HISTAMINE AND H_1-HISTAMINE RECEPTORS FASTER VENOUS CIRCULATION

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Abstract

The study has analysed the action of histamine in the rabbit venous system and evaluated its potential role in contraction during increased venous pressure. We have found that a great variety exists in histamine sensitivity and H_1-histamine receptor expression in various types of rabbit veins. Veins of the extremities (saphenous vein, femoral vein, axillary vein) and abdomen (common iliac vein, inferior vena cava) responded to histamine by a prominent, concentration-dependent force generation, whereas great thoracic veins (subclavian vein, superior vena cavas, intrathoracic part of inferior vena cava) and a pelvic vein (external iliac vein) exhibited slight sensitivity to exogenous histamine. The lack of reactivity to histamine-was not due to increased activity of NOS or heme oxygenase-1. H_1-histamine receptor expression of veins correlated well with the histamine-induced contractions. Voltage-dependent calcium channels mediated mainly the histamine-induced force generation of saphenous vein, whereas it did not act in the inferior vena cava. In contrast, the receptor-operated channels were not involved in this response in either vein. Tyrosine phosphorylation occurred markedly in response to histamine in the saphenous vein, but not in the inferior vena cava. Histamine induced a prominent Rho kinase activation in both vessels. Protein kinase C and MAPK were not implicated in the histamine-induced intracellular calcium sensitization. Importantly, transient clamping of the femoral vein in animals caused a short-term constriction, which was inhibited by H_1-histamine receptor antagonist *in vivo*. Furthermore, a significantly greater histamine immunopositivity was detected in veins after stretching compared to the resting state. We conclude that histamine receptor density adapts to the actual requirements of the circulation, and histamine liberated by the venous wall during increased venous pressure contributes to the contraction of vessels, providing a force for the venous return. **Key words:** venous regulation, histamine, regional differences, H_1-histamine receptors, Rho kinase, tyrosine phosphorylation.

Introduction

Research of cardiovascular regulation is a well-established area of physiological science. However, the role of veins in this process has never received much emphasis, although about 70% of the circulating blood volume is distributed in the venous system [1-2]. The main driving force of venous return to the heart was based on the venous pump mechanism. This comprises muscle pumps in extremities, thoraco-abdominal pump *vis a fronte* and *vis a tergo* mechanisms of the heart and myogenic mechanism of veins [3-7].

It is known that veins are sensitive to a variety of neurotransmitters and autacoids and these can be produced in the wall of the veins [2, 7]. Veins have been described as eliciting contractile responses to norepinephrine [2, 8-12], epinephrine [13-18] angiotensin II [17-24], and endothelin [25-28]. In addition, purinergic mediators such as ATP are also capable of inducing significant venoconstrictions [29, 30]. However, to the best of our knowledge, there are no convincing experimental data for the role of these substances – with the exception of adrenergic neurotransmitters – in the involvement of physiology and pathophysiology of venous return [2, 31]. Vasoactivity of histamine is also well known, and it is described that this autacoid has a prominent action not only in arteries, but in veins as well [32-40].

The aim of our study was to analyse the overall mechanical activity of histamine in the rabbit venous system and to evaluate whether histamine has a potential role in venous contraction during increased venous pressure. We have found that a great variety exists in the histamine sensitivity and H_1-histamine receptor expression in various types of rabbit veins, and that histamine receptor density adapts to the actual and local requirements of the circulation. On the basis of our results, it can be supposed that histamine liberated by the venous wall during increased venous pressure can play a significant role in the mechanism of venous return.

Materials and Methods

Animals

Male New Zeeland (Lab-Nyul Kft, Godollo, Hungary) white rabbits were used in the study (weight: 3.2-3.8 kg). The experimental protocol has been approved by the Animal Care and Use Committee of University of Debrecen. Animals were kept in metal cages at room temperature and 12-hour light cycle. Animals had access to standard granulated food with the addition of fresh vegetables.

Contractility Measurements

Animals were sacrificed by a single bonus injection of sodium pentobarbital (60 mg/kg) into a marginal ear vein. Various veins were dissected by microscopic control and excess connective tissue excised. Vascular specimens were cut into rings approximately 4 mm long. Special care was taken to avoid contact with the luminal surface of the rings in order to preserve the endothelium. The endothelium was removed in some rings, where the luminal surface of vessels was rubbed for 10-20 seconds with a wooden stick to damage the endothelium. Rings were vertically mounted on two stainless steel triangular clips, the lower clip being attached to a moveable support, and the upper clip to a force displacement transducer in a 10-ml organ bath containing Krebs solution (37^{o}C), the composition of which was (mmol/l): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; NaH_2PO_4, 1.0; $MgCl_2$, 1.2; $NaHCO_3$, 24.9 and glucose 11.5 with pH 7.4 when gassed with 95% O_2 and 5% CO_2 (pH 7.4). Tension of vascular strips was measured isometrically by transducer (SG-01D, Experimetria, Budapest, Hungary), and the ouput fed to a potentiometric recorder (SP-K2V, Riken Denshi, Tokyo, Japan). Specimens were given an initial tension of 10 mN and allowed to stabilize this tension for at least 2 hours. Following the equilibration period, preparations were exposed to 10 μM histamine (Sigma) to reach a steady level of tension. Integrity

or removal of endothelium was monitored functionally by the quality of responses to acetylcholine (0.1 – 10 μM, Sigma). Following a 30-minute wash-out period, a cumulative concentration-response (E/[A]) curve was constructed to histamine (1 nM-100 μM). At the completion of the experiments, tissues were lightly blotted, measured and weighed. The cross-sectional area of each preparation was calculated using the following formula: cross-sectional area (mm^2) = weight (mg)/(length (mm) x density (mg/mm^3)). The density of the vessels was assumed to be 1.05 mg/mm^3. Responses to histamine were then calculated as the increase in tension (mN) in response to each concentration of agonist/cross-sectional area of tissue (mm^2).

