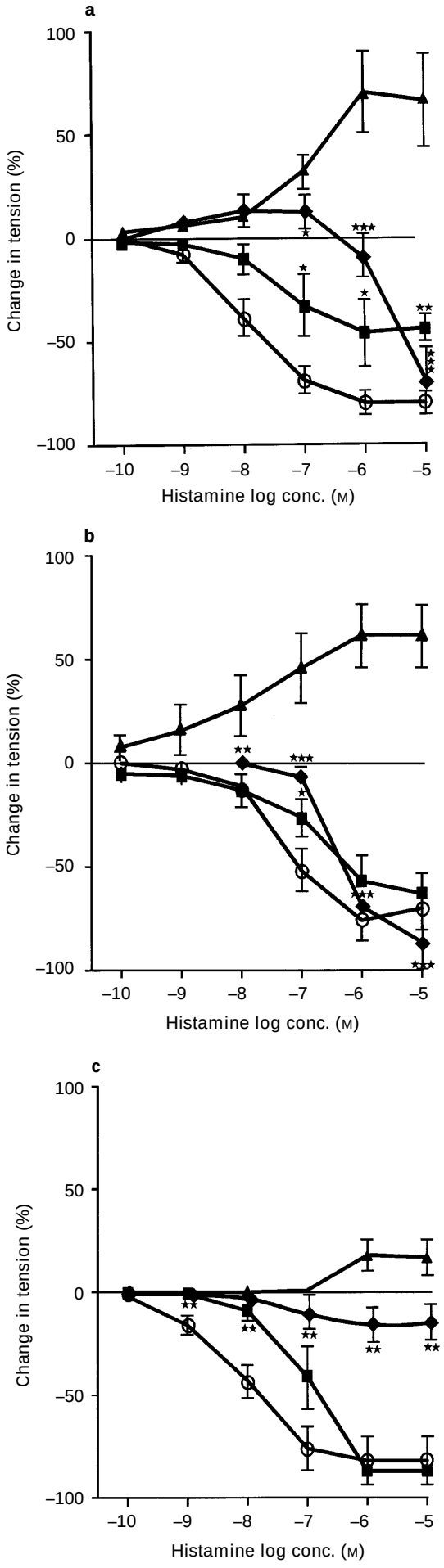
The drugs used were obtained from the following sources: acetylcholine, cimetidine, histamine dihydrochloride, indomethacin, N^G-nitro-L-arginine methyl ester (L-NAME), noradrenaline hydrochloride (Sigma Chemical, St Louis, MO, U.S.A.), mepyramine maleate (May and Baker Ltd., Dagenham, U.K.). A stock solution was prepared by dissolving the drugs in distilled water. Indomethacin was dissolved in 70% ethanol. All drugs were just before the experiment further diluted in buffer solution. The concentrations are expressed as a final molar concentration in the tissue bath.

Reverse transcriptase - polymerase chain reaction

Isolation of total RNA Human cerebral, temporal and meningeal arteries were snap frozen in liquid nitrogen immediately after acquisition. Total cellular RNA was extracted with the TRIzol reagent (GIBCO BRL, Life Technologies, Sweden). This TRIzol reagent, a mono-phasic solution of phenol, guanidine isothiocyanate, buffer and solubilizing agents, is an improvement to the single-step RNA isolation method developed by Chomczynski and Sacchi (1987). Frozen tissues were homogenized with 1 ml of TRIzol reagent until completely disrupted at room temperature, with the microprobe of a power homogenizer (Polytron Kinematica AG, Model PT 1200, Labora, Sweden) for 30 to 60 s. The homogenates were mixed with chloroform and centrifuged at $12\,000 \times g$ for 15 min at 4°C. The aqueous phase containing RNA was transferred to a fresh tube and then the RNA was precipitated from the aqueous phase by the addition of isopropanol. Samples were incubated at room temperature for 10 min and centrifuged at $12\,000 \times g$ for 10 min at 4°C.

