Histamine sensitivity of mesenteric afferent nerves in the rat jejunum

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port. We have therefore characterized the action of histamine on jejunal afferent discharge in the anesthetized rat. Whole nerve mesenteric afferent discharge was recorded in conjunction with intestinal pressure in response to a range of histamine agonists and antagonists. Histamine at 2, 4, and 8 µmol/kg (iv) evoked a dose-dependent biphasic increase in afferent discharge together with a biphasic rise in intestinal pressure. However, these two events were mediated indepen-
dently, since nifedipine (1 mg/kg) substantially reduced the intestinal pressure increase but not the afferent discharge. These responses were completely inhibited by pyrilamine (5 mg/kg), but unaffected by ranitidine (5 mg/kg) or thiopera-
dime (2 mg/kg). Neither the selective H2 receptor agonist dimaprit nor the selective H3 receptor agonist R-α-methylhistamine caused any modulation of afferent discharge. We conclude that histamine stimulates an H1 receptor-mediated increase in mesenteric afferent discharge that is independent of intestinal motor events. This suggests that histamine potentially acts as a mediator in mast cell-to-afferent nerve communication in the small intestine.

METHODS

Experiments were performed on adult Hooded-Lister rats (250–350 g), which were housed under standardized condi-
tions (regular rat chow, free access to water, 12:12-h light–dark cycle, lights on at 6 AM). The institutional guidelines for the use and care of animals were followed throughout the study. Animals were withdrawn from food but not water for 12 h before the experiment. Deep anesthesia was induced by an intraperitoneal injection of pentobarbital sodium (60 mg/kg Nembutal; Sanofi Santé Animale, Libourne-Cedex, France). Through a neck incision, the trachea was cannulated to facilitate spontaneous breathing. The right external and internal jugular veins were cannulated separately to enable administration of different drugs and maintenance of anesthesia (~10 mg/kg pentobarbital sodium every 30 min). The left carotid artery was cannulated to monitor arterial blood pressure. Body temperature was maintained at 37°C by a thermostatically controlled heated operating table.

Abdominal surgery. After a midline laparotomy, the cecum was removed to augment space in the abdominal cavity. A 10-cm loop of jejunum beginning at the ligament of Treitz was cannulated at both ends with two Portex tubes (5 mm OD). The remaining free ends of the cannula were exteriorized through stab incisions in the right side of the abdominal wall. This setup allowed intestinal pressure recording by a pressure transducer (custom built, G&J Electronics, Toronto, ON, Canada). The abdominal wall at the borders of the laparotomy was sewn to a circular metal ring to create a well. The abdominal cavity was filled with colorless, light liquid paraf-
fin prewarmed to 37°C.

Nerve preparation and recording. A single neurovascular bundle located between 2 and 8 cm distal to the ligament of Treitz was isolated from the surrounding connective tissue
and placed on a black plastic platform. With the aid of a viewing microscope (Wild M3Z, Heerburg, Switzerland), a mesenteric nerve was exposed by dissection of the overlying fat and surrounding blood vessels. The nerve was then severed at the proximal end of the bundle –10–15 mm from the jejunal wall and wrapped around one arm of a bipolar platinum electrode. A sliver of connective tissue was wrapped around the second arm, serving as an indifferent electrode. The electrodes were connected to a differential amplifier (DAM 50, World Precision Instruments, Sarasota, FL) and filtered with a bandwidth of 300 Hz to 1 kHz. The signal was relayed to a spike processor (D-130, Digitimer, Welwyn Garden City, UK) that served to count action potentials per time unit with a variable trigger level. The whole nerve signal was displayed on a digital oscilloscope (TDS 310, Tektronix, Cologne, Germany) and registered by a DAT recorder (DTC 60 ES, Sony, Cologne, Germany). In parallel, spike frequency and arterial blood pressure were continuously recorded with a computer system (1401plus, Cambridge Electronic Design, Cambridge, UK) installed on a personal computer and running software (Spike2, version 4.79, Cambridge Electronic Design).

