Br. J. Pharmac. (1979), **67**, 587–590

A PHARMACOLOGICAL ANALYSIS OF THE CONTRACTILE ACTION OF HISTAMINE UPON THE ILEAL REGION OF THE ISOLATED, BLOOD-PERFUSED SMALL INTESTINE OF THE RAT

K. SAKAI

Department of Pharmacology, Research Laboratories, Chugai Pharmaceutical Co., Ltd., Takada, Toshima-ku, Tokyo 171, Japan

1 The mode of action of histamine was investigated on the rat isolated small intestine perfused at a fixed flow rate through the superior mesenteric artery with blood from the carotid artery of a heparinized donor rat. Compounds were closely injected into the superior mesenteric artery.

2 Increasing doses (1 to 100 μg) of histamine caused a monophasic fast contraction of the ileum.

3 The fast contraction was abolished by tetrodotoxin, hexamethonium, morphine or mepyramine, but was not prevented by atropine.

4 The present study indicates that histamine produces the fast contraction of the ileum by primarily acting on the myenteric nerve plexus involving cholinergic interneurones.

Introduction

It has been reported that histamine has almost no effect on the longitudinal muscle strips of rat ileum (Sakai, Shiraki, Tatsumi & Tsuji, 1979) and colon (Ulrich, 1965), unlike the guinea-pig ileum (Rocha e Silva, Valle & Picarelli, 1953; Brownlee & Johnson, 1963; Day & Vane, 1963; Sakai *et al.*, 1979). However, these studies were carried out on isolated muscle strip preparations bathed in physiological salt solutions. Recently, attempts were made to perfuse the rat isolated small intestine through the superior mesenteric artery with arterial blood from a donor rat and to record the intestinal muscle tone (Sakai, Akima & Shiraki, 1979). The study revealed certain differences in the mode of action of 5-hydroxytryptamine (5-HT) between the isolated blood-perfused intestinal preparation of the rat and rat isolated ileal strip bathed in physiological salt solution (Sakai *et al.*, 1979); the action of 5-HT injected intra-arterially to the blood-perfused intestinal preparation was primarily to stimulate neuronal elements there, while that of 5-HT given to isolated ileal strips bathed in physiological salt solution was exerted on ileal smooth muscle. In view of this, it seemed to be of interest to examine the effect of histamine injected into the mesenteric artery on the ileal region of the isolated, blood-perfused small intestine of the rat.

Methods

Male Sprague-Dawley rats were anaesthetized with sodium pentobarbitone (65 mg/kg i.p.). Details of the preparation involving a cross-circulation technique have been described in full (Sakai *et al.*, 1979).

The recipient rat (about 150 g) was deprived of food overnight before the experiment but water was allowed *ad libitum*. Under anaesthesia, the abdomen was opened in the midline and the intestine was gently exteriorized. Both ends of the intestine were ligated and cut off, proximally at the junction between the pylorus and duodenum and distally at the ileum about 10 cm above the caecum. The isolated small intestine was perfused at a fixed flow rate through the superior mesenteric artery with heparinized blood (37°C) from the carotid artery of a donor rat (550 to 700 g) by means of a peristaltic pump (Mitsumi Science, SJ-1210); the flow rate was precalibrated and re-checked at the end of the experiment. The donor was not ventilated artificially. A square wave electromagnetic flowmeter (Nihon Kohden, MF-25) was used for the measurement of the mesenteric blood inflow. The blood pressure of the donor and the mean perfusion pressure were measured with pressure transducers (Nihon Kohden, MPU-0.5). The venous outflow from the portal vein was returned to the donor through a reservoir by gravity.

A small opening was made in the wall of the ileum about 20 cm from its end and through the opening a water-filled balloon 3 to 5 mm long and made of thin rubber, was inserted into the lumen of the intestine in the direction of the duodenum. The amount of water filling the balloon was adjusted initially to give resting intraluminal pressures ranging between 2 and 7 cmH_2O. The pressure of the ileal region was

0007-1188/79/120587-04 $01.00

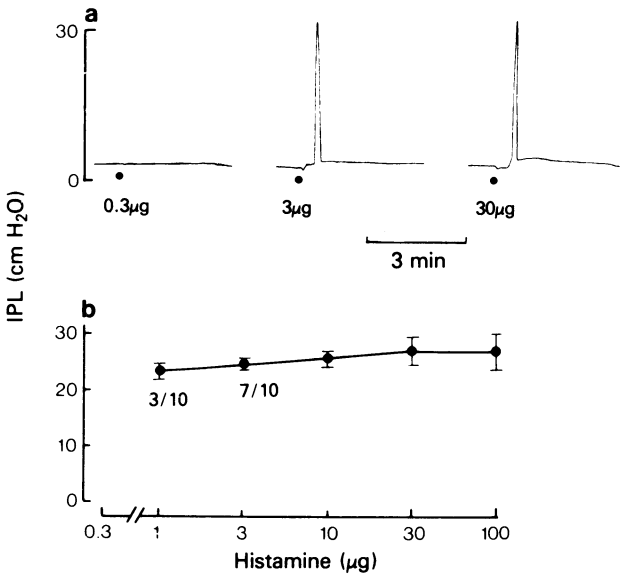

Figure 1 Rat ileal responses to increasing doses of histamine. ILP, intraluminal pressure. (a) Original recordings of responses to histamine. (b) Dose-response curve for the amplitude of the fast contractile responses to histamine. Doses were increased by a factor of about 3, and they were given at intervals of at least 5 min. Vertical bars represent s.e. mean, $n = 10$ except for 1 μg ($n = 3$) and 3 μg ($n = 7$)-administered groups. The number of preparations out of the total tested, in which ileal contractile response to histamine occurred, is shown. Minimum effective dose of histamine inducing the ileal contraction was 1 μg. There were no significant differences between the values from each group.

measured by means of a pressure transducer (Nihon Kohden, LPU-0.1). All recordings were made continuously on an ink-writing rectigraph (TOA Electronics, EPR-3T). The isolated intestine was covered with cellophane to prevent it from drying.