The RNA pellet was finally washed with 70% ice-cold ethanol, air-dried, dissolved in 20 μl of diethylpyrocarbonate-treated water and stored at −20°C until use. The purity and yield of total RNA was determinated spectrophotometrically by measurement of optical density of an aliquot at 260 nm and 280 nm, by use of a DU-65 spectrophotometer (Beckman Instruments, Sweden). The ratio of absorption (260:280) of all preparations was between 1.6 and 1.8. Finally, samples were subjected to gel electrophoresis and stained with ethidium bromide to prove the integrity of the 18 and 28 S ribosomal RNAs.

Removal of genomic DNA from RNA samples In order to eliminate any residual contaminating DNA, duplicate tubes containing 1 μg of total RNA were pretreated with 1 unit of



Amplification Grade DNase I (GIBCO BRL, Life Technologies, Sweden) in 1 × DNase I reaction buffer, in the presence of 20 units of RNase inhibitor (Perkin Elmer, Sweden). RNase free water was added to each tube to a final volume of 10 μl and samples were incubated for 15 min at room temperature. One microlitre of 25 mM EDTA solution (pH 8.0) was added to each tube and samples were heated for 10 min at 65°C to inactivate the DNase I and thereafter immediately cooled on ice.

Reverse transcriptase-polymerase chain reaction (RT-PCR) Synthesis of first strand cDNA and subsequent polymerase chain reaction (PCR) amplification was carried out by use of the GeneAmp RNA PCR kit reagents (Perkin-Elmer AB, Sweden) in a PCR DNA thermal cycler (Perkin-Elmer, Sweden).

DNase-treated RNA samples were reverse transcribed to cDNA in a 20 μl reaction volume in the presence of 1 × PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3), 5 mM $MgCl_2$, 1 mM of deoxyribonucleoside triphosphate (dNTP), 50 pmol of oligo (dT) primers and 50 units of M-MLV (Moloney Murine Leukemia Virus) reverse transcriptase. The samples were incubated at room temperature for 10 min, at 42°C for 15 min, then inactivated at 99°C for 5 min and immediately cooled on ice.

Appropriate sense and antisense primer oligonucleotides were designed from the human sequences derived from GenBank accession number X76786 and M64799 corresponding to human histamine H_1- and histamine H_2-receptors, and by computer analysis with the OLIGO primer analysis software, version 4.0. The oligonucleotide primers used in the PCR to amplify a 497 bp and 330 bp fragment corresponding to a region of human histamine H_1- and H_2-receptors, respectively, were as follows: For the histamine H_1-receptor, sense primer 5′ TGGTCACAGTAGGGCTCAAC 3′ corresponding to nucleotides 116–135 and antisense primer 5′ CAAGGTGGG-CAGGTA-GAAGT 3′ complementary to nucleotides 593–612. For the histamine H_2-receptor, sense primer 5′ TCGTGTCCTTGGCTAT-CAC 3′ corresponding to nucleotides 170–188 and antisense primer 5′ CCTTGCTGG-TCTCGTTCCT 3′ complementary to nucleotides 481–499.

The PCR amplification reaction was composed to 2 μl of the first strand cDNA reaction mixture and 48 μl master mix containing 1 × PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3), 1 mM $MgCl_2$, 25 pmol of each specific sense and antisense specific primers for human histamine H_1-receptor or for histamine H_2-receptor, and 2.25 units of AmpliTaq DNA polymerase. The reaction mixture was overlaid with mineral oil (Perkin-Elmer, Sweden) and then subjected to 25–40 cycles of PCR amplification. After an initial denaturation step at 94°C for 2 min, the cycle profile included denaturation for 1 min at 94°C and annealing for 1 min at 58°C. After the final cycle, the temperature was maintained at 72°C for 7 min to allow completion of synthesis of amplified products. To determine if the amplification product came exclusively from the RNA, a reverse transcriptase negative reaction was run where the enzyme was replaced by RNA-ase free water for each sample. To establish the optimal number of cycles for the RT-PCR assay a