Histamine, dimaprit, nifedipine, bethanecol, ranitidine, pyrilamine, and DMSO were purchased from Research Biochemicals International (Natick, MA). Sodium nitroprusside (SNP) was bought from Schwarz Pharma (Monheim, Germany). Stock solutions were made up to the following concentrations: 10–5 mol/ml histamine, dimaprit, and R-α-methylhistamine; 160 nmol/ml 2m5HT; 0.5 µmol/ml bethanecol; 0.1 mg/ml SNP; 1 mg/ml nifedipine; 2 mg/kg thioperamide; 5 mg/ml ranitidine; and 5 mg/ml pyrilamine. All solutions were made up in normal saline except nifedipine (in 25% DMSO). For injection, stock solutions were not diluted except for 2m5HT, which was diluted with normal saline 1:10.

Experimental protocols. All compounds were given intravenously with a minimum interval of 5 min. After a 20-min baseline recording for signal stabilization, a test dose of 2m5HT at 160 nmol/kg was applied to establish the sensitivity of the nerve preparation. Histamine was then administered at 2, 4, and 8 µmol/kg (n = 15). Before the experiment was terminated, nerve sensitivity was again verified by an injection of 2m5HT.

The effects of different histamine receptor antagonists or vehicle on the histamine response were each investigated in six experiments. After consecutive injections of 2, 4, and 8 µmol/kg histamine, either vehicle (for time controls) or one of the following receptor antagonists was administered: the H1 receptor antagonist pyrilamine (5 mg/kg), the H2 receptor antagonist ranitidine (5 mg/kg), or the H3 receptor antagonist thioperamide (2 mg/kg). Ten minutes after administration of vehicle or antagonist, the three histamine doses were repeated. Test doses of 2m5HT were given before and after each experiment, as mentioned previously. In two separate groups of four experiments, the selective H2 receptor agonist dimaprit or the H3 receptor agonist R-α-methylhistamine was administered at doses of 2, 4, and 8 µmol/kg. In these experiments, nerve sensitivities to histamine and 2m5HT were determined after the agonists had been tested.

As a control for any afferent responses secondary to the blood pressure fall induced by histamine, SNP was administered at a dose (0.1 mg/kg) that produced a comparable hypotension to that obtained with histamine (n = 12). To determine the contribution of intestinal motor responses to afferent nerve discharge, the responses to histamine (8 µmol/kg), 2m5HT (160 nmol/kg), and bethanecol (0.5 µmol/kg) were compared before and 10 min after either vehicle (time control, n = 6) or nifedipine (1 mg/kg, n = 6) were administered.

Analysis. Whole nerve discharge frequencies in response to intravenous substances were determined by the CED computer system off-line from the digitized raw nerve signal. All impulses were counted as they matched templates previously defined by the Spike2 software (see Ref. 14 for details). Thus erroneous discharge frequencies due to baseline shifts were avoided. Nerve discharge responses to compounds were characterized in terms of peak discharge frequency (impulses–1) minus baseline discharge frequency, response duration, and latency between injection and response onset. The area under the response curve (AUC; total number of impulses) was determined after subtraction of baseline discharge.

Statistics. Paired t-tests were used to statistically compare data within single experimental groups. The unpaired t-test was used when data from groups of animals treated with either histamine receptor antagonists or nifedipine were compared with their respective vehicle-treated group. P < 0.05 was taken to indicate statistical significance. Data are presented as means ± SE. In Fig. 4, in which data was normalized to the time-matched controls, statistical comparisons were performed on the raw data.

RESULTS

Afferent nerve discharge following histamine. Histamine evoked a powerful and, in the majority of cases, a biphasic increase in afferent nerve discharge frequency. It was apparent from the multiunit raw nerve signal that the majority of afferents, recognized by their variable spike amplitudes, responded to histamine. In addition to the discharge frequency of spontaneously active fibers being augmented by histamine, nonspontaneously active fibers were also recruited, and they contributed to the overall response observed. The afferent nerve response to histamine was accompanied by a decrease in arterial blood pressure and was generally followed by a biphasic increase in intestinal pressure (Fig. 1).

