HISTAMINE AND H₁-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 H₁-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 cava, 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. H₁-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 H₁-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, H₁-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 H1-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°C), the composition of which was (mmol/l): NaCl, 118; KCl, 4.7; CaCl₂, 2.5; NaH₂PO₄, 1.0; MgCl₂, 1.2; NaHCO₃, 24.9 and glucose 11.5 with pH 7.4 when gassed with 95% O₂ and 5% CO₂ (pH 7.4). Tension of vascular strips was measured isometrically by transducer (SG-01D, Experimetria, Budapest, Hungary), and the output 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 (mm²) = weight (mg)/(length (mm) x density (mg/mm³)). The density of the vessels was assumed to be 1.05 mg/mm³. Responses to histamine were then calculated as the increase in tension (mN) in response to each concentration of agonist/cross-sectional area of tissue (mm²).

**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-H₁-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 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 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 immunopositive pixels was based on specific colour of immunostaining. A mask was applied for immunopositive pixels and the number of positive pixels was counted in the region of the given endothelium section. Data are presented as percentage of immunopositive pixels in the selected region.

**Ultrasonic Echo-Tracking**

The experiments were performed in 12 New Zealand 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° 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 H1-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: $E=\frac{E_{max}[A]^n}{[A]^n+EC50^n}$, where E denotes the effect, $E_{max}$ is the asymptote, [A] is the concentration of the agonist, EC50 is the concentration producing a half-maximal response, and n is the midpoint slope parameter.

The data are expressed as means ± S.E.M. The EC50 values were expressed as their negative base 10 logarithms (pD2 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 H1-Histamine Receptor Proteins**

H₁-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 H₁-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 cava, 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. H₁-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 H₁-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. H1-, H2- and H3-receptors. We have analysed only the role of H1-histamine receptors, as H2 and H3 antagonists have very slight or negligible action on the H1-histamine receptor-induced contractions (39, 47, Galajda and Szentmiklosi: unpublished data). In our experiments, regional differences in H1-histamine receptor expression and signaling mechanism were also shown. Histamine-induced contractions and H1-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 Ca2+ 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\textsuperscript{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\textsuperscript{2+} sensitization in both veins. In our experiments, 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\textsubscript{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\textsubscript{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.
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References

1. Rothe CF. Venous system: physiology of the capacitance vessels. In: J.T. Shepherd and F. M. Abboud (Eds.) HANDBOOK OF PHYSIOLOGY: THE CARDIOVASCULAR SYSTEM, PERIPHERAL CIRCULATION. Washington, D.C.: Am. Physiol. Soc., Sect. 2, Vol. III, Chap. 13, 1983. pp. 397-452.

2. Pang CCY. Autonomic control of the venous system in health and disease. Effects of drugs. Pharmacol Ther. 2001; 90:179-230.

3. Donegan JF. The physiology of veins. J Physiol (Lond). 1921; 55:226–245.

4. Ludbrook J. The musculovenous pumps of the human lower limb. Am Heart J. 1966; 71:635–641.

5. Noordergraaf A. Veins. In: CIRCULATORY SYSTEM DYNAMICS. New York: Academic Press; 1978. pp. 157–181.

6. Rothe CF. Physiology of venous return. An unappreciated boost to the heart. Arch Intern Med. 1986;146:977-82.

7. Monos E, Berczi V, Nadasy G. Local control of veins: biomechanical, metabolic, and humoral aspects. Physiol Rev. 1995;75:611-66.

8. Burch GE, DePasquale NP. The effect of norepinephrine on the digital veins. Am Heart J. 1960; 60:915-923.

9. Cohen ML, Wiley KS. Comparison of arteries with longitudinal and circular venous muscle from the rat. Am J Physiol. 1977;232:H131-9.
10. **Sticht FD, Wood WB, Cardoso SS et al.** Effect of prazosin on veins: Inhibition of norepinephrine venoconstriction in dog forelimb. *Drug Development Research*. 1983; 3:393-488.

11. **Hohmann M, Keve TM, Osol G. et al.** Norepinephrine sensitivity of mesenteric veins in pregnant rats. *Am J Physiol*. 1990; 259:R753-759.