Figure 1 Relaxant effects of increasing concentrations of histamine in precontracted (NA, 3×10^{-6} M) human (a) cerebral, (b) middle meningeal and (c) temporal arteries with (○) and without (■) endothelium. In quiescent arteries without endothelium the presence of cimetidine (10^{-5} M) (▲) reversed the relaxation into a contraction that was blocked by mepyramine (10^{-7} M) (◆). Values given represents mean and vertical lines show s.e.mean, $n = 4–15$. Statistical evaluation comparing responses to histamine in arteries with endothelium to arteries without endothelium or to arteries without endothelium with cimetidine to arteries without endothelium with cimetidine and mepyramine was performed by the Mann-Whitney U-test, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

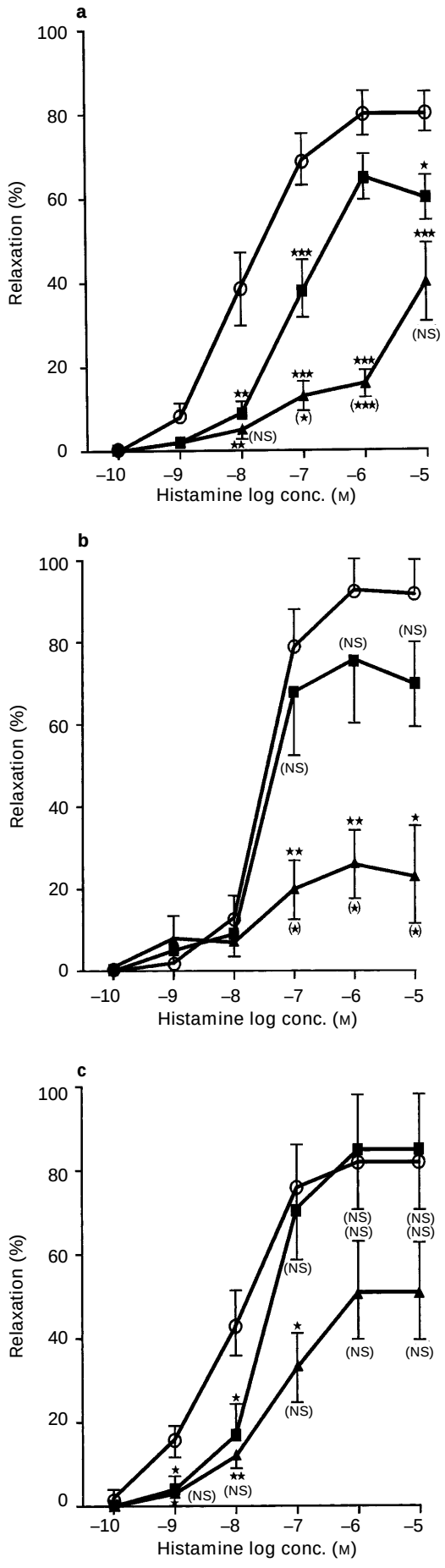


Figure 2 Relaxant effect of increasing concentrations of histamine in precontracted (NA, 3×10^{-6} M) human (a) cerebral, (b) middle meningeal and (c) temporal arteries in the absence (○) and in the presence of L-NAME 3×10^{-5} M (■) and L-NAME 3×10^{-5} M plus cimetidine 10^{-5} M (▲). Values given represents mean and vertical lines show ± s.e.mean, $n=6-15$. Statistical evaluation was performed by Kruskal Wallis non-parametric ANOVA test followed by Mann-Whitney U-test, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. Stars without parentheses indicate statistical significance as compared to control. Stars in parentheses indicate significance between relaxant responses to histamine in presence of L-NAME with and without cimetidine.

control experiment was performed with cycle numbers ranging from 25 to 40. For the qualitative purpose of our study 40 cycles appeared to be most suitable.

