Histamine H<sub>3</sub> receptors modulate reactive hyperemia in rat gut.

Reactive hyperemia (RH) is an abrupt blood flow increase following release from mechanical occlusion of an artery, with restoration of intra-arterial pressure. The mechanism of this postocclusion increase in blood flow in the gut is multifactorial. Relaxation of intestinal resistance vessels, observed during RH, may involve myogenic, metabolic, hormonal and neurogenic factors. Evidence exists that histamine is an important endogenous mediator of various functions of the gut, including blood flow. The vascular effects of histamine in the intestinal circulation are due its agonistic action on histamine H<sub>1</sub>, H<sub>2</sub> and H<sub>3</sub> receptors. In the present study the hypothesis was tested that peripheral histamine H<sub>3</sub> receptors are involved in the mediation of RH in the intestinal circulation. In anesthetized rats, anterior mesenteric artery blood flow (MBF) was determined with ultrasonic Doppler flowmeter, and arterial pressure (AP) was determined with a transducer. The increase in the volume of blood accumulating during RH (RH-volume), the peak increase of arterial blood flow (RH-peak response) and the duration of the hyperemia (RH-duration) were used to quantify RH after occluding the anterior mesenteric artery for 30, 60 and 120 s. Hyperemia parameters were determined before and after administration of the selective histamine H<sub>3</sub> receptor antagonist clobenpropit. Pretreatment with clobenpropit was without any effect on control MBF and AP but significantly reduced most of RH responses. These findings support the hypothesis that histamine H<sub>3</sub> receptors do not play any role in the control of intestinal vasculature at basal conditions but these receptors participate in the intestinal hyperemic reaction in response to complete temporal intestinal ischemia.

Key words: histamine H<sub>3</sub> receptors, intestinal reactive hyperemia
Reactive hyperemia in the gut (RH) is a local vascular response that occurs following release from occlusion of the mesenteric artery with restoration of intra-arterial pressure. In this response arterial blood flow to the gut increases rapidly and overshoots the preocclusion value (1-3). The mechanism of this postocclusion intestinal hyperemia is complex although myogenic, metabolic and neurogenic mediators of this vascular response have been proposed. A myogenic mechanism for RH presupposes relaxation of vascular smooth muscle in response to lowered transmural pressure during time of arterial occlusion (1, 3). A metabolic mechanism for RH presumes the release and accumulation of vasodilator substances, such as hydrogen ions, potassium, adenosine, bradykinin and prostaglandins (1-4).

In earlier study, we demonstrated that the magnitude of mesenteric RH is modulated by peripheral adrenergic nerves and their receptors. Stimulation of alpha-adrenergic receptors appears to restrict RH, and stimulation of beta-adrenoreceptors appears to enhance RH (5). Our previous experimental findings also suggest that there is a sensory nerve-dependent component of RH in the mesenteric circulation of rats. However, there is no direct evidence that this modulation is due to either the presence of a resting sensory influence or to an increase in activation of sensory afferents during mesenteric artery occlusions. It is possible that tissue hypoxia and local production of metabolites during the occlusion period may stimulate the sensory fibers to release vasodilatatory peptides (6).

Pawlik et al. and Miller et al. (7, 8) have presented evidence that the intrarterial infusion of histamine into the canine superior mesenteric circulation elicited a significant vasodilatory response which appeared to have two components, namely a transient spike with subsequent fade and a later more sustained and stable dilation of lesser amplitude. We further observed that tripelennamine, a classic H₁-receptor antagonist, effectively inhibited only the early, transient spike while metiamide, an H₂-receptor antagonist, inhibited the later stable vasodilatory response. These findings suggested that both H₁ and H₂ histamine receptors were present in the canine superior mesenteric artery.

Originally the histamine H₃ receptors were discovered as presynaptic inhibitory receptors on histaminergic nerve terminals in the brain (9). Since then histamine H₃ receptors have been identified in a wide variety of tissues and organs including the gastrointestinal tract (10-12). Ishikawa and Sperelakis (13) first reported that stimulation of H₃ receptors inhibited sympathetic neurotransmission in adrenergic postganglionic neurons and induced vasodilation in the guinea pig mesenteric arteries. Malinowska and Slicker (14) found a marked inhibition of vasopressor response to exogenous electrical stimulation of perivascular sympathetic nerves after H₃ receptors stimulation. Subsequently vascular presynaptic H₃ receptors have been identified by Molderings et al. (15) in the
human saphenous vein, by Rizzo et al. (16) in the pulmonary artery of the guinea pig and by Hey et al (17) in anesthetized normotensive rats. In vitro studies by Kim et al. (18) 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.