12. **Zhang L, Dyer DC.** Characterization of alpha-adrenoceptors mediating contraction in isolated ovine umbilical vein. *Eur J Pharmacol*. 1991;197:63-7.

13. **Errasti AE, Werneck de Avellar MC, Daray FM, et al.** Human umbilical vein vasoconstriction induced by epinephrine acting on alpha1B-adrenoceptor subtype. *Am J Obstet Gynecol* 2003;189:1472-80.

14. **Miller JW, Streeter DH.** Vascular responsiveness to norepinephrine in sympatheticotonic orthostatic intolerance. *J Lab Clin Med*. 1990;115:549-58.

15. **Nilsson H.** Adrenergic nervous control of resistance and capacitance vessels. Studies on isolated blood vessels from the rat. *Acta Physiol Scand* Suppl. 1985;541:1-34.

16. **Arneklo-Nobin B, Owman C.** Adrenergic and serotoninergic mechanisms in human hand arteries and veins studied by fluorescence histochemistry and in vitro pharmacology. *Blood Vessels*. 1985;22:1-12.

17. **Savino EA, Taquini AC, Jr.** Effects of adrenaline, angiotensin, potassium and calcium on lanthanum treated portal veins. *Arch Int Pharmacodyn Ther*. 1977;226:100-8.

18. **Mantel R, Taquini AC, Jr., Savino EA.** Effects of adrenaline, angiotensin and calcium on spontaneously active and potassium-depolarized rat portal veins. *Arch Int Physiol Biochim*. 1975;83:211-9.

19. **Gurzu B, Costuleanu M, Slatineanu SM, Ciobanu A, Petrescu G.** Are multiple angiotensin receptor types involved in angiotensin (1-7) actions on isolated rat portal vein. *J Renin Angiotensin Aldosterone Syst*. 2005;6:90-5.

20. **Fernandes L, Loiola RA, Tostes RC, Nigro D, Fortes ZB, de Carvalho MH.** Angiotensin II-induced venoconstriction involves both AT1 and AT2 receptors and is counterbalanced by nitric oxide. *Peptides*. 2005;26:2458-63.

21. **Li Q, Feenstra M, Pfaffendorf M, Eijsman L, van Zwieten PA.** Comparative vasoconstrictor effects of angiotensin II, III, and IV in human isolated saphenous vein. *J Cardiovasc Pharmacol*. 1997;29:451-6.

22. **Pelet C, Mironneau C, Rakotoarisona L, Neuilly G.** Angiotensin II receptor subtypes and contractile responses in portal vein smooth muscle. *Eur J Pharmacol*. 1995;279:15-24.

23. **Zhang JS, Van Meel JC, Pfaffendorf M, Van Zwieten PA.** Characterization of the angiotensin II-receptor subtype in the longitudinal smooth muscle of the rat portal vein. *Naunyn Schmiedebergs Arch Pharmacol*. 1993;347:220-4.
24. Blair-West JR, McKenzie JS, McKinley MJ. The actions of angiotensin II on the isolated portal vein of the rat. *Eur J Pharmacol.* 1971;15:221-30.

25. Bogoni G, Rizzi A, Calo G, Campobasso C, D'Orleans-Juste P, Regoli D. Characterization of endothelin receptors in the human umbilical artery and vein. *Br J Pharmacol.* 1996;119:1600-4.

26. Mawatari K, Komori K, Kuma S, Yamamura S, Ishii T, Sugimachi K. Effects of serotonin and endothelin on the smooth muscle cells of autogenous vein grafts. *Br J Surg.* 1997;84:1419-24.

27. Wang HG, Shibamoto T, Miyahara T. Endothelin-1 selectively contracts portal vein through both ETA and ETB receptors in isolated rabbit liver. *Am J Physiol.* 1997;273:G1036-43.

28. Rizzi A, Calo G, Battistini B, Regoli D. Contractile activity of endothelins and their precursors in human umbilical artery and vein: identification of distinct endothelin-converting enzyme activities. *J Cardiovasc Pharmacol.* 1998;31 Suppl 1:S58-61.