Electrophoretic analysis Ten microlitres from each PCR amplified product were electrophoresed in a 1.5% agarose gel (GIBCO BRL, Life Technologies, Sweden), containing 0.5 μg ml^{-1} ethidium bromide (Sigma E 1510), in 1 × TBE buffer (89 mM TRIS-borate, 2 mM EDTA, pH 8.0) at 5 V cm^{-1} for 1½ h. This analysis was performed in a 20 × 10 cm Midicell, Model EC 350 (E-C Apparatus Corporation; Techtum Lab AB, Sweden). A 100 bp DNA ladder (Promega, SDS, Sweden) was run in the outside lane to confirm the molecular size of the amplification product.

DNA-sequencing The identity of the PCR-products was verified by sequencing. Approximately 80 ng of gel-purified PCR-product was directly sequenced with both forward and reverse primers by use of the Amplitaq FS cycle sequencing kit (Perkin-Elmer) on a DNA thermocycler (Perkin-Elmer) according to the manufacturers protocol. After the reaction the samples were purified and run on an ABI Prism 310 DNA sequencer. The resulting sequences were finally compared to the published sequences of histamine H_1- and H_2-receptors by the Genetics Computer Group Sequence Analysis Software Package (version 8.1).

Results

Vasomotor studies

Cerebral arteries Histamine, given in increasing concentrations to the *in vitro* bath (10^{-10} to 10^{-5} M), induced a concentration-dependent relaxation of precontracted cerebral arteries ($I_{max}=87\pm4\%$ [91%], $pIC_{50}=7.84\pm0.17$ [7.92], $n=15$). In endothelium-denuded arteries histamine caused relaxation with a maximum response and a pIC_{50} value that were significantly ($P<0.05$) lower than in those with intact endothelium ($I_{max}=66\pm8\%$ [69%], $pIC_{50}=6.67\pm0.39$ [6.80], $n=6$). In quiescent arteries without endothelium and in the presence of cimetidine (10^{-5} M), histamine induced a concentration-dependent contraction ($E_{max}=77\pm16\%$ [69%], $pEC_{50}=7.16\pm0.46$ [6.81], $n=4$) (Figure 1a). The addition of mepyramine (10^{-7} M) caused a slight contraction in three out of five vessel segments ($E_{max}=14\pm7\%$ [17%], $pEC_{50}=9.10\pm0.48$ [9.13], $n=3$) followed by a relaxation in all five segments ($I_{max}=81\pm15\%$ [70%], $pIC_{50}=5.86\pm0.14$ [5.94], $n=5$).

The nitric oxide synthase (NOS) inhibitor L-NAME (3×10^{-5} M) significantly ($P<0.05$) reduced the relaxation and pIC_{50} induced by histamine ($I_{max}=71\pm4\%$ [73%], $pIC_{50}=7.13\pm0.11$ [7.00], $n=11$). The addition of cimetidine (10^{-5} M) displaced the concentration-response curve further to the right and reduced the relaxation significantly ($P<0.05$) at histamine concentrations between 10^{-8} M and 10^{-6} M ($I_{max}=54\pm9\%$ [68], $pIC_{50}=6.57\pm0.40$ [6.36], $n=10$) (Figure 2a). Administration of L-arginine (10^{-4} M) at the end of the experiments with L-NAME or L-NAME plus cimetidine restored the histamine-induced relaxation significantly ($P<0.05$) ($56\pm4\%$ [56%] to $72\pm6\%$ [75%], $n=7$ and $43\pm13\%$ [48%] to $63\pm14\%$ [72%], $n=4$, respectively).

In the presence of indomethacin (3×10^{-6} M) low concentrations of histamine ($10^{-10}-10^{-8}$ M) induced a slight contraction (Figure 3). The contraction was followed by a relaxation that occurred at the same concentrations as in the absence of indomethacin. This pattern of response was seen in cerebral arteries with endothelium as well as without endothelium.