Western Blotting

Tissue samples were collected in lysis buffer [20 mM Tris-HCl, pH 7.4, 5 mM EGTA, 1 mM 4-(2-aminoethyl) benzensulfonyl fluoride, protease inhibitor cocktail diluted 1:100, all from Sigma] and the protein content of samples was measured by a BCA protein assay (Pierce, Rockford, IL, USA) [41-43]. The samples were subjected to sodium dodecyl sulfate-polyacrylamide gelelectrophoresis (7.5 % gels were loaded with 30 μg protein per lane), transferred to BioBond nitrocellulose membranes (Whatman, Maidstone, England). After the membranes were blocked with 5% nonfat milk in phosphate-buffered saline for 1 h, the blots were probed with anti-Histamine or anti-H_1-histamine receptor rabbit primary antibodies (1:200, Santa Cruz). Horseradish peroxidase-polymer-conjugated, goat anti-rabbit antibodies (1:1000, BioRad) were used as the secondary antibody and the immunoreactive bands were visualized by SuperSignal® West Pico Chemiluminescent Substrate-enhanced chemiluminescence (Pierce). Immunoblots were then subjected to densitometric analysis using an Intelligent Dark Box (Fuji, Tokyo, Japan) and the Image Pro Plus 4.5.0 software (Media Cybernetics, Silver Spring, MD, USA). To assess equal loading, membranes were stripped in 200 ml of 50 mM TRIS-HCl buffer

(pH 7.5) containing 2% SDS and 0.1 %-mercaptoethanol (all from Sigma) at 65°C for 1 hour and were re-probed with a rabbit cytochrome-C (CytC) antibody (Santa Cruz) followed by a similar visualization procedure as described above.

Immunohistochemistry

According to the slight modification of the original method described in Anichtchik *et al.* [44] and Rinne *et al.* [45] after excision, the samples of veins were immediately immersed in the fixative, 4% l-ethyl-3.3 (dimethylaminopropyl) carbodiimide (EDAC, Sigma, St. Louis, MO, USA) in 0.1M phosphate buffer, pH 7.0 for 2 days followed by fixation in 4% paraformaldehyde (Sigma), embedded in paraffin, and processed for immunohistochemistry. The expression of histamine was determined by horseradish-peroxidase (HRP) based method using diaminobenzidine (DAB) as a chromogene. In brief, paraffin-embedded sections (5 μm), after antigen retrieval [with 1mg/ml protease (Sigma Aldrich, St. Louis, MO, USA) diluted in TRIS-HCl], endogenous peroxidase activity was blocked with peroxidase blocking solution (DAKO, Glostrup, Denmark) (10 minutes, RT). Non-specific binding was prevented by incubating the sections with Protein Block Serum Free Reagent (DAKO, 5 min, RT). The tissue sections were then incubated overnight at 4°C with the primary anti-Histamine H1-receptor antibody (1:50, Santa Cruz, Biotechnology, Santa Cruz, CA, USA). Sections were then incubated with a rabbit anti-goat HRP-polymer-conjugated secondary antibody (1:1000, BioRad). Immunoreactions were finally visualized using DAB-substrate Histamine H_1-receptor antibody (1:50, Santa Cruz, Biotechnology, Santa Cruz, CA, USA). Sections were then incubated with a rabbit anti-goat HRP-polymer-conjugated secondary antibody (1:1000, BioRad). Immunoreactions were finally visualized using DAB-substrate μ (EnVision kit, DAKO) and the sections were counterstained by hematoxylin (Sigma-Aldrich) and mounted in aqueous mounting medium (DAKO).

In addition, for negative controls of the labeling procedure, primary antibodies were omitted from the procedure. Immunohistochemical images were captured and digitalized using an RT Spot Colour CCD camera (Diagnostic Instruments Inc., Sterling Heights, MI, USA) integrated on a Nikon Eclipse 600 fluorescence and light microscope (Nikon, Tokyo, Japan). Analysis of images was carried out using Image Pro Plus 4.5 software. Identification of immunpositive pixels was based on specific colour of immunostaining. A mask was applied for immunpositive pixels and the number of positive pixels was counted in the region of the given endothelium section. Data are presented as percentage of immunpositive pixels in the selected region.

Ultrasonic Echo-Tracking

The experiments were performed in 12 New Zeeland rabbits weighing 3.2-3.8 kg, anesthetized with sodium pentobarbital 30 mg/kg i.v. into the marginal ear vein. The animals were placed on a heated table to maintain constant body temperature (39.0 ± 0.4^{o} C). A 1cm segment of right femoral vein was prepared just below the Poupart's ligament and a tourniquet was placed gently around the vessel. After preparation of the vessel, a non-invasive ultrasound probe was positioned in the distal portion of femoral vein for measuring its internal diameter by an ultrasonic echo-tracking device (ATL HDI 5000) C8-5 transducer with 5-8MHz probe scan frequency. Using a Doppler mode, the probe was positioned by the characteristic sound of the femoral vein and its focal zone was close to the center of the vein. Signals of the venous walls were tagged by an electronic tracker, allowing the continuous measurement of the venous diameter. After measuring the resting internal diameter of femoral vein, a 15-second occlusion was applied, followed by release of the vessel clamp. One group of animals (n=6) was treated by i.v. administration of 4 mg/kg pyrilamine (a H_1-histamine receptor blocker;

Sigma) before clamping. In the other group of animals (controls, n=5), PBS was administered as a solvent.

Data analysis

Agonist-induced changes in mechanical activity were expressed as the percentage reduction of pre-drug contractile force. Individual E/[A] curve data were fitted by means of a least-square iterative computer program to a logistic function of the following form: $E=E_{max}[A]^{n}_{H}/[A]^{n}_{H}+[EC_{50}]^{n}_{H}$, where E denotes the effect, E_{max} is the asymptote, [A] is the concentration of the agonist, EC_{50} is the concentration producing a half-maximal response, and n_H is the midpoint slope parameter.

The data are expressed as means ± S.E.M. The EC_{50} values were expressed as their negative base 10 logarithms (pD_2 values) throughout the text. Multiple comparisons between the experimental groups were performed by one way analysis of variance (ANOVA) with a Newman-Keuls post hoc test. Statistical significance was evaluated by student's *t*-test, where appropriate; $P < 0.05$ was taken as the level of significance.

Results

Regional Variability of Histamine-Induced Contractions

Histamine (10 pM – 100 μM) evoked a concentration-dependent increase of contractile force, but E_{max} of E/[A] was significantly different in various types of veins. Veins have substantial histamine sensitivity in the lower (saphenous vein, femoral vein) and upper extremities (axillary vein), as well as in the abdomen (common iliac vein, inferior vena cava). In contrast, the thoracic

veins (thoracic part of inferior vena cava, right and left superior vena cavas, subclavian vein) and one pelvic vein, external iliac vein were virtually insensitive to histamine (Fig. 1-3).