29. Ziganshin AU, Khaziakhmetov DF, Ziganshina LE, Khaziakhmetova VN, Dzhordzhikiya RK, Burnstock G. Contractile activity of human greater saphenous vein mediated by P2-receptors. *Bull Exp Biol Med.* 2003;135:23-5.

30. Ralevic V, Burnstock G. Roles of P2-purinoceptors in the cardiovascular system. *Circulation.* 1991;84:1-14.

31. Monos E, Lorant M, Dornyei G, Berczi V, Nadasy G. Long-term adaptation mechanisms in extremity veins supporting orthostatic tolerance. *News Physiol Sci.* 2003;18:210-4.

32. Okamura T, Yamazaki M, Toda N. Responses to histamine and acetylcholine in isolated monkey mesenteric veins versus arteries. *Cardiovasc Res.* 1994;28:667-72.

33. Mikkelsen E, Sakr AM, Jespersen LT. Studies on the effect of histamine in isolated human pulmonary arteries and veins. *Acta Pharmacol Toxicol (Copenh).* 1984;54:86-93.

34. Schoeffter P, Godfraind T. Histamine receptors in the smooth muscle of human internal mammary artery and saphenous vein. *Pharmacol Toxicol.* 1989;64:64-71.

35. Diana JN, Schwinghamer J, Young S. Direct effect of histamine on arterial and venous resistance in isolated dog hindlimb. *Am J Physiol.* 1968;214:494-505.

36. Cook DA, Macleod KM. Responses of rabbit portal vein to histamine. *Br J Pharmacol.* 1978;62:165-70.

37. Toshimitsu Y, Uchida K, Kojima S, Shimo Y. Histamine responses mediated via H1- and H2-receptors in the isolated portal vein of the dog. *J Pharm Pharmacol.* 1984;36:404-5.
38. Oriowo MA, Bevan JA. Characterization of histamine H1 and H2 receptors in the rabbit isolated ovarian artery and vein. *J Cardiovasc Pharmacol.* 1987;10:76-81.

39. Tsuru H, Kohno S, Iwata M, Shigei T. Characterization of histamine receptors in isolated rabbit veins. *J Pharmacol Exp Ther.* 1987;243:696-702.

40. Bergner M, Graser T, Handschuk L, Tiedt N. Vasomotor tone of isolated porcine coronary veins in response to acetylcholine, noradrenaline, and histamine. *Biomed Biochim Acta.* 1988;47:775-9.

41. Bodo E, Kovacs I, Telek A, et al. Vanilloid receptor-1 (VR1) is widely expressed on various epithelial and mesenchymal cell types of human skin. *J Invest Dermatol.* 2004;123:410-3.

42. Bodo E, Biro T, Telek A, et al. A hot new twist to hair biology: involvement of vanilloid receptor-1 (VR1/TRPV1) signaling in human hair growth control. *Am J Pathol.* 2005;166:985-98.

43. Biro T, Maurer M, Modarres S, et al. Characterization of functional vanilloid receptors expressed by mast cells. *Blood.* 1998;91:1332-40.

44. Anichtchik OV, Rinne JO, Kalimo H, Panula P. An altered histaminergic innervation of the substantia nigra in Parkinson's disease. *Exp Neurol.* 2000;163:20-30.

45. Rinne JO, Anichtchik OV, Eriksson KS, et al. Increased brain histamine levels in Parkinson's disease but not in multiple system atrophy. *J Neurochem.* 2002;81:954-60.

46. Berczi V, Greene AS, Dornyei G, et al. Venous myogenic tone: studies in human and canine vessels. *Am J Physiol Heart Circ Physiol.* 263: H315–H320, 1992.

47. Champion HC, Bivalacqua TJ, Lambert DG, Abassi RA, Kadowitz PJ. Analysis of vasoconstrictor responses to histamine in the hindlimb vascular bed of the rabbit. *Am J Physiol.* 1999;277:R1179-87.

48. Fukata Y, Amano M, Kaibuchi K. Rho-Rho-kinase pathway in smooth muscle contraction and cytoskeletal reorganization of non-muscle cells. *Trends Pharmacol Sci.* 2001;22:32-9.