Smit et al. (19) have shown that stimulation of prejunctional histamine H₃ receptors inhibit electrically evoked endogenous noradrenaline overflow in the portal vein of rats.

Our recent experimental evidence (22) suggests that histamine H₃ receptors when stimulated play a role in the control of blood flow and oxygen uptake in the rat gut. Thus, the present knowledge indicates that histamine H₃ receptors may modulate vascular autoregulatory responses including reactive hyperemia. Therefore the aim of this study was to evaluate the role of histamine H₃ receptors in the regulation of intestinal RH.

**MATERIAL AND METHODS**

Experiments were performed on 54 Wistar rats of both sexes weighing 270-320 grams. Animals were fasted, but were allowed access to water for 24 h before the onset of experiments. Animals were anesthetized with ketamine (75 mg/kg) administered intraperitoneally. Body temperature was maintained at 37.5° by warming rats with a heating pad, controlled by a rectal thermistor and regulator (Fine Science Tools TR - 100). The trachea was cannulated, and the animals were artificially ventilated with room air using a positive pressure respirator (Ugo Basile). Mean systemic arterial pressure (AP) was monitored via saline - filled cannula inserted into the right carotid artery and connected to a strain gauge transducer (Statham). Another carotid artery was used to introduce a catheter into the thoracic aorta for intra-arterial (i.a.) injection of drugs. A saline filled catheter was placed in the right jugular vein for administering required supplemental anesthetic and for continuous saline infusion (0.5 ml/h).

A midline laparotomy was performed to expose the main trunk of the anterior mesenteric artery for placement of a ultrasonic flow probe (RS₁) (1.5 i.d.) on the vessel to estimate the mean mesenteric blood flow (MBF). MBF was determined with the use of directional Ultrasonic Doppler flowmeter (T206 Transonic system - Ithaca). The probe was positioned 3-4 mm distal to the origin of the anterior mesenteric artery to avoid turbulence beneath the probe. The recorded blood flow was expressed in ml/min. Mechanical zero flow was established during each experiment with a stainless steel, vascular clamp for occlusion of the anterior mesenteric artery, placed distal to the flow probe. Continuous recordings of AP and MBF were made on PC computer using Windows 98 based program. Experimental procedures conducted during the study conform to guidelines of Animals Research Committee of Jagiellonian University.

The experimental animals were divided into four groups and the effects of blockade of peripheral histamine H₃ receptors on basal intestinal blood flow and intestinal RH responses were examined. In separate group of rats, complete arterial occlusion was performed for 30, 60, and 120 s during control and experimental periods. The peak MBF during RH was determined, as well as the preceding control MBF value before occlusion. In addition, the duration and the volume of RH were measured from the chart recordings. The duration of RH was measured in seconds after release from occlusion, from the point in time when MBF exceeded the basal value until MBF had returned.
to the basal value. The volume of excess blood accumulating during RH was measured as the area under the RH curve, whose base is a horizontal line reflecting the preocclusion MBF. A measurement system was used to determine the area and convert measurements into milliliters of blood.

After completion of the surgical preparation, the circulation was allowed to stabilize for 30 min.

In the first group the effects of intra-arterial administered H₃ receptor blocker clobenpropit on AP and MBF were examined.

In the second group the effects of H₃ receptor agonist imetit on AP and MBF were examined.

In the third group the effectiveness of H₃ receptors antagonism was tested. In this group the effects of imetit on AP and MBF were examined after pretreatment with clobenpropit.

In the fourth group the three subsequent periods of arterial occlusions were performed before and within 15 min after H₃ receptor blockade with clobenpropit.

Statistical methods: AP, MBF, volume, peak and duration of RH data have been presented as means ± S.E.M. with n = 10-12 per each experimental group. Statistical analysis was performed using Student's t test for either paired data with a confidence limit of less than 5%.

RESULTS

Control mean MBF and arterial pressure values in four experimental groups were 8 ± 1 ml/min and 97 ± 18 mm Hg, respectively.

In the first group the effect of intra-arterially administered clobenpropit (4 µmol/kg i.a.) on basal MBF and AP was estimated. Blockade of histamine H₃ receptors per se did not significantly change either MBF or AP (Fig. 1).

