INVolvement of CENTral and PeripherAL Histamine H₃ Receptors in the control of the Vascular Tone and Oxygen Uptake in the MeSenteric circulation of the Rat

Data concerning cardiovascular effects of peripherally and centrally located histamine H₃ receptor stimulation are contradictory, and despite excessive studies their role in the control of the cardiovascular function have not been cleared yet. Effect of histamine H₃ receptors activation have been attributed to modulation of sympathetic system activity but exact role of peripherally and centrally located histamine H₃ receptors stimulation in the modulation of vascular tone of the mesentery and intestinal metabolism remains unexplored. Aim of the present study was to evaluate the role of centrally and peripherally located histamine H₃ receptors in the modulation of vascular tone of the mesentery and metabolic activity of intestinal tissue. In anesthetized rats total mesenteric blood flow (MBF), mucosal intestinal blood flow (LDBF), intestinal oxygen uptake (VO₂) and arterial pressure (AP) were determined. Intestinal arterial conductance (C) was also calculated. Administration of the selective histamine H₃ receptor agonist imetit (10 µmol/kg i.a) evoked marked changes in hemodynamic and metabolic parameters; MBF, LDBF, C and VO₂ were significantly increased, whereas AP was significantly decreased. Pretreatment with histamine H₃ receptor antagonist clobenpropit (4 µmol/kg i.a.) abolished imetit-induced circulatory and oxygen uptake responses. Clobenpropit (4 µmol/kg i.a.) alone failed to affect the MBF, LDBF, AP, C and VO₂ values. Central administration of imetit (0.1 µmol i.c.v.) markedly increased AP and decreased MBF, LDBF, C and VO₂. Pretreatment with histamine H₃ receptor antagonist clobenpropit (0.4 µmol i.c.v.) diminished circulatory and metabolic responses to centrally injected imetit. Central histamine H₃ receptors blockade by clobenpropit evoked no significant changes in the mesenteric arterial and mucosal microcirculatory blood flow, intestinal metabolism and mean arterial pressure. We conclude that, peripheral histamine H₃ receptors when stimulated decreases vasoconstrictory tone of the mesenteric artery and precapillary structures and evokes increase of intestinal oxygen uptake. This might be in part due to inhibition of sympathetic postganglionic fibers vasopressor activity. Central histamine H₃ receptor stimulation activates vasoconstrictory sympathetic adrenergic system with possible involvement of other, presumably non-histaminergic receptors system. At basal conditions neither central
nor peripheral histamine H₃ receptors are involved in the control of mesenteric macro-
and microcirculation and intestinal oxygen consumption.  

Key words: histamine H₃ receptors, mesenteric blood flow, intestinal oxygen uptake