Meningeal arteries In precontracted arteries histamine induced a concentration-dependent relaxation ($I_{max}=81\pm8\%$ [80%], $pIC_{50}=7.15\pm0.11$ [7.29], $n=15$). Removal of the endothelium only induced a slight rightward non-significant ($P>0.05$) shift of the relaxation-response curve ($I_{max}=68\pm8\%$ [60%], $pIC_{50}=6.86\pm0.24$ [6.66], $n=11$). As in cerebral arteries, quiescent meningeal arteries without endothelium and in the presence cimetidine (10^{-5} M) reacted with a strong contraction ($E_{max}=86\pm10\%$ [94%], $pEC_{50}=7.53\pm0.48$ [6.85], $n=7$) (Figure 1b). The additional administration of mepyramine (10^{-7} M) reversed the contraction seen in the presence of cimetidine into a relaxation ($I_{max}=84\pm21\%$ [89%], $pIC_{50}=6.28\pm0.30$ [6.51], $n=5$).

There was no significant difference either in the maximum relaxant response or in the potency to histamine in the absence and presence of L-NAME (3×10^{-5} M) (I_{max} control: $94\pm11\%$ [87], L-NAME: $88\pm15\%$ [88%]; pIC_{50} was 7.51 ± 0.06 [7.48]) ($n=8$) without L-NAME and 7.46 ± 0.11 [7.51] ($n=9$) with L-NAME present. The addition of cimetidine (10^{-5} M) together with L-NAME induced a significant ($P<0.01$) reduction in relaxation and a slight rightward shift of the concentration-response curve to histamine ($I_{max}=39\pm9\%$ [32%], $pIC_{50}=7.14\pm0.47$ [6.99], $n=9$).

Temporal arteries Precontracted temporal arteries reacted with relaxation upon histamine administration in increasing concentrations ($I_{max}=85\pm10\%$ [98%], $pIC_{50}=8.14\pm0.19$ [8.06], $n=11$). Removal of the endothelium induced a significant ($P<0.01$) rightward shift of the concentration-response curve but no reduction in I_{max} ($I_{max}=87\pm3\%$ [89%], $pIC_{50}=7.01\pm0.21$ [6.69], $n=5$). In the presence of cimetidine the relaxation was reversed to a contraction in quiescent arteries ($E_{max}=29\pm8\%$ [24%], $pEC_{50}=6.77\pm0.35$ [6.52], $n=12$). This contraction was abolished by additional blockade with mepyramine (10^{-7} M) and even turned into a relaxation in four of nine vessel segments ($I_{max}=14\pm10\%$ [0%], $pIC_{50}=7.42\pm0.40$ [7.51], $n=9$).

L-NAME significantly ($P<0.05$) shifted the histamine-induced concentration-response curve in precontracted arteries to the right but did not reduce I_{max} ($I_{max}=93\pm11\%$ [96%], $pIC_{50}=7.43\pm0.10$ [7.43], $n=7$). The additional blockade with cimetidine (10^{-5} M) slightly reduced the histamine-induced relaxation ($P>0.05$) and slightly increased ($P>0.05$) the rightward shift of the concentration-response curve ($I_{max}=62\pm10\%$ [68%], $pIC_{50}=6.95\pm0.17$ [6.88], $n=6$).

Reverse transcriptase-polymerase chain reaction

Agarose gel electrophoresis of the PCR products from human cerebral, meningeal and temporal arteries with and without endothelium, demonstrated products of the expected size corresponding to mRNA encoding both H_1-receptors (497 base pairs) and H_2-receptors (330 base pairs) (Figure 4). Human placenta was used as a positive control. DNase was successfully used to eliminate any contaminating DNA, since no bands were detected in negative controls where the reverse transcriptase enzyme was omitted in the first strand cDNA reaction. The amplified PCR-products were sequenced and compared to the published sequences of histamine H_1- and H_2-receptors and showed 100% homology. Our experiments show expression of mRNAs encoding histamine H_1- and H_2-receptors in cerebral, middle meningeal and temporal arteries. Both receptor subtypes were detectable even after removal of the endothelium.