Role of NO or CO Liberation in the Histamine Resistance

To analyse the action of a possibly increased nitric oxide or carbon monoxide release in the reduced histamine-induced contraction of special veins, after constructing the first concentration-response curve and a washout period, external iliac veins (n=5) and right superior vena cavas (n=6) were exposed to 100 μM N^G-nitro-L-arginine (L-NOARG) to block NOS activity. After the blocking of NOS enzymes, no significant differences were observed in histamine-induced responses of both veins. Similar results were detected if the above specimens were incubated by the inhibitor of heme oxygenase-1 Zn-protoporphyrine IX (ZnPP) of 20 μM (data not shown).

Immunoblotting of H_1-Histamine Receptor Proteins

H_1-histamine receptors were expressed in high density in saphenous vein and inferior vena cava, whereas expression level of these receptors was low in internal iliac vein and right and left superior vena cavas (Fig. 4).

Differences in Signaling Mechanism of Histamine-Sensitive Veins

In these experiments, the signaling of two high histamine-sensitive veins (saphenous vein, inferior vena cava) was compared. The possible role of membrane calcium channels (VOC and

ROC), intracellular calcium stores, and enzymes involved in calcium sensitization (Rho-kinase, tyrosine kinase, MAPK, protein kinase C) was studied. In saphenous vein, experiments with nifedipine and SKF-96365 demonstrated that histamine activates mainly voltage-operated calcium channels, whereas the role of ROC is negligible. In the inferior vena cava, the roles of both types of calcium channels are minor in mechanical activity induced by histamine (Fig. 5).

For investigating the role of the possible implication of Ca^{2+} sensitization pathways, we studied the action of various protein kinases previously known to be involved in Ca^{2+} sensitization process. Thus the effects of inhibitors of Rho kinase, protein kinase C, MAPK and tyrosine kinase were investigated. Rho kinase inhibitors (50 μM HA-1077 or Y-27632 10 μM) all virtually completely inhibited the histamine-induced force generation in both saphenous vein and inferior vena cava ring preparations (Fig. 6). Tyrosine phosphorylation was studied by application of genistein 50 μM. Strong difference was observed between the two types of vessels in the genistein-induced inhibition of histamine contraction. In saphenous veins, genistein induced a 43% inhibition, whereas it was practically ineffective in inferior vena cava indicating the diverse implication of tyrosine phosphorylation in histamine's actions in the above vessels. The inhibition of protein kinase C by calphostin 0.2 μM or inhibition of MAPK by PD-098059 10 μM did not influence significantly the development of force in both saphenous veins and the inferior vena cava (data not shown).

Effects of Transient Clamping/Release on the Femoral Vein *in vivo*

In anesthetized rabbits, the femoral vein just below the Poupart's ligand was clamped for 20 seconds, followed by a release. In controls, after clamping, an immediate slight constriction was

monitored by an echo-tracking device. This constriction became more prominent after releasing the clamping, then returned to the pre-clamping value. In another group of animals, 4 mg/kg pyrilamine, a specific H_1-histamine receptor blocker was injected into the marginal ear vein. This induced an initial dilation of the femoral vein, which then returned to the control value. When clamping, the femoral vein was not constricted, but dilated and this venodilation became pronounced after the release of the clamp (Fig. 8).

Effect of stretch of femoral vein on the histamine distribution and content

Rabbits were anesthetized and saphenous vein and inferior vena cava were prepared. One part of the proximal region of these veins was cut and immersed in 4% EDAC, embedded in paraffin, and processed for immunohistochemistry (non-stretched veins). A silicon catheter was inserted into the proximal end of the veins and PBS was injected into the vein by a 80-100Hgmm controlled pressure for 20 seconds. After this procedure, the intact part of vein was excised and used for immunohistochemistry following the above protocol (stretched veins). Histamine staining was more intensive on stretched saphenous vein and inferior vena cava than on corresponding controls suggesting a causative relationship between stretching and histamine release (Fig. 9).

Study of Histamine Liberation from Mast Cells

In isolated saphenous veins, experiments were carried out to study the possible cellular basis of histamine release. The mast cell activator compound, 48/80, at a concentration of 300 μM, was

not able to induce contraction both in endothelium intact and denuded saphenous vein rings (data not shown).

Discussion

The major findings of this study are the followings: (i) Histamine-sensitivity of rabbit veins shows an extreme variability. Veins of the extremities (saphenous vein, femoral vein, axillary vein) and abdominal veins (common iliac vein, inferior vena cava) respond to histamine by a prominent, concentration-dependent force generation, whereas great thoracic veins (subclavian vein, superior vena cavas, intrathoracic parts of the inferior vena cava) and a pelvic vein (external iliac vein) displayed minimum sensitivity to exogenous histamine. The concentration-response curve for histamine (ii) was not influenced by mechanical deprivation of endothelial layer. The low histamine sensitivity (iii) of selected veins (external iliac vein, superior vena cava) was not changed by the inhibition of NOS or heme oxigenase-1 enzyme by L-NOARG or Zn protoporphyrine-IX, respectively. H_1-histamine receptor expression (iv) of the selected high and low histamine-sensitivity veins correlated well with the histamine-induced force generation, and (v) there are some differences in excitation-contraction coupling between the histamine-reactive vessels saphenous vein and inferior vena cava. While voltage-dependent calcium channels (VOC) play a significant role in the histamine-induced force generation of saphenous vein, they do not in the inferior vena cava. The role of receptor-operated channels (ROC) is very slight in both types of veins. Tyrosine phosphorylation is profoundly involved in the saphenous vein, but not in the inferior vena cava. Histamine induces a prominent Rho kinase activation in both vessels. Protein kinase C and MAPK are not implicated in the histamine-induced intracellular calcium sensitization in any of the vessels studied. *In vivo* experiments (vi) of transient clamping of the femoral vein caused a short-term constriction followed by a moderate dilation. Following the blockade of H_1-histamine receptors, no constriction was demonstrated, and (vii) in veins with great sensitivity to histamine (saphenous vein,

inferior vena cava) a significantly greater histamine immunopositivity was detected after stretching than in resting state. As a pharmacological approach, it seems (viii) that after stretched conditions, histamine release probably is not originated from mast cells, but the origin of its release from endothelial or non-mast cells cannot be excluded.