INTRODUCTION

Vascular bed of mesenteric circulation remains under precise neurogenic control in which extrinsic adrenergic fibers predominate. Especially vascular tone of resistance vessels involved in the modulation of blood flow magnitude and metabolic activity of intestinal tissue is tonically influenced by sympathetic outflow. Existence of presynaptic inhibitory H₃ receptors located on postganglionic adrenergic fibers and their possible importance in the cardiovascular system regulation was first reported by Ishikawa and Spherlakis (1). They demonstrated in vitro vasodilatatory effects of H₃ receptor selective agonists on the guinea pig mesenteric artery. Observed vascular effects attributed to inhibition of sympathetic neurotransmission in adrenergic postganglionic fibers innervating resistance vessels was then confirmed by results of in vivo studies performed on anesthetized pithed and spontaneously hypertensive rats by Malinowska and Slicker (2). They found a marked inhibition of vasopressor response to exogenous electrical stimulation of perivascular sympathetic nerves after H₃ receptors stimulation by R-α-methyl histamine (RαMH). Subsequently vascular presynaptic H₃ receptors have been identified by Moldergins et al. (3) in the human saphenous vein, by Rizzo et al. (4) in the pulmonary artery of the guinea pig and by Hey et al. (5) in anesthetized normotensive rats. In vitro studies by Kim et al. (6) provided evidence for the presence of postjunctional histamine H₃ receptors at the level of resistance vessels because their selective activation was responsible for marked decrease of vascular tone in the vasculature of the mesentery. Regarding heart functions under H₃ receptors stimulation in vivo, H₃ receptors were found to inhibit neurogenic cardioaccelerator responses to exogenous electrical stimulation of the adrenergic fibers in the pithed rats (2). Studies performed on anesthetized guinea pigs confirmed the absence of compensatory tachycardia accompanying hypotensive responses to H₃ receptor stimulation by highest RαMH doses. Hey et al. (5) proved that RαMH lowers basal heat rate and peripheral resistance. But in contrast to those findings, studies performed on anesthetized normotensive rats did not confirm any importance of exogenous selective peripheral stimulation of H₃ receptors in the control of cardiovascular parameters (7 - 9). Moreover, many authors described short lasting cardiodepressor effects of RαMH administration suggesting that it could be attributed to other than H₃ receptors sites of stimulation (10, 11). In the light of above mentioned discrepancies, we undertook the present study to elucidate the role of peripheral histamine H₃ receptors under basal conditions in the control of
the mesenteric macro- and micro-circulation in the anesthetized rats. Further aim was to assess the possible role of peripherally located H₃ receptors in the modulation of the intestinal tissue metabolism.

Histamine H₃ receptors were originally discovered as inhibitory autoreceptors by Arrang et al. (12) and latter as heteroreceptors in in vitro studies on rat brain cortical slices. Clapham and Clipatrick (13) postulated that H₃ receptors regulate the release of various neurotransmitters in neurons present in the central nervous system. Presence of histaminergic neurons was confirmed in the tuberomamillary nucleus in the posterior hypothalamus where they influence activity of the structures related to control of cardiovascular system (14 - 15). Szwartz et al. (16) described pressor effect of intraventricularly injected histamine which evoked short lasting but marked increase of blood pressure with concomitant tachycardia in anesthetized normotensive rats. Finch (17) reported pressor effect of centrally administered histamine on cardiovascular system in anesthetized rats, suggesting its mediation by central sympathetic mechanisms. These observations were made using a non-selective histaminergic receptors agonist - histamine and can be only cautiously extrapolated to effects of selective H₃ receptors activity. According to Kwiecień et al. (18) intracerebroventricular administration of the selective H₃ agonist (R-α-methyl histamine) evoked marked increase in the gastric blood flow with the accompanying fall of acid output and enhanced gastroprotection. These effects were attributed to the elevated adrenergic activity. Interactions between histaminergic and non-histaminergic neurons at the level of the central nervous system are not clear and there are no data concerning their possible importance in the control of mesenteric circulation and intestinal metabolic activity in the rat. Therefore, the second aim of our study was to evaluate the role of centrally located H₃ receptors in the control of vascular tone in the mesenteric vasculature and its importance in the modulation of oxygen consumption as indicator of intestinal tissue metabolic activity.

MATERIAL AND METHODS

Experiments were performed on 70 Wistar rats of both sexes weighing 300 - 350 grams. Procedures conducted during the investigation conform to guidelines of Animals Research Committee of Jagiellonian University. Animals were kept in cages at room temperature and fasted for 24 hours before experiment with free access to water. Changes in the metabolic and hemodynamic parameters were evaluated in the six experimental groups; obtained results were compared to control estimated from the group of untreated animals. Rats were anesthetized with ketamine 75 mg/kg i.p. (Spofa, Prague) then placed on heating pad, body temperature was maintained at 37°C and monitored by rectal thermistor and regulator (FST TR-100). After induction of anesthesia rats underwent catheterization of left carotid artery by cannula filled with saline and connected with electromanometer (Statham) for continuous blood pressure (AP) recording (expressed in mmHg), whereas heart rate was calculated from the phasic blood flow. Right carotid artery was cannulated for infusion of pharmacological agents. Right jugular vein was catheterized for continuous saline (0.5 ml/h) and supplemental anesthetic administration.
Middle laparotomy was performed and the main trunk of the anterior mesenteric artery was exposed where ultrasonic probe (RS1) (2 mm i.d.) was gently placed and mesenteric blood flow (MBF) was determined with use of directional Ultrasonic Doppler flowmeter. (T206 Transonic systems - Ithaca). The recorded data were expressed in ml/min. Microcirculatory intestinal mucosal blood flow (LDBF) was determined by laser Doppler flowmetry (Periflux 4001 Master, Perimed, Sweden). A fiberoptic probe was positioned against the surface of the intestinal mucosa and secured on the animal to prevent any movement of the tip of the probe. The change in LDBF was calculated in terms of percentage of control. AP, MBF, LDBF, HR and AVO2 were continuously monitored and recorded on PC computer using Windows 98 based PlowPress program.