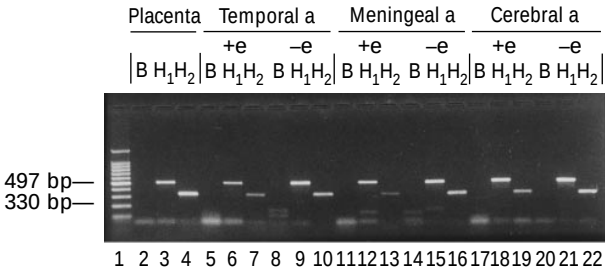


Figure 4 Gel electrophoresis of RT-PCR reaction products of mRNA fragments corresponding to human histamine H_1 (497 bp) and histamine H_2 (330 bp) receptor transcripts. As a positive control: human uterine placenta (lanes 3 and 4). H_1-mRNA and H_2-mRNA in human temporal artery with endothelium (+e, lane 6 and 7) and without endothelium (−e, lane 9 and 10). H_1-mRNA and H_2-mRNA in human meningeal artery with endothelium (lane 12 and 13) and without endothelium (lane 15 and 16). H_1-mRNA and H_2-mRNA in human cerebral artery with endothelium (lane 18 and 19) and without endothelium (lane 21 and 22). As a negative control, no amplification product occurred when reverse transcriptase was omitted in the first-strand cDNA reaction (lanes 2, 5, 8, 11, 14, 17 and 20). Promega's 100 bp DNA Ladder (Promega, SDS, Sweden) was run to confirm molecular size of the amplification product (lane 1).

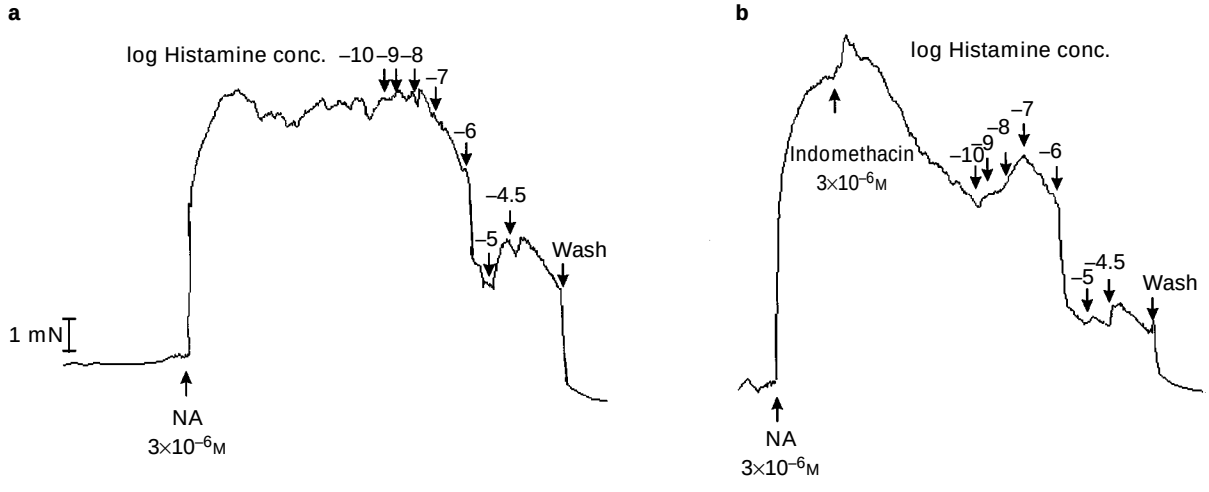


Figure 3 The response to histamine ($10^{-10}-3\times10^{-5}$ M) in arteries with endothelium in the absence (a) and presence (b) of 3×10^{-6} M indomethacin in noradrenaline (NA) precontracted human cerebral arteries.