It is an important question in the physiology and pathophysiology whether active vasoconstriction plays an important role in venous return and raising of end-diastolic volume. It is generally accepted that in orthostasis, the increase of myogenic tone and an increment of sympathetic tone have a great significance in the regulation of venous blood flow [2, 6, 7, 46]. To the best of our knowledge, our present paper is the first to describe the potential role of histamine as an autacoid in the active vasoconstriction of veins, emphasizing the involvement of histamine in the venous return. We have shown that by increasing the venous pressure by clamping of the proximal part of the saphenous vein, a possible histamine-induced transient vasoconstriction develops. Histamine liberation from the vessel wall was convincingly documented by immunohistology, where after stretching of saphenous vein and inferior vena cava a strong increase in histamine immunoreactivity can be detected. The overall results of the present study emphasize that the venous network is extremely plastic at the point of view of histamine-induced regulation. In the proximal parts of the body, in extremities, but also in the abdomen, the histamine receptor expression and the maximum contractile response to histamine are very high, while in the thorax (because of the changes of intrathoracic pressure) the veins are virtually insensitive to histamine. Regional variabilities for the sensitivity of rabbit veins were described earlier by Tsuru et al. [39], but these authors did not detect such practically inactive veins, as our present study did. This variability is physiologically quite logical, because the thoraco-abdominal venous pump is effectively operating and no additional venoconstriction is needed for venous return in this region. It is not completely understood that the insensitivity of the external iliac vein operates in the pelvic part. It might ensure a reservoir or buffer function preventing the simultaneous constriction of all

veins in the lower extremities and the abdomen, providing as a "fountain pump" and making the venous return continuous. It is mentioned that in this region of the body, the plasticity of the histamine-sensitivity is noteworthy. In some experiments, we observed arteriolisation of the external iliac vein when forming a side-to-side anastomosis between the femoral vein and artery, the external iliac vein became highly sensitive to histamine. In addition, we have observed that under certain physiological and pathological circumstances (post-partum period, intrapelvic tumors), a clear-cut shift can be observed from the external iliac vein toward common iliac vein, i.e. the external iliac vein responds well to histamine, but the common iliac artery lost its histamine-sensitivity (Galajda and Szentmiklósi: unpublished data). This phenomenon can be high significant, mainly in women during pregnancy.

Histamine is an endogenous substance that is widely distributed in various tissues and has been involved in a variety of physiological and pathophysiological processes. This autacoid is implicated in the initial phase of an anaphylactic reaction and acts on various smooth muscles including vascular. The biological action of histamine operates by three different surface receptors, i.e. H_1-, H_2- and H_3-receptors. We have analysed only the role of H_1-histamine receptors, as H_2 and H_3 antagonists have very slight or negligible action on the H_1-histamine receptor-induced contractions (39, 47, Galajda and Szentmiklosi: unpublished data). In our experiments, regional differences in H_1-histamine receptor expression and signaling mechanism were also shown. Histamine-induced contractions and H_1-histamine receptor expressions are well correlated in sensitive and non-sensitive veins. As far as the regional variability of signaling mechanism is concerned, the role of the activation of voltage-operated calcium channels is significant in histamine-induced saphenous vein contraction, but not in inferior vena cava. A special emphasis was placed on the analysis of histamine-related intracellular calcium sensitizing mechanisms. In vessels, a variety of protein kinases are implicated in Ca^{2+} sensitization mechanisms: Rho kinase, protein kinase C, mitogen-activated protein kinase and tyrosine phosphorylation [48-51]. In our

study, tyrosine phosphorylation had a significant role in the Ca^{2+} sensitization mechanism underlying the histamine-induced contractions of saphenous vein, but no involvement was detected in inferior vena cava. Activation of Rho kinase was strongly implicated in the histamine-induced Ca^{2+} sensitization in both veins. In our experiements, endothelium did not play any role in the histamine-induced contraction. In histamine-insensitive veins, the slight reactivity of the specimens can be due mainly to low H_1-histamine receptor density, and no role of a possible increased liberation of NO or CO was supposed. However, we did not investigate the potential role of peroxynitrate as an oxidative and nitrosative stressor, but we can not rule out its possible contribution to histamine-induced venorelaxation [52].

In histochemical studies, we convincingly demonstrated that stretching of the veins induces a remarkable histamine release in the vascular tissue. The source of histamine is possibly not the vascular mast cells, but in this case liberation of non-mast cell histamine [53] could be suggested. *In vivo* experiments by inducing venoconstriction during clamping of the femoral vein, and the fact that this process could be reversed by pyrilamine, a selective H_1-histamine receptor blocker, confirmed our theory that depending on where the vein wall is stressed, this induces histamine liberation followed by a venoconstriction. This process might be a "helper" in addition to the musculovenous pump and the myogenic tone.

It should be emphasized that these results could also be observed in human veins obtained from multidonors and the variability of the histamine-sensitivity, histamine receptor expression, and excitation-contraction coupling of various veins are also operating (Galajda and Szentmiklósi: unpublished results). Furthermore, detailed studies are needed to clarify the physiological and pathophysiological significance of these results, but on the basis of our study it can be suggested that histamine release after stretching the vein wall may play a significant role in the regulation of venous circulation and widen the implication of this autacoid in a variety of physiological processes.

There is no conflict of interest.

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Legend for Figures

Figure 1. Histamine-induced contraction in isolated rabbit vessels. Saphenous vein (open circle) (n=12), femoral vein (full circle) (n=7), external iliac vein (open square) (n=9). The histamine-induced increases in tension are normalized for vessel cross-sectional area, and absolute values of the increase in tension are shown. Data represent mean ± S.E.M.

Figure 2. Histamine-induced contraction in isolated rabbit vessels. Abdominal part (open circle) of inferior vena cava (n=10), common iliac vein (full circle) (n=5), intrathoracic part (open square) of inferior vena cava (n=7). The histamine-induced increases in tension are normalized for vessel cross-sectional area, and absolute values of the increase in tension are shown. Data represent mean ± S.E.M.