Mean intestinal vascular conductance (C) was calculated as the quotient of MBF divided by AP and was expressed as ml/mmHg. C was used as a measure of mesenteric vascular tone in order to facilitate comparison between vascular responses to different agents when simultaneous changes in the arterial pressure and mesenteric blood flow have occurred.

Intestinal metabolism was assessed from intestinal oxygen uptake (VO2) that was calculated as a product of arterio-venous oxygen difference in the mesenteric circulation (AVO2) and MBF. AVO2 was determined from whole blood samples obtained from aorta and intestinal arcade veins. Oxygen content in blood samples was determined with use of AVOximeter (A-VOX 1000 E, Texas) and the results were expressed in O2 ml per 100 ml of the whole blood. The significance of changes in measured parameters from control was determined using Student's t test for either paired data with a confidence limit of 0<0.05. Percentage differences in specific parameters were compared with control calculated as a mean average ± S.E.M. with n = 6 - 8 per each experimental group.

**Drugs used for experimentation**

For assessment of the role of peripheral H3 receptors in the control of mesenteric vasculature, agonist and blocker of those receptors were administered intra-aortally (i.a.) via catheter inserted to the abdominal aorta through carotid artery. Selective H3 receptor agonist; imetit (dihydrobromide) (Sigma) was injected in a dose of 10 µmol/kg (i.a.). To determine the possible involvement of peripheral receptors in control of the mesenteric vascular tone and intestinal metabolism under basal conditions the selective H3 receptor blocker; clobenpropit (Sigma) was administered in the dose of 4 µmol/kg (i.a.). Drugs were dissolved in saline and injected as the single bolus in the volume of 0.3 ml.

Central effects of H3 receptor stimulation or blockade were examined with using of intracerebroventricularly (i.c.v.) administered imetit injected by the micro syringe in the bolus of 5 µl volume at the dose of 0.1 µmol per injection or clobenpropit 0.4 µmol per injection. For intracerebroventricular instillation of both agents the fine needle was inserted through dura mater after a perforation of skull bones with use of a fine drill of 0.2 mm diameter. The sites for drilling holes in the skull bones to the lateral ventricles were determined as follow: 9 mm anterior to the frontal interaural zero plane and 3 mm lateral to the sagittal zero plane. Zubrzycka et al. (19).

As the control for centrally administered agents 5 µl saline bolus was injected to the lateral ventricle.

**RESULTS**

Basal hemodynamic an metabolic parameters measured in the group of control rats were as follow: AP; 98±15mmHg, MBF; 9±3ml/min, HR; 286±60 b/min, arterio-venous oxygen difference (AVO2) was: 4.5±0.5 ml O2. Intestinal oxygen
uptake (VO₂) was 4.9±0.7 ml O₂/min/100g of tissue and the mean basal LDBF was 258±23 PU.

As mentioned in the Material and Methods, the animals were divided to six groups and the effects of blockade or stimulation of peripheral or central histamine H₃ receptors were examined.