![Fig. 1. Effects of peripherally administered clobenpropit and imetit after clobenpropit on mesenteric blood flow and mean arterial pressure. Single asterisk indicates significant (p<0.05) difference form control. Cross indicates significant (p<0.05) difference from imetit alone.](image-url)
In the second group imetit was injected intra arterially in a dose 10 µmol/kg. After imetit administration the significant increase of MBF by 28 ± 11% (p<0.05) and the significant decrease of AP by 22 ± 8% (p<0.05) were observed. Imetit-induced changes in MBF and AP lasted up to 30 min (Fig. 1).

In the third group MBF and AP responses to i.a. administered imetit were observed 10 minutes after selective H₃ receptor antagonism with clobenpropit administered as noted above. Histamine - H₃ receptors blockade significantly diminished intestinal hyperemic and general depressor responses induced by imetit (Fig. 1).

In the fourth group the RH responses to 30, 60, and 120 s arterial occlusions were performed before and after histamine H₃ receptors antagonism with clobenpropit. Figure 2 shows the recording from typical 30, 60, and 120 s occlusions. In control conditions, we found that the RH volume, the peak response and the duration increased progressively from 30 to 60 to 120 s occlusions and release (Figs. 2-5). Occlusion lasting 30 s (before pretreatment with clobenpropit) evoked a 65 ± 21% increase in RH volume, whereas 60 and 120 s duration of arterial occlusion induced increase in RH volume by 87 ± 29% and 110 ± 28%, respectively (Fig. 3). The control RH peak response of the intestinal vasculature to release from 30, 60, and 120 s occlusions of arterial inflow were characterized by 88 ± 19%, 118 ± 25% and by 128 ± 24% increase of peak flow, respectively (Fig. 4). Duration of RH after 30, 60 and 120 s periods of arterial occlusion was 86 ± 5, 125 ± 8 and 189 ± 9 s, respectively. Linear increments in RH; volume, peak flow and duration were observed in this group, as the period of occlusion was prolonged (Figs. 2-5). The control responses of the
Fig. 3. RH - volume values after occlusions of 30, 60, and 120 s before and after histamine H₃ receptor blockade with clobenpropit. There was a significant (p<0.05) attenuation of volume after administration of clobenpropit for 60- and 120 s time period of occlusions.

Fig. 4. Effect of H₃ receptor blockade with clobenpropit on RH peak response for 3 periods of occlusion. Clobenpropit significantly (p<0.05) reduced peak response for 60 and 120 s occlusions.
intestinal circulation to release from 30, 60, and 120 s occlusions of arterial inflow were similar to those reported previously (3, 5, 6).

In this experimental group the effects of arterial occlusion before and after histamine H$_3$ receptor blockade with clobenpropit were observed and RH responses appear in Figs. 2-5. A response pattern similar to that of control was observed; however, clobenpropit significantly reduced certain RH responses to 60, and 120 s of mesenteric arterial occlusions compared with these responses before clobenpropit. Thus there were significant reductions of RH volume (Fig. 3), the RH peak response (Fig. 4), and the duration of RH (Fig. 5).

DISCUSSION

Histamine has well documented physiological and pharmacological action on the stomach and intestines. This amine has long been known to play principal role in regulating gastric acid secretion (10). Histamine when applied peripherally protects gastric mucosa against gastric lesions induced by various damaging factors (11). The evidence collected so far indicates undoubtedly that histamine is involved in the modulation of gastric mucosal and intestinal blood flow which plays an essential role in the maintenance of physiological function of the gastrointestinal tract.

![Figure 5](image_url)
In the previous studies by Pawlik et al. (7) and Miller et al. (8) evidence has been presented that \( H_1 \) and \( H_2 \) receptors to histamine are present in the canine mesenteric circulation. These receptors when stimulated elicited a significant intestinal vasodilatory response. Obuchowicz et al. (22) originally demonstrated the involvement of histamine \( H_3 \) receptors in the control of the mesenteric circulation and intestinal oxygen uptake. Evidence from above mentioned studies indicate that at basal conditions peripheral histamine \( H_3 \) receptors do not play any role in the control of mesenteric circulation, whereas, when activated decreases the basal tonic vasoconstriction of mesenteric resistance vessels and precapillary sphincters and evokes increase of intestinal tissue oxygen uptake. A cumulated knowledge indicates that histamine plays a role as local modulator of intestinal vasodilation. It was, therefore, of interest to determine whether or not histamine \( H_3 \) receptors participate in intestinal RH.