The characteristics of the histamine response were defined from the detailed analysis of 15 experiments. Doses of 2, 4, and 8 µmol/kg evoked a dose-dependent increase in afferent nerve discharge that was reduced during a consecutive series of histamine administration at identical doses (Fig. 2). At a dose of 8 µmol/kg, the overall response was 527 ± 140 impulses (AUC) and occurred with a latency of 3.4 ± 0.3 s. The response duration was 28.7 ± 1.5 s. In 12 of 15 experiments, a biphasic response pattern was observed. The first peak discharge frequency was 31 ± 3.5 impulses/s and occurred 4.1 ± 0.2 s after histamine injection, i.e., within 1 s of the response onset. The second peak was generally larger, 57 ± 6.9 impulses/s, and occurred after 13.1 ± 1.1 s.

Histamine caused a profound fall in blood pressure. To evaluate any influence that this particular effect may have had on afferent nerve discharge, the response was compared with that following SNP (0.1 mg/kg, n = 12) administration. The fall in mean blood pressure was greater after SNP than after 4 µmol/kg histamine administration (52 ± 2.1 vs. 43 ± 2.4 mmHg, P < 0.05).
In contrast, the concomitant nerve discharge (AUC) was 117 ± 43.9 impulses for SNP vs. 530 ± 186 impulses after histamine (P < 0.05). In addition, the response latency was shorter for histamine, with 3.9 ± 0.4 vs. 55 ± 3.1 s for SNP (P < 0.001). Thus a small increase in discharge could be attributed to the fall in blood pressure, but this occurred well after the rapid histamine response had subsided (Fig. 3).

Intestinal motor events. Increases in intestinal pressure induced by 8 µmol/kg histamine were analyzed in 12 experiments when a biphasic response was obtained. The first peak was 1.3 ± 0.5 cmH2O above baseline pressure and occurred 9.8 ± 0.7 s after the histamine injection, whereas the second pressure peak was 1.7 ± 0.3 cmH2O and occurred after 48.7 ± 6.0 s. Thus the pressure peaks always followed the peaks in afferent nerve discharge (both P < 0.001).

The first peak in intestinal pressure following histamine was reduced after nifedipine (n = 6) but not after vehicle (n = 6) administration. The reduction of the second peak following nifedipine pretreatment was not significant compared with the time-controlled vehicle administration. In contrast, afferent nerve discharge to histamine was similar after either nifedipine or vehicle. 2m5HT and bethanechol were administered as a comparison, and both agents caused an increase in intestinal pressure of 1.1 ± 0.3 and 3.8 ± 0.8 cmH2O, respectively, with a peak discharge frequency of 62 ± 9.1 and 48 ± 4.0 impulses/s. Nifedipine did not alter the afferent nerve response to 2m5HT. In contrast, the afferent nerve response following bethanechol was reduced after nifedipine as was the concomitant increase in intestinal pressure (Fig. 4).
The H1 receptor antagonist pyrilamine compared with time-matched control experiments responses to 2-, 4-, and 8-µmol/kg doses of histamine were selective histamine receptor antagonists on the response. Statistical comparisons were performed on the raw data, with \( P < 0.05 \), \( \* P < 0.001 \) vs. time control. Response to histamine (8 µmol/kg) was separated into the 1st and 2nd peak following administration. Afferent and intestinal pressure responses to bethanechol and 2-methyl-5-hydroxytryptamine (2m5HT) are shown for comparison. Note that only the afferent response to bethanechol was significantly reduced, whereas the pressure rise following all agents was attenuated, although that during the second series of histamine was not significantly reduced.

Effect of histamine receptor antagonists. The effects of selective histamine receptor antagonists on the responses to 2-, 4-, and 8-µmol/kg doses of histamine were compared with time-matched control experiments (Fig. 5). The H1 receptor antagonist pyrilamine (5 mg/kg) completely abolished both the afferent and intrajejunal pressure responses to all three doses of histamine compared with the time controls (\( P < 0.001 \)). In contrast, the responses to histamine were unchanged after administration of the H2 and the H3 receptor antagonists ranitidine (5 mg/kg, not significant) and thioperamide (2 mg/kg, not significant).