In the first group the effect of intra arterially administered imetit (10 µmol/kg i.a.) was estimated. After imetit administration the significant increase of MBF by 33±9% (p<0.05) which lasted up to 20 min. and the significant decrease of AP by 16±5% (p<0.05) which lasted up to 20 min. were observed. Mesenteric artery tone was diminished as vascular conductance was significantly increased by 20±6% (p<0.05). Microcirculatory blood flow was increased by 19±4% (p<0.05). Arterio-venous oxygen difference was increased by 16±5% (p<0.05), which gave the significant rise in the metabolism by 27±3% (p<0.05).

In the second group, the effects of i.a. administered H₃ receptor blocker clobenpropit 4 µmol/kg were examined. No changes in the MBF, AP, HR and in C were observed and the intestinal metabolism was also not altered since arterio-venous oxygen difference was not influenced.

In the third group, the results of imetit (10 µmol/kg i.a.), after pretreatment with H₃ receptor selective blocker (clobenpropit - 4 µmol/kg i.v) were examined. No changes of vascular tone in the mesenteric artery were observed, as MBF and AP were unchanged in comparison to control values. Microcirculatory blood flow was unaffected as well. Intestinal metabolism was unchanged because no alterations in the arterio-venous oxygen difference were observed.

In the fourth group, an intracerebroventricular (i.c.v.) application of imetit evoked cardiopressor response followed by the marked increase in AP by 38±7% (p<0.05) and accompanied by tachycardia 390±40. Mesenteric artery tone was significantly elevated as C was decreased by 52±11% (p<0.05), MBF was significantly decreased by 25±3% (p<0.05), accompanied by the decrease in microcirculatory blood flow by 35±11% (p<0.05). Arterio-venous oxygen difference did not show a marked changes but intestinal oxygen uptake was significantly decreased by 18± 6% (p<0.05).

In the fifth group the effect of central administration of H₃ receptor blocker - clobenpropit (0.4 µmol i.c.v.) was examined. Clobenpropit failed to significantly affect the AP and HR. Vascular tone of the mesenteric artery was decreased since C was increased by 15±3%. Total and microcirculatory blood flow values were increased by 8±2% and 18±5% (p<0.05), respectively. Arterio-venous oxygen difference was not changed in comparison to control values.

In the sixth group, the effect of central administration of imetit given to animals earlier pretreated with clobenpropit (0.4 µmol/kg) was examined. In these rats, an increase of AP by 19±4% (p<0.05) not accompanied by tachycardia was observed. Mesenteric artery tone was significantly increased as vascular conductance was decreased by 38±10% (p<0.05). Mesenteric and microcirculatory blood flow were decreased by 11±3 and 16±4%, respectively.
Intestinal metabolism examined from the arterio-venous oxygen difference and total blood flow were decreased in comparison to control (9±2%), however, this change failed to reach statistical significance.

**DISCUSSION**

In a previous studies by Pawlik et al. (20) and Miller et al. (21), the intra-arterial infusion of histamine into the canine superior mesenteric circulation elicited a significant vasodilatatory response, which consisted of two components namely a transient spike with subsequent fade and a later, more sustained and stable, vasodilation of lesser amplitude. We further observed that tripelenamine a classic H\(_1\)-receptor antagonist effectively inhibited only the early, transient spike while metiamide, an H\(_2\)-receptor antagonist, inhibited the later stable vasodilatory response. These findings suggested that both H\(_1\) and H\(_2\) receptors to histamine were present in the same local circulatory bed.

Ishikawa and Sperelakis (1) originally demonstrated the existence of histamine H\(_3\) receptors in the perivascular sympathetic nerves. The predication of histamine H\(_3\) receptors depends upon the specificity of drugs that antagonize different responses to this amine in different organs. Additional support for the
The presence of H₃ receptors comes from the availability of agonists for this receptor that selectively evoke known actions of histamine or its analogues in different organs. We tested such specificity and selectivity in rats using clobenpropit and imetit. Our results suggest the presence of H₃ receptors in the vasculature of the gut in this species.