49. Walsh MP. Calmodulin and the regulation of smooth muscle contraction. *Mol Cell Biochem.* 1994;135:21-41.

50. Somlyo AP, Wu X, Walker LA, Somlyo AV. Pharmacomechanical coupling: the role of calcium, G-proteins, kinases and phosphatases. *Rev Physiol Biochem Pharmacol.* 1999;134:201-34.

51. Hughes AD, Wijetunge S. Role of tyrosine phosphorylation in excitation-contraction coupling in vascular smooth muscle. *Acta Physiol Scand.* 1998;164:457-69.
52. Ferdinandy P. Peroxynitrite: just an oxidative/nitrosative stressor or a physiological regulator as well? Br J Pharmacol. 2006;148:1-3.

53. Gruetter CA, Bailey B, Easterling L, Szarek JL. Release of non-mast cell histamine from rat aorta. Life Sci. 2002;70:1709-17.
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 H₁ immunopositive bands at ~55 kDa of a representative immunoblot and corresponding CytC staining. B: Optical density of H₁ immunopositive bands, normalized to CytC. n≥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
Fig. 3

Contractile force (mN/mm²) vs. -log (mol/l) histamine

- The x-axis represents -log (mol/l) histamine, ranging from 8 to 3.
- The y-axis represents contractile force (mN/mm²), ranging from 0 to 20.

The graph shows a peak in contractile force at around -log (mol/l) 6, indicating a significant response to histamine at this concentration.
Fig. 4

A

H1

CytC

B

Optical Density (Mean of V. cava inferiors=100%)

v. cava inf.  v. saphena  v. cava sup.  v. iliac ext.

26
Fig. 5

![Graph showing contractile force percentages for different treatments.](graph.png)
Fig. 6

![Bar chart showing contractile force % for histamine at 3 μM under different conditions. The bars represent untreated (HA-1077 50 μM), treated with HA-1077 50 μM, Y27632 5 μM, and Y27632 5 μM + Y27632 5 μM. The chart includes statistical significance symbols (***).]
Fig. 7

Histamine 3 μM

H-1077 50 μM

Histamine 3 μM
Fig. 9

**V. saphena**

- **Stretched**
  - Immunopositive pixels: 32.26%
- **Non-stretched**
  - Immunopositive pixels: 19.6%

**V. cava inferior**

- **Stretched**
  - Immunopositive pixels: 40.09%
- **Non-stretched**
  - Immunopositive pixels: 13.6%
**Table 1.** Effect of histamine on contractility parameters in isolated ring preparations of various rabbit veins

|        | Vein                                     | n  | pD₂       | Eₘₐₓ     | P (Eₘₐₓ)       |
|--------|------------------------------------------|----|-----------|-----------|---------------|
| 1.     | Inferior vena cava (abdominal part)      | 10 | 6.11 ± 0.10 | 17.8 ± 1.1 | > 0.05        |
| 2      | Saphenous vein                           | 12 | 5.73 ± 0.04 | 16.2 ± 0.4 | > 0.05        |
| 3      | Axillary vein                            |  5 | 6.07 ± 0.12 | 14.7 ± 0.3 | > 0.05        |
| 4      | Femoral vein                             |  7 | 6.03 ± 0.08 | 10.7 ± 0.8 | < 0.05        |
| 5      | Common iliac vein                        |  5 | 6.29 ± 0.11 |  9.5 ± 0.6 | > 0.05        |
| 6      | Inferior vena cava (thoracic part)       |  7 | 5.57 ± 0.06 |  1.5 ± 0.1 | < 0.01        |
| 7      | Right superior vena cava                 |  7 | 5.77 ± 0.16 |  0.6 ± 0.1 | < 0.05        |
| 8      | Subclavian vein                          |  5 | 5.10 ± 2.60 |  0.5 ± 0.7 | > 0.05        |
| 9      | External iliac vein                      |  9 | 5.74 ± 0.07 |  0.3 ± 0.01| > 0.05        |

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
pD₂: negative base 10 logarithms of EC₅₀ values
Eₘₐₓ: maximum effect (mN/mm²)
P: statistical significance