Figure 3. Histamine-induced contraction in isolated rabbit vessels. Axillary vein (open circle) (n=5), right superior vena cava (full circle) (n=7), subclavian vein (open square) (n=5). The histamine-induced increases in tension are normalized for vessel cross-sectional area, and absolute values of the increase in tension are shown. Data represent mean ± S.E.M.

Figure 4. H1 histamine receptor expression in various rabbit veins. A: Specific H_1 immunopositive bands at ~55 kDa of a representative immunoblot and corresponding CytC staining. B: Optical density of H_1 immunpositive bands, normalized to CytC. $n \geq 3$ in each group

Figure 5. Role of voltage-operated (nifedipine-treatment) and receptor operated (SKF-96365 treatment) channels in the histamine-induced contraction of rabbit saphenous vein (grid columns; n= both 6) and abdominal part of inferior vena cava (hatched columns; n= both 5). Contractile force is expressed in % of control. *** $P < 0.001$

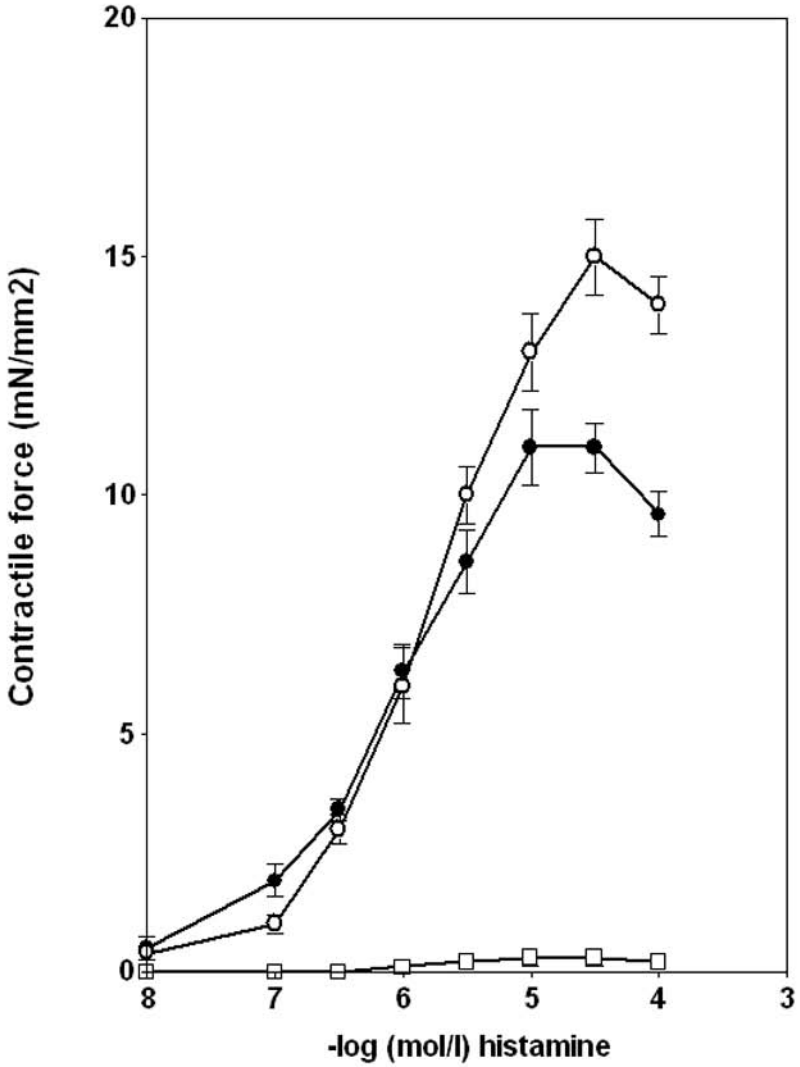
Figure 6. Effects of inhibition of Rho kinase by 50 μM HA-1077 (n= both 5) or 5 μM Y-27632 (n=both 4) on the histamine-induced contraction in rabbit saphenous vein (grid columns) and abdominal part of inferior vena cava (hatched columns). Contractile force is expressed in % of control. *** $P < 0.001$

Figure 7. Original record of the action of Rho kinase inhibition (50 μM H-1077) on the histamine-induced force generation in rabbit saphenous vein.

Figure 8. Typical clamping/release studies in anesthetized rabbit under control condition and after administration of 4 mg/kg i.v. pyrilamine.

Figure 9. Histamine immunostaining in stretched and non-stretched veins of rabbit. Histamine was visualised using DAB chromogen (brown color). Immunopositive pixels were selected and counted on images using Image Pro Plus 4.5 software. NC – negative control.

Fig. 1


Contractile force (mN/mm2)
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15
10
5
0
8
7
6
5
4
3
-log (mol/l) histamine

Fig. 2

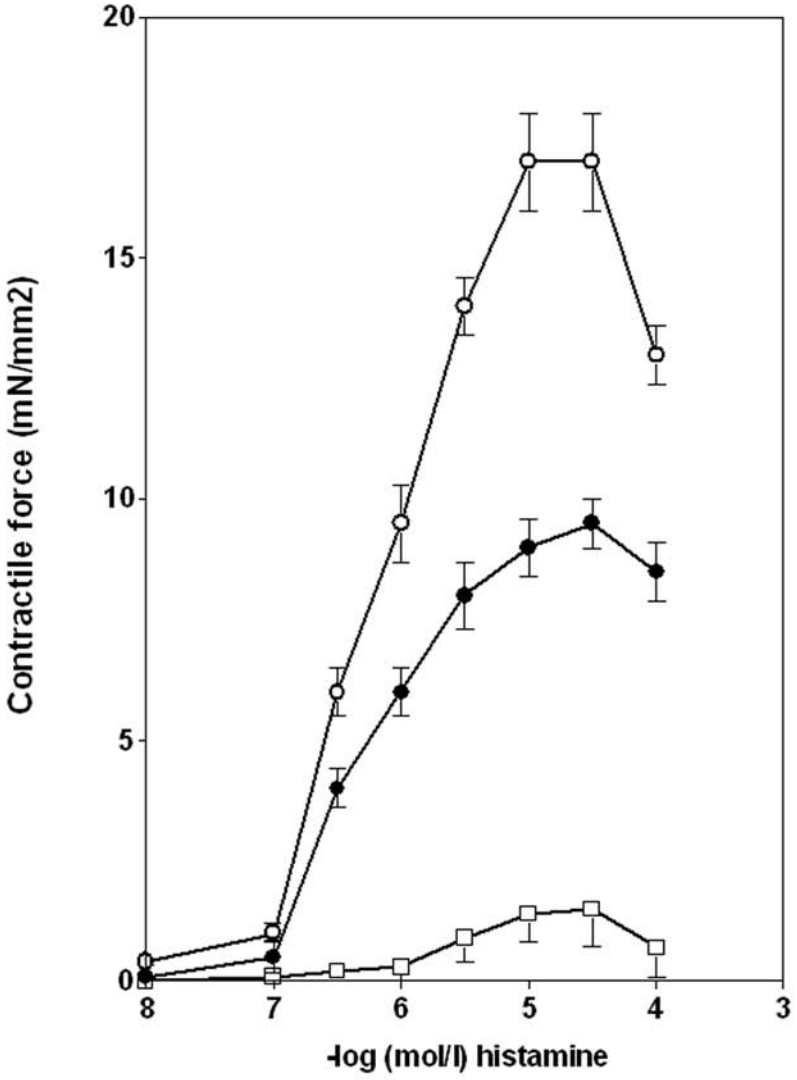
20
15
10
5
0
Contractile force (mN/mm2)
8
7
6
5
4
3
-log (mol/l) histamine