We found that peripherally administered selective H₃ receptors agonist imetit elicited a significant vasodilator response of the rat superior mesenteric artery with concomitant increase in mesenteric blood flow (Fig. 1). The observed vascular response was accompanied by significant increase in intestinal oxygen uptake (Fig. 4). During imetit-induced intestinal vascular and metabolic responses the significant decrease of the mean arterial blood pressure was observed without any changes in heart rate. The above hemodynamic and metabolic responses lasted about twenty minutes.

An accepted measurement of the nutrient circulation can be obtained using the local laser Doppler flowmetry. In the present study histamine H₃ receptors ligand increased intestinal microcirculatory blood flow to the greater extent than that seen in total MBF. The observed changes of the blood flow distribution in the vascular compartments of the intestinal wall may be due to greater population of H₃ receptors in the mucosal-submucosal layer of intestine. This is, at least in part, related to increased activity of perivascular postganglionic adrenergic fibers and

Fig. 2. Effects of peripheral or central application of imetit, clobenpropit and imetit after clobenpropit on systemic arterial pressure. Asterisk indicates significant difference from control. Cross indicates significant difference from imetit alone.
might be attributed not only to $\alpha_1$ but also to $\alpha_2$ adrenoreceptors activation by presynaptically released norepinephrine as this subclass of adrenoreceptors predominate on smooth muscle cells over locations on vascular epithelium in the resistance vessels. (22). Our findings with peripherally administered imetit are partially in agreement with observations of Coruzzi et al. (9) who also observed an imetit-induced decrease in arterial pressure (Fig. 2). Thus, it seems that observed decrease of mean arterial pressure due to activation of peripheral H$_3$ receptors, at least in part, could be the consequence of the decrease in mesenteric vascular resistance or the increase in mesenteric vascular conductance (Fig. 3).

Thus, our study provide an evidence for the presence of H$_3$ receptors in the mesenteric circulation, however, the localization of these receptors to particular smooth muscle structures, vascular endothelial cells or perivascular adrenergic nerves can only be speculated upon our data.

Nevertheless, our results confirm and extend findings of other investigators who also identified H$_3$ histamine receptors in other peripheral tissues. The peripheral H$_3$ receptors to histamine have been identified by Malinowska and Slicker (2, 23), Hey et al. (5), McLeod et al. (8), Coruzzi et al. (9) and Kwiecień et al. (18). In the present study we have also demonstrated that activation of mesenteric H$_3$ receptors increases intestinal oxygen consumption (Fig. 4), which suggests the presence of H$_3$ receptors on precapillary sphincters as well. The
mechanism by which \( H_3 \) receptors induced increase in oxygen consumption could be related to precapillary sphincters relaxation and increase in intestinal capillary density and improvement in oxygen diffusion parameters. This microcirculatory effects could be also the consequence of inhibition of the tonic adrenergic vasoconstrictory influences by histamine receptor blockers as shown by Pawlik et al. (24) and Shepheard et al. (25). Another explanation for the observed vasodilatation could be the increase in the generation of nitric oxide (NO) which is known as potent dilator of precapillary sphincteric smooth muscles and endogenous factor which controls intestinal oxygen consumption via microcirculatory mechanism (26). However, exact localization of \( H_3 \) receptors to specific sites in the mesenteric circulation requires further elucidation.

Moreover, in the present study, we did not confirm any role of peripheral \( H_3 \) receptors in the basal regulation of vascular tone in the mesentery and involvement of those receptors in the control of arterial pressure in the normotensive anesthetized rat (Fig. 2).

Findings from the first part of the present study, however, pertain to the effects of exogenously administered imetit but do not permit to conclude that endogenously stimulated \( H_3 \) receptors play a physiological role in the control of blood flow to the gut.