Discussion

Human cerebral, middle meningeal and temporal arteries are equipped with relaxant histamine H_1- and H_2-receptors as shown by *in vitro* pharmacology and RT-PCR. In the cerebral artery the H_1-receptors appear to predominate while the H_2-receptor predominates in the temporal artery. In the middle meningeal artery both the H_1- and the H_2-receptors seems to be of importance for histamine-induced relaxations. H_3-receptors, originally characterized as inhibitory presynaptic receptors controlling histamine release in the brain (Arrang *et al*., 1983), have in animal experiments been found on perivascular nerve terminals and may induce vasodilatation via inhibition of sympathetic tone (Ishikawa & Sperelakis, 1987). In the rabbit middle cerebral artery, activation of endothelial H_3-receptors cause vasodilatation via release of NO and of a prostanoid (Ea-Kim *et al*., 1992). There are no data on the existence of H_3-receptors in the human cranial circulation, but preliminary *in vitro* experiments with selective agonists and antagonists have not given support for any significant H_3-receptor mechanism in intracranial arteries (unpublished results). Hopefully the H_3-receptor is cloned in the near future, which will be of great aid in the localization of this receptor in the vasculature.

In the present study we examined the role of the endothelium and of NO in histamine induced responses and studied possible contractile histamine receptors. Furthermore, we used reverse transcription of total RNA and subsequent polymerase chain reaction (PCR) in order to demonstrate the presence of mRNA encoding H_1- and H_2-receptors in human cerebral, meningeal and temporal arteries with and without endothelium.

RT-PCR

For the amplification of histamine H_1- and H_2-receptor cDNA we designed new sets of primers based on the known nucleotide sequences for the human histamine receptors (GenBank accession number X76786 and M64799). The DNA nucleotide sequences encoding these receptors are intronless, which provides a special problem since the samples therefore must be free from DNA contamination. This was achieved by using RNase free DNase and confirmed by replacing the reverse transcriptase by RNase free water in synthesis of first strand cDNA.

The presence of both H_1- and H_2-receptors in cerebral, meningeal and temporal arteries was confirmed by the gel electrophoresis of the PCR products (Figure 3). This was further verified by sequencing of the products, which showed 100% homology with the published nucleotide sequences.

As can be seen in Figure 4 mRNA encoding the H_1- and H_2-receptors in cerebral, meningeal and temporal arteries remain despite removal of the endothelium. This is the first time that mRNA encoding the histamine H_1- and H_2-receptors has been demonstrated in human cranial vessels and the findings support the view that both H_1- and H_2-receptors are present in smooth muscle in these human arteries. The finding that both receptors are expressed in the smooth muscle cells agrees well with the pharmacological experiments.

Vasomotor studies

The relaxant response (I_{max}) to histamine in precontracted human cerebral arteries was reduced by removal of the endothelium. The concentration-response curve was shifted to the right in all three cranial arteries. However, it was not significantly shifted in the human meningeal artery. This indicates the existence of a notable relaxant endothelial histamine receptor in the human cerebral and temporal arteries. The remaining relaxation after endothelium removal shows the existence of a relaxant smooth muscle receptor. Blockade of the H_2-receptor by cimetidine in endothelium denuded arteries changed this relaxation into a contraction, demonstrating that the relaxant smooth muscle receptor is of the H_2-type. The presence of a contractile smooth muscle H_1-receptor was supported by the additional blockade with the H_1-receptor antagonist mepyramine, abolishing the contraction. This provides new information for human meningeal and temporal arteries and is in concert with previous findings on human cerebral arteries (Toda, 1990). There are results indicating that human large cranial arteries respond to histamine with contraction *in vitro*, by use of spirally cut segments (Toda, 1990), whereas the use of transcranial Doppler revealed dilatation of