Fig. 3

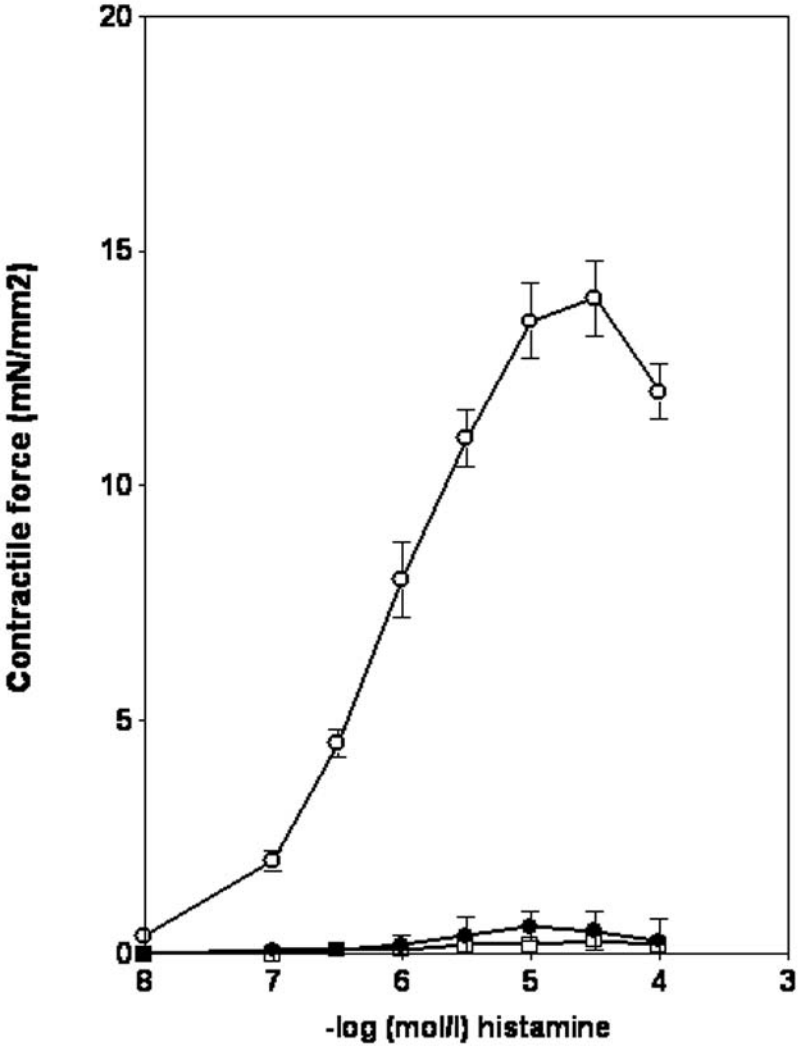
Contractile force (mN/mm2)
20
15
10
5
0
8
7
6
5
4
3
-log (mol/l) histamine

Fig. 4

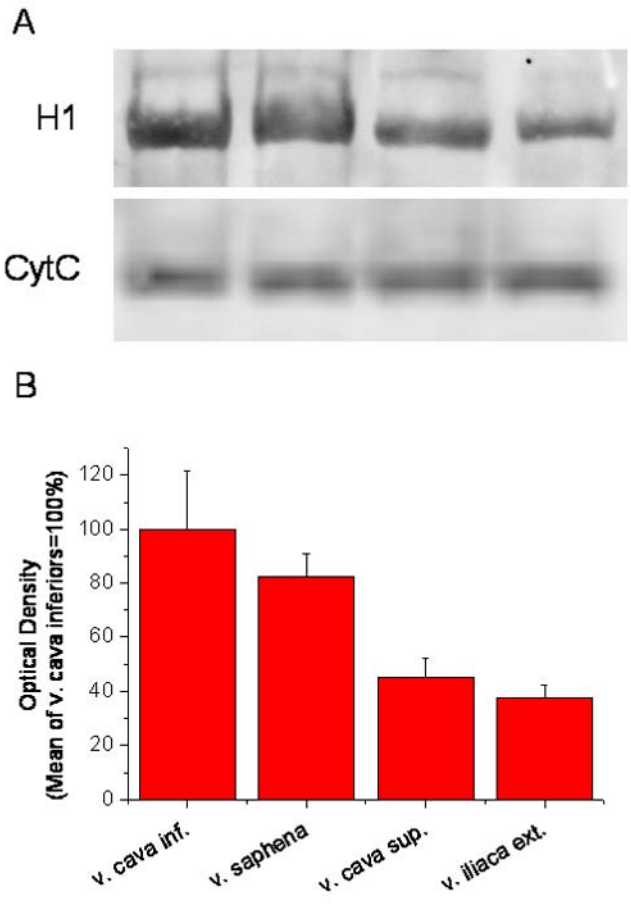
A
H1
CytC
B
Optical Density
(Mean of v. cava inferiors=100%)
120
100
80
60
40
20
0
v. cava inf.
v. saphena
v. cava sup.
v. iliaca ext.