![Graph](image)

**Fig. 4.** Effects of peripheral and central administration of imetit, clobenpropit and imetit after clobenpropit on intestinal oxygen uptake. Asterisk indicates significant difference from control. Cross indicates significant difference from imetit alone.
In the present study we also found that activation of central H₃ receptors induced significant increase of vascular tone in the mesentery with concomitant decrease of mesenteric blood flow and a reduction in the intestinal tissue oxygenation and significant rise of arterial blood pressure. The characteristics of the changes in the mesenteric and systemic circulation which appeared after activation of central H₃ receptors with i.c.v. injected imetit are partially consistent with previous reports by our group (27). We found that activation of central H₃ receptors by imetit elicits mesenteric vasoconstrictory response. We also found that centrally administered imetit evokes significant decrease in intestinal oxygen consumption. The observed decrease in C and in VO₂ in our present study suggests that stimulation of central H₃ receptors induced indiscriminate contraction of arteriolar and precapillary sphincteric smooth muscles in the mesenteric circulation thereby decreasing total intestinal blood flow by constricting arterioles and reducing the nutrient circulation by closing capillaries. This notion is in agreement with previous reports from our laboratory showing that acute activation of sympathetic postganglionic perivascular nerves and numerous endo- and exogenous vasoconstrictors evoke the same pattern of responses in the mesenteric circulation (24, 25, 28). In the present investigation we did not confirm any physiological meaning of centrally located H₃ receptors
at the basal conditions in the modulation of cardiovascular centers which control
the systemic vascular tone and heart rate in the normotensive anesthetized rat.

However, contradictory findings concerning the effects of H₃ receptor
blockade comes from literature. Godlewski et al. (11) reported vasopressor
responses in the normotensive rats but Corruzzi et al. (9) found dose-related
decrease of heart rate and blood pressure after H₃ receptors blockade with
clobenpropit and thioperamid.

Imetit administered centrally after H₃ receptors blockade was without any
effect on cardiac and vascular local mesenteric and general pressure responses,
indicating high selectivity of agonist and antagonist towards H₃ histamine
receptors (29). It has been also suggested (11, 23, 30) that H₃ receptors agonists
may demonstrate a weak sympathicomimetic effect via activation of postsynaptic
α₂ adrenoreceptor. Ganellin et al. (31) and Vollinga et al. (32) questioned
selectivity of imetit and suggested other possible sites of its action than H₃
receptor activation and our present data are in keeping with this hypothesis. The
results of present study demonstrate that central histamine H₃ receptors are mainly
involved in observed action of imetit on the mesenteric circulation.

Central administration of imetit evoked significant increase of arterial pressure
and this vasopressor effect was accompanied by tachycardia and marked increase
of vascular tone in the mesentery. This pressor effect is attributed to the increase
of peripheral sympathetic tone due to central activation of adrenergic neurons via
stimulation of H₃ receptors in the brain of the rat (15, 33). Although central
histaminergic system is clearly involved in the previously mentioned circulatory
effects, the exact mechanism of its relation to the peripheral adrenergic activation
is not quite recognized. Many doubts exist about the participation of H₃ receptors
in the responses to centrally applied histamine analogs. According to our
observations animals pretreated with centrally applied H₃ receptor blocker
exhibited the pressor circulatory response to H₃ receptor agonist, however its
magnitude was low and observed changes were not significant. Based on these
observations, we suggest that the central actions of imetit could be partially
mediated by other centrally located histaminergic receptors subtypes but also
involvement of the nonhistaminergic mechanism could not be excluded as was
proposed by Malinowska et al. (34). Experiments performed with use of R-a-
methyl histamine suggest involvement of α₂ adrenoreceptors (2, 11), although
these findings could not be directly extrapolated to effects of imetit.

In conclusion, the present study shows that peripheral activation of histamine
H₃ receptors decreases the basal tonic vasoconstriction of mesenteric resistance
vessels and precapillary sphincters and evokes increase of intestinal tissue
metabolism. Whereas, stimulation of the central H₃ receptors evokes mesenteric
vasoconstriction and decrease in intestinal oxygen uptake probably via peripheral
activation of adrenergic postganglionic perivascular nerves. At basal conditions
peripheral and central H₃ receptors are not involved in the control of mesenteric
circulation and intestinal tissue oxygenation in the rat.
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