Table 1 Histamine receptors in human cranial blood vessels

Cranial artery	*Receptor*	*Response*	*Location*	Reference
Cerebral (small)	H_1	R	E	Present study
	H_2	R	S	
	H_1	C	S	
Cerebral (small)	H_2	R	ND	Edvinsson *et al*., 1976
Cerebral (small)	H_1	R	ND	Ottosson *et al*., 1988
	H_2	R	ND	
Cerebral (small)	H_1	R	E	Toda, 1990
	H_2	R	S	
	H_1	C	S	
Cerebral (trunk)	H_1	C	S	Toda, 1990
Cerebral (large)	H_1	C	ND	Takagi *et al*., 1993
	H_2	R	E	
Meningeal	H_1	R	E	Present study
	H_2	R	S	
	H_1	C	S	
Meningeal	H_1	R	ND	Ottosson *et al*., 1991
	H_2	R	ND	
Temporal	H_1	R	E	Present study
	H_2	R	S	
	H_1	C	S	
Temporal	H_2	R	ND	Glover *et al*., 1973
Temporal	H_2	R	ND	Hardebo *et al*., 1980
Temporal	H_2	R	ND	Ottosson *et al*., 1989
	H_1	R	ND	
Opthalmic	H_1	R	E	Haeflinger *et al*., 1992
	H_2	R	S	

R = relaxant, C = contractile, S = smooth muscle, E = endothelium, ND = not determined.

human intracranial arteries upon histamine administration *in vivo* (unpublished results). This might be explained by the blood-brain barrier preventing histamine infusion from affecting the contractile smooth muscle histamine-receptors, and that the endothelium is intact *in vivo*.

The present and previous studies of human cranial arteries are summarized in Table 1. In many of the previous studies, in contrast to the present, the locations of the different receptors were not determined. The presence of both H_1- and H_2-receptors in the cranial arteries seems well established, and the only conflicting result is that of a relaxant H_2 receptor coupled to the endothelium in cerebral arteries (Takagi *et al*., 1993). However, this study concerned large cerebral arteries and most of the vasculature demonstrated arteriosclerotic changes, which is in contrast to the small calibre arteries used in the present study which were macroscopically free from any arteriosclerosis.

The existence of both contractile and relaxant H_1-receptors can also explain previous difficulties in showing competitive antagonism for mepyramine at the H_1-receptor in meningeal and temporal arteries (Ottosson *et al*., 1989; 1991).

Effector mechanisms for the relaxant endothelial histamine-receptor were also examined. The reduction of the histamine-induced relaxation in precontracted cerebral arteries and the rightward shift of the concentration-response curves by the nitric oxide synthase inhibitor L-NAME in cerebral and temporal arteries, suggests the presence of NO-coupled relaxant receptors (Figure 2a–c). L-NAME in the presence of cimetidine further reduced the relaxant effect and the rightward shift of the concentration-response curves in all three arterial regions, suggesting that this relaxant receptor is of the H_1-type.

The addition of indomethacin did not block the relaxation induced by histamine. The presence of indomethacin caused a slight contraction at low histamine concentrations. Conceivably low concentrations of histamine could act on the formation of dilator prostaglandins. The same response was found in arteries without endothelium indicating that the mechanism of action is not associated with the endothelium.

The dilator H_2-receptor, acting independently of the endothelium/NO, has in previous studies been shown to be coupled to activation of adenylyl cyclase and increased levels of cyclic AMP in the vessel walls (Johnson, 1992; Leurs *et al*., 1995).

The finding of an endothelial H_1-receptor coupled to the production of NO is of particular interest to the study of headache pathogenesis. Infusion of histamine or nitroglycerin causes an immediate headache followed by a delayed migraine attack in migraineurs (Thomsen *et al*., 1994). The histamine-induced delayed migraine attack is abolished by pretreatment with the H_1-antagonist mepyramine (Lassen *et al*., 1995). This has led to the proposal that migraine may be caused by increased amounts of NO or a mechanism triggering the release of NO (Olesen *et al*., 1994).

The presently observed location and type of receptors may be of importance also for other pathological events. Factors like mechanical stress can release histamine from mast cells and injure the endothelium. In such conditions histamine will not bind to the endothelial relaxant H_1-receptors but to the smooth muscle cells, increasing the risk for vasospasm.

In summary, the response of human precontracted cerebral, meningeal and temporal arteries to histamine *in vitro* is relaxation. This relaxation is partially mediated via an endothelial H_1-receptor coupled to the production of NO and partially via a H_2-receptor associated with the smooth muscle cells. In addition there is evidence for a contractile H_1-receptor at the smooth muscle cells in these arteries which is uncovered after endothelial removal.

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