In order to determine the influence of \( H_3 \) receptors on mesenteric RH responses, the physiological role of \( H_3 \) receptors in the control of resting vascular tone of the mesenteric vasculature was determined. We found that peripherally administered selective \( H_3 \) receptors blocker clobenpropit was without any effect on basal MBF and AP. In the present investigations we also demonstrated that pharmacological activation of \( H_3 \) receptors with imetit induced significant increase of MBF with concomitant decrease of AP. Pretreatment with clobenpropit abolished above imetit - induced local and general circulatory responses (Fig. 1). The current findings are consistent with our previous data (22) and observations of other authors (20, 21) showing that at basal conditions peripheral \( H_3 \) receptors are not involved in the control of cardiovascular system.

The main object of our experiments was to examine the role of the histamine \( H_3 \) receptors in modulating intestinal RH. Our findings support the hypothesis that these receptors do influence the magnitude of RH.

In control rats, RH was characterized by the increase in MBF that reached peak values within 10 s after release from occlusion and returned to basal values over the next 1-2 min. The magnitude of the volume and duration of hyperemia, as well as peak MBF value, was related to the duration of arterial occlusion. These observations agree with previous reports related to RH (1, 3, 4, 5, 6). In addition, we found that there were significant changes in the duration of RH that corresponded to those of RH volume in our experimental groups. Similarly, we also observed that changes in the volume of excess blood in RH paralleled the changes in the duration of RH in most of the comparisons in this experimental rat model. Thus the combination of MBF and duration provided the most sensitive parameters of change in RH. Finally, to explore the participation of histamine \( H_3 \) receptors in intestinal RH responses we employed pharmacological intervention which is know to inhibit activity of \( H_3 \) receptors. With our preparation, we could not restrict drug action to the gut, inasmuch as such restriction would have required full isolation of the mesenteric circulation,
continual removal of the venous effluent, use of donor rat blood, and intra-arterial administration of drugs.

Our findings indicate that peripheral intestinal histamine H₃ receptors play an important role in modulating the magnitude of RH in our experimental model. Thus, RH volume, the duration and the peak were decreased by H₃ receptor antagonism with clobenpropit. Our findings with clobenpropit suggest that temporal mesenteric artery occlusion stimulate the process of intestinal mast cell activation and release of endogenous histamine which then stimulate H₃ receptors and augments RH responses. In our experimental model local intestinal histamine release is probably the consequence of hypoxic stress - induced mast cell degranulation (23).

The vascular mechanism of H₃ receptors-induced augmentation of intestinal RH responses could be related to direct relaxatory effect on smooth muscles of intestinal resistance vessels and precapillary sphincters. In our previous study we have demonstrated that the magnitude of RH responses is modulated by peripheral adrenergic nerves and their receptors. Basal tonic activity and stimulation of sympathetic perivascular nerves appears to restrict RH. In the light of our earlier reports (7) and the latter findings of Ishikawa and Sperelakis (13) and Smit et al. (19) it is also possible that endogenous histamine, when released, modulates intestinal RH responses indirectly via inhibition of the tonic neurogenic vasoconstrictory influences through activation of presynaptic H₃-receptors on noradrenergic nerve endings in the intestinal vasculature. Our preceding experimental findings suggest that there is a sensory nerve - dependent component of RH responses in the intestinal circulation in rats (6). It is also possible that intestinal tissue hypoxia and local production of metabolites during the occlusion period may stimulate the sensory C neurones to release vasodilatory peptides. Available evidence indicates that CGRP can be released near splanchnic vessels by the stimulation of histamine H₃ receptors located on afferent C neurones. Subsequently the peptide induces vasodilation of the intestinal vasculature through interaction with specific receptors on the vessel wall (6).

Our present findings support the view that histamine H₃ receptors activation is one of several interacting mechanisms involved in the modulation of autoregulatory vascular response in the gut such as RH.

REFERENCES


Received: July 10, 2004
Accepted: August 2, 2004

Author’s address: Prof. Wiesław W. Pawlik, M.D., Department of Physiology, Jagiellonian University Medical College, ul. Grzegorzecka 16, 31-531 Cracow, Poland, Tel. (48-12) 4211106; fax (48-12)4222014.
E-mail: mppawlik@cyf-kr.edu.pl