Fig. 5

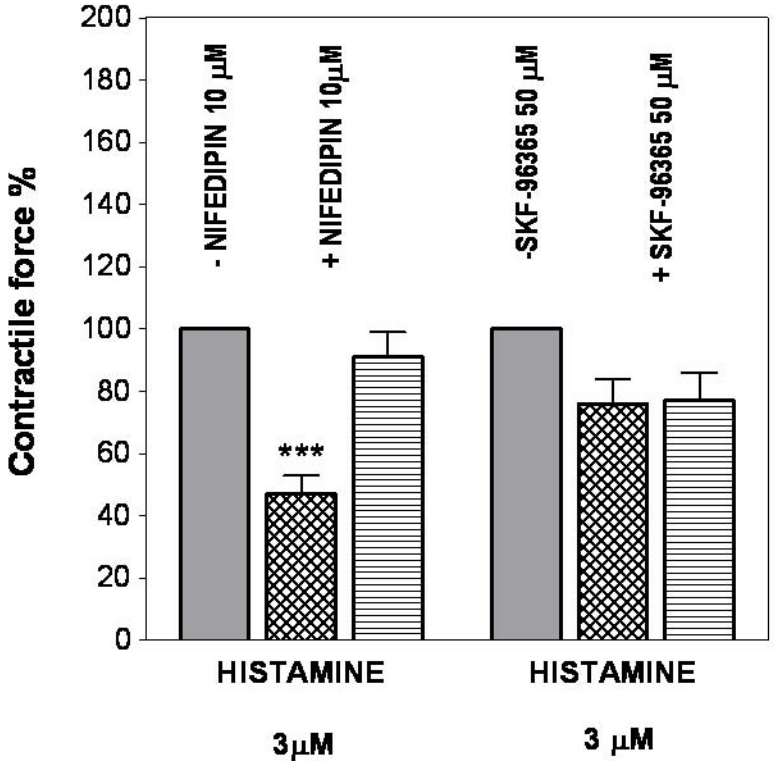
Contractile force %
200
180
160
140
120
100
80
60
40
20
0
- NIFEDIPIN 10 μM
+ NIFEDIPIN 10μM
-SKF-96365 50 μM
+ SKF-96365 50 μM
***
HISTAMINE
3μM
HISTAMINE
3 μM

Fig. 6

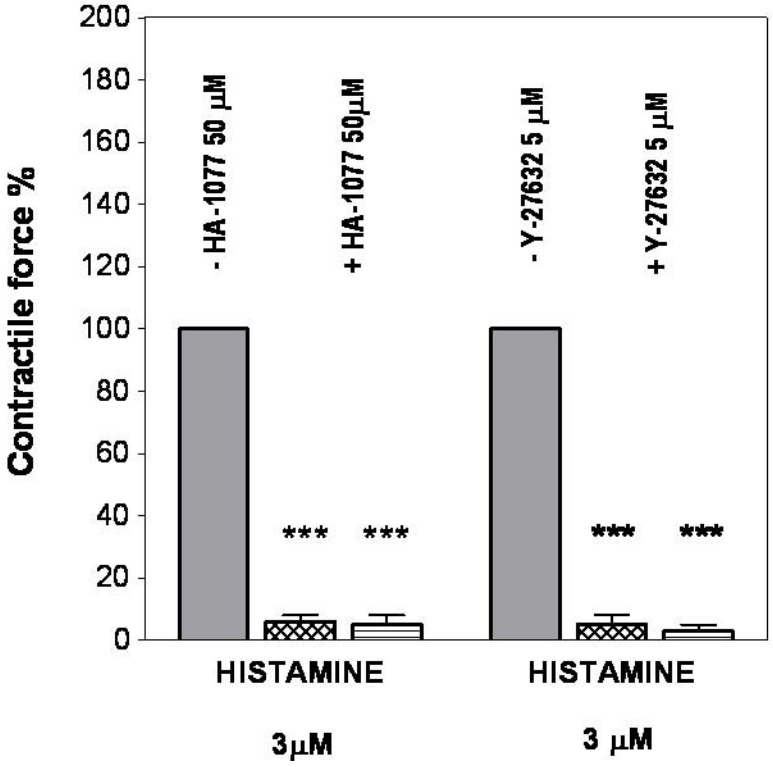
Contractile force %
200
180
160
140
120
100
80
60
40
20
0
- HA-1077 50 μM
+ HA-1077 50μM
- Y-27632 5 μM
+ Y-27632 5 μM
***
***
***
***
HISTAMINE
3μM
HISTAMINE
3 μM