Effects of selective histamine receptor agonists. The afferent and intrajejunal pressure responses to 2, 4 and 8 µmol/kg of the selective H2 receptor agonist dimaprit and the selective H3 receptor agonist R-α-methylhistamine were evaluated in four experiments each (Fig. 2). No change in afferent nerve discharge or intrajejunal pressure was observed after either dimaprit or R-α-methylhistamine administration. However, decreases in mean blood pressures of 26 ± 3.5 and 25 ± 4.0 mmHg were observed after doses of 8 µmol/kg dimaprit and 8 µmol/kg R-α-methylhistamine. In all experiments, sensitivity of the afferent nerve bundle was confirmed by administration of 2m5HT (160 nmol/kg) and histamine (8 µmol/kg) after agonist injection.

**DISCUSSION**

We studied the effects of the mast cell mediator histamine on mesenteric afferent nerve fibers in the rat proximal jejunum in vivo. Histamine evoked a dose-dependent increase in afferent nerve discharge that was typically biphasic at a dose of 8 µmol/kg. After one series of histamine applications, the second series tended to induce lower responses, indicating that desensitization of the response occurred. This observation necessitated the use of time-matched control experiments. The H2 receptor agonist pyrilamine completely abolished the afferent nerve response to histamine, whereas the H2 receptor antagonist ranitidine and the H3 receptor antagonist thioperamide did not. Correspondingly, neither the selective H2 receptor agonist dimaprit nor the selective H3 receptor agonist R-α-methylhistamine caused any modulation of afferent nerve discharge. These observations indicate that the afferent nerve response following systemic histamine is selectively mediated via the H1 receptor. However, this is at variance with the study by Akoev and colleagues (1) in which both H1 and H2 receptors were implicated in the response to histamine in the cat. This discrepancy could reflect a species difference; however, in their study (1), the decreased response to histamine in the presence of antagonists was not quantified and the potential for desensitization of the response following repeated administration of histamine was not assessed.

H1 receptors have previously been described in rat nervous tissue (22) and dorsal root ganglion neurons of other species (2, 21). We are, however, unaware of any study that characterized histamine receptor subclases on intestinal nerve fibers in rat. Consequently, there is no evidence for H1 receptors on intestinal afferent nerve terminals. Without this evidence, it is uncertain whether the increase in afferent nerve discharge following histamine is due to a direct histamine effect on afferent nerve terminals or an indirect effect. However, the rapidness of the response corresponding to the circulating delay from injection site and intestine is consistent...
with a direct action. Despite this uncertainty, our data demonstrate that the receptor that mediates the afferent nerve response is the H₁ receptor. Notably, in the guinea pig, histamine stimulates enteric neurons via H₂ receptors (10). H₂ or H₃ receptors may simply not be present in the rat enteric nervous system or on afferent intestinal nerve terminals as a result of species differences. However, an alternative explanation may relate to the differential expression of receptors on enteric neurons compared with extrinsic afferents. In the case of serotonin (5-HT) sensitivity, it has been recently demonstrated that, whereas extrinsic afferents are stimulated by 5-HT₃ receptors (14), enteric sensory neurons may respond to 5-HT via 5-HT₂ receptors (15). It may be that extrinsic and enteric reflex circuits are equipped with different subsets of receptors to the same mediator.

We further explored the hypothesis that the histamine-induced increase in afferent nerve discharge may be secondary to other histamine in vivo. Histamine caused a fall in systemic blood pressure. Therefore, we investigated whether this decrease in blood pressure gave rise to a subsequent increase in afferent nerve discharge, potentially by inducing ischemia in the gut, which has been shown to stimulate enteric afferents via prostaglandin synthesis (18). This possibility, however, was ruled out because a comparable blood pressure fall in the absence of histamine only caused a minimal increase in afferent nerve discharge. In addition, this minimal response was delayed compared with the histamine response.