Fig. 7

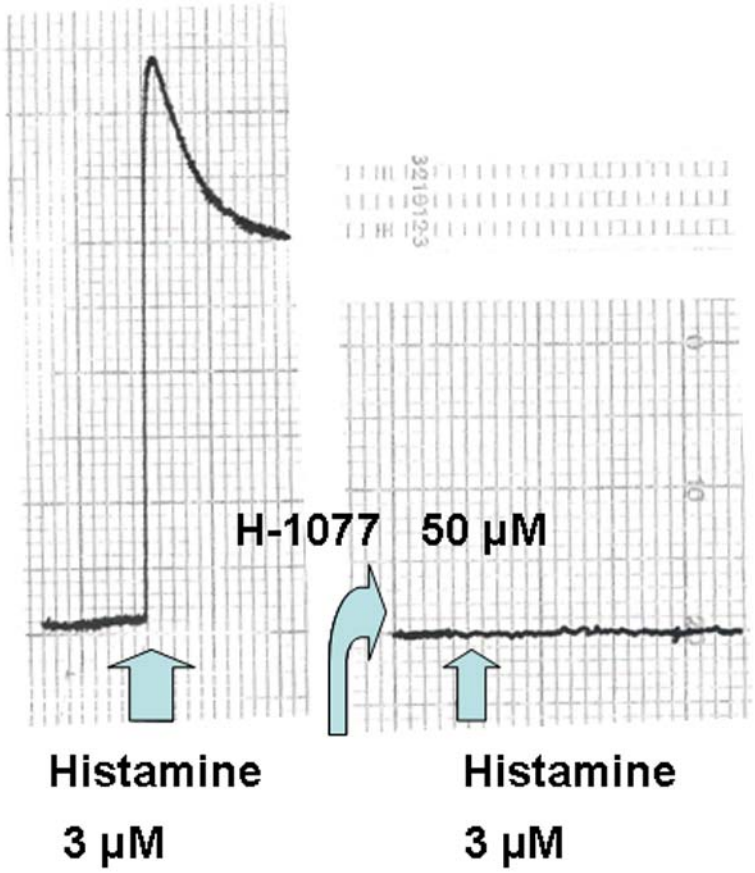

Fig. 8

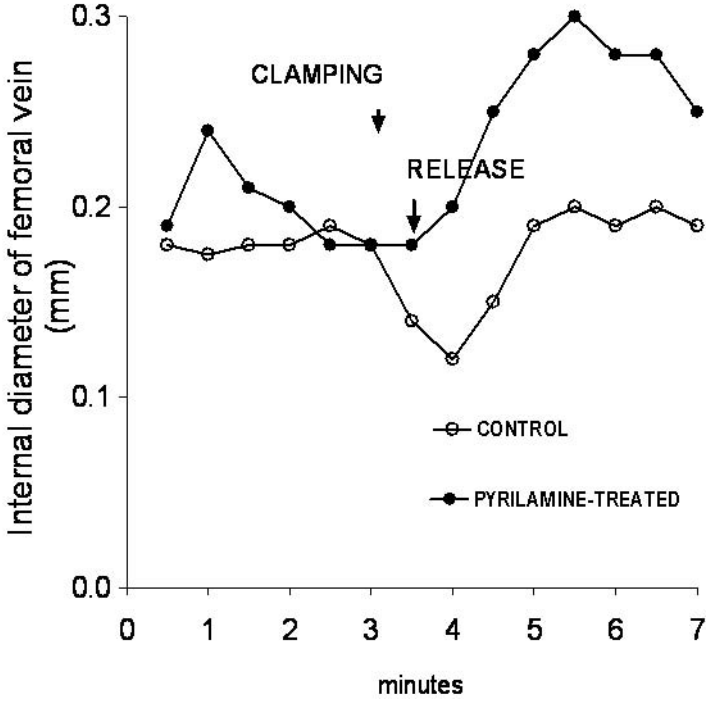
0.3
0.2
0.1
0.0
Internal diameter of femoral vein (mm)
CLAMPING
RELEASE
CONTROL
PYRILAMINE-TREATED
0
1
2
3
4
5
6
7
minutes

Fig. 9

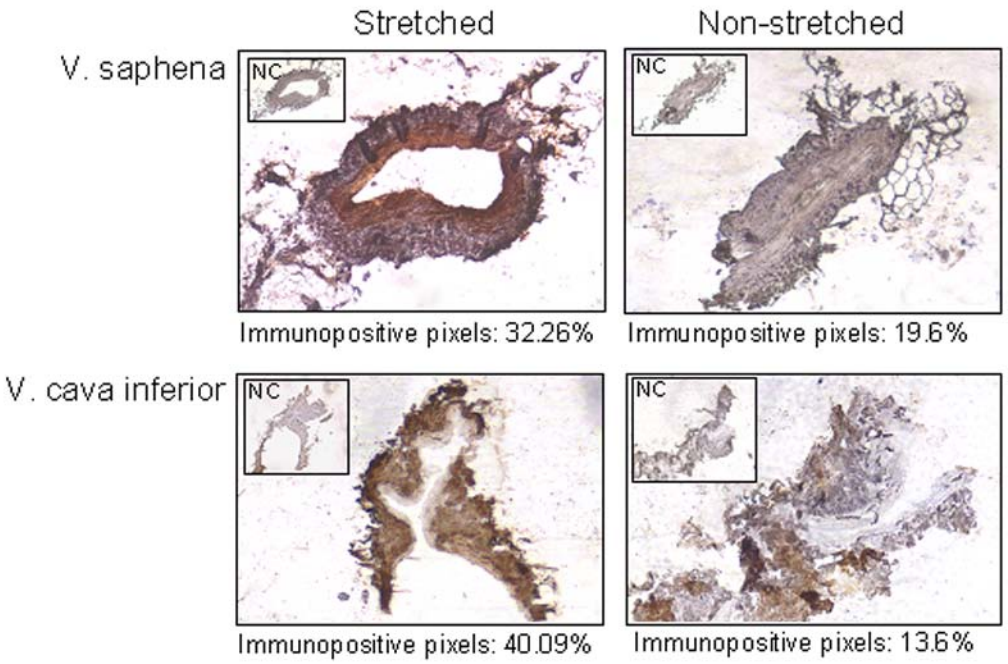
Stretched
Non-stretched
V. saphena
NC
NC
Immunopositive pixels: 32.26%
Immunopositive pixels: 19.6%
V. cava inferior
NC
NC
Immunopositive pixels: 40.09%
Immunopositive pixels: 13.6%

Table 1. Effect of histamine on contractility parameters in isolated ring preparations of various rabbit veins

	Vein	n	pD_2	E_{max}	*P* (E_{max})
1.	Inferior vena cava (abdominal part)	10	6.11 ± 0.10	17.8 ± 1.1	
2	Saphenous vein	12	5.73 ± 0.04	16.2 ± 0.4	> 0.05 1 *vs*. 2
3	Axillary vein	5	6.07 ± 0.12	14.7 ± 0.3	>0.05 2 *vs*. 3
4	Femoral vein	7	6.03 ± 0.08	10.7 ± 0.8	<0.05 3 *vs* 4
5	Common iliac vein	5	6.29 ± 0.11	9.5 ± 0.6	>0.05 4 *vs*. 5
6	Inferior vena cava (thoracic part)	7	5.57 ± 0.06	1.5 ± 0.1	<0.01 5 *vs*. 6
7	Right superior vena cava	7	5.77 ± 0.16	0.6 ± 0.1	<0.05 6 *vs*. 7
8	Subclavian vein	5	5.10 ± 2.60	0.5 ± 0.7	>0.05 7 *vs*. 8
9	External iliac vein	9	5.74 ± 0.07	0.3 ± 0.01	>0.05 8 *vs*. 9

Data are mean values ± S.E.M.
Analysis of statistical significance was performed using variance analysis (ANOVA) with the Newman-Keuls *post hoc* test.
n: number of experiments
pD_2: negative base 10 logarithms of EC_{50} values
E_{max}: maximum effect (mN/mm2)
P: statistical significance