Smooth muscle contraction is another well-known histamine effect that may have affected afferent nerve discharge indirectly (25). In our study, histamine administration typically caused a biphasic increase in afferent nerve discharge, which was followed by a biphasic pressure increase. Although the time course of the events suggests that stimulation of afferent nerves occurred before the intestinal motor response, this delay in the intraluminal pressure recording may also be related to a delay in pressure signal transduction. Therefore, we studied afferent nerve discharge following histamine before and after nifedipine administration. Nifedipine as an L-type Ca²⁺ channel antagonist and smooth muscle relaxant substantially reduces intestinal motor responses (19). However, the afferent response to histamine after nifedipine remained comparable to the time-matched control despite a reduction in the contractile response, which was significant for the first pressure peak only. This is largely because of the variable extent to which the second component of the contractile response was attenuated. Nevertheless, there were occasions after nifedipine administration when a robust afferent response occurred with only a minimal rise in pressure. This suggests that afferent nerve discharge following histamine is independent of intestinal motor events. The muscarinic agonist bethanechol was administered as a positive control, which caused an increase in afferent nerve discharge that is entirely secondary to smooth muscle contraction (6, 14) and a subsequent intraluminal pressure increase. The indirect action of bethanechol on afferent discharge was evident because the afferent response was reduced after nifedipine together with a decrease in intraluminal pressure. The effect of nifedipine on pressure, however, exceeded that on afferent discharge. This may be explained by a predominance of low-threshold mechanosensitive afferents in the mesenteric bundles and a nonlinear relationship between pressure and afferent discharge. In contrast to bethanechol, 2m5HT had mainly a direct effect on afferent nerve terminals via the 5-HT₃ receptor (14) and was used as a second control. Although the pressure response to 2m5HT was attenuated, the afferent discharge was maintained. The lack of sensitivity of the afferent histamine response to nifedipine resembled the 2m5HT sensitivity. This is consistent with a direct effect on the afferent discharge. The contractile responses to bethanechol, 2m5HT, and histamine were not equally influenced by nifedipine with the second component of the histamine response being least susceptible. The H₁ receptor-mediated contraction may have a greater dependence on Ca²⁺ released from intracellular stores than from external sources (16).

These experiments suggest that the afferent nerve response and the intestinal motor responses to histamine are coincidental. Nevertheless, the associated intestinal pressure increase is likely to also stimulate some mechanosensitive afferent nerve fibers. However, the contribution of these relatively weak contractions to the overall increase in afferent nerve discharge seems to be rather small. With our experimental setup, we were unable to determine whether the intestinal pressure increase following histamine was a direct effect on gut smooth muscle or a reflex-induced contraction due to the stimulation of afferent nerve fibers. This question was addressed by Sakai (25) who studied intraluminal pressure increase following dose arterial histamine injection in a segment of isolated rat small intestine (25). With this preparation, a biphasic pressure increase was described that corresponded to the motor pattern observed in our study. This similarly suggested that the intestinal pressure increase after histamine injection was a direct effect by intestinal structures rather than a reflex contraction via the central nervous system (CNS), which would be elicited by stimulation of extrinsic afferent nerve fibers.

Our study demonstrates that histamine stimulates afferent mesenteric nerve fibers in rat proximal jejunum. Histamine is one of an array of mediators that are released during mast cell activation, e.g., during type I hypersensitivity reactions (12). We have previously shown that the mast cell mediator 5-HT stimulates vagal afferent nerve fibers originating in the mucosa of the gastrointestinal tract (14). In addition, some limited data show that other mast cell mediators such as prostaglandin E₂ may also modulate intestinal afferents (1). Consequently, data are accumulating that suggest that mast cells may sensitize afferent nerve fibers by the release of various mediators. Such a signal transduction pathway from mast cell to nerve may be pivotal in the capability of the organism to detect...
potentially hazardous materials in the gut lumen. Subsequent central processing of this information, which is transmitted by intestinal afferents, may trigger not only protective reflexes but also an efferent modulation of immune cells (26).

The small intestine is supplied by two types of afferent nerve fibers that project to the CNS in either vagal or splanchnic nerves. In addition, mesenteric nerves contain intestinal afferent fibers projecting to the prevertebral ganglia (20). In the present study, we did not determine in which of these afferent populations the sensitivity to histamine resides. It remains to be determined which type of fiber is stimulated by systemic histamine and where in the gut wall the endings of these fibers are located. The presented data, however, allow us to conclude that histamine stimulates afferent intestinal nerve fibers exclusively via H$_1$ receptors. The response in afferent nerve discharge does not appear to be secondary to the concomitant intestinal motor response or the fall in blood pressure. Thus histamine has the potential to participate in signal transduction from mucosal mast cells to the CNS in the small intestine.

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