Drugs used were histamine dihydrochloride (Wako Junyaku), 5-hydroxytryptamine creatinine sulphate (5-HT, Sigma), carbachol chloride (Tokyo Kasei), tetrodotoxin (TTX) and morphine hydrochloride (both Sankyo), hexamethonium bromide (C_6, Yamanouchi), atropine sulphate (Takeda) and mepyramine maleate (Merck, Sharp & Dohme). Drugs were freshly prepared with 0.9 % w/v NaCl solution (saline) and 0.01 ml of each solution was injected in a period of 4 s into the superior mesenteric artery through individual microsyringes (Jintan Terumo Co.).

Values in the text are means ± s.e. mean (unless otherwise noted). Student's *t* test was used for statistical analyses. A *P* value of 0.05 or less was considered statistically significant.

Results

Basal values of main parameters under resting conditions

Experiments were carried out on 35 preparations. Mean perfusion pressure was set at a value slightly lower than the mean systemic blood pressure of the donor at the onset of perfusion with a flow rate of about 3.5 ml/min. Shortly after the start of perfusion the pressure rose slightly, but fell subsequently to reach a new steady-state level. Then, the pressure was re-adjusted to nearly 100 mmHg and, thereafter, remained almost constant for about 3 h. Thus, a stable situation of each preparation was established within 20 min of the onset of perfusion. At this stage, the measured main parameters were as follows: mean perfusion pressure, 90.5 ± 1.3 mmHg; mesenteric blood inflow, 3.9 ± 0.1 ml/min; intraluminal pressure, 3.8 ± 0.2 cmH_2O; mesenteric vascular resistance, 185.6 ± 2.7 (10^4.dyne.s.cm^{-5}); mean systemic blood pressure of the donor, 115.2 ± 3.5 mmHg.

Ileal response to increasing doses of histamine

Histamine in doses of 0.3 to 100 μg was injected into the superior mesenteric artery. A monophasic fast contraction of the ileum, which was followed occasionally by a rise in tone, reproducibly occurred with single injections of 1 to 100 μg of histamine (Figure 1a). Even if the doses of histamine were administered intravenously to the donor, they did not give any changes in the intraluminal and perfusion pressures of the isolated perfused intestine. The

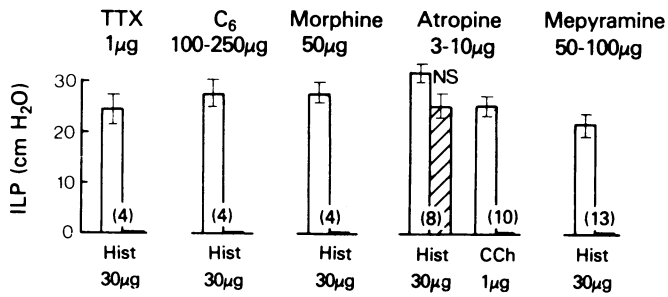

Figure 2 Effects of various blocking agents on the fast contractile response of the ileum to histamine (Hist). CCh = carbachol; C_6 = hexamethonium. Vertical bars represent s.e. mean, numbers of experiments are given in parentheses. Open columns, before treatment; hatched columns, after treatment. NS, no significant difference between the corresponding values before and after the treatment with blocking agents. ILP, intraluminal pressure.

present study was designed to examine the mechanism for the induction of the fast contractile response to histamine. As seen in Figure 1b, the dose-response relationship to histamine for the amplitude of the fast contractile response was linear; once a response was elicited, an increase in dose of histamine failed to cause a further increase in the amplitude of the contractile response. Thus, the dose-response relationship for the amplitude of the response was flat. However, when the frequency of occurrence of the response was plotted against the dose, the dose-incidence relation was sigmoid for histamine in the dose range 0.3 to 10 μg.

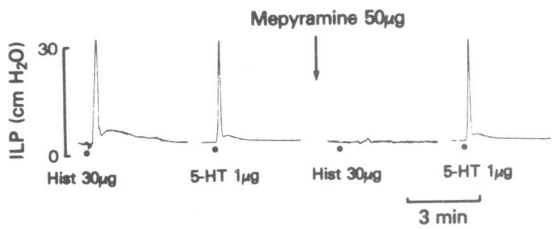

Figure 3 Effect of mepyramine on the contractile responses of the ileum to histamine (Hist) and 5-hydroxytryptamine (5-HT). ILP, intraluminal pressure.

Effects of various blocking agents on the ileal contractile response to histamine

Summarized results are shown in Figure 2. The fast contraction in response to 30 μg of histamine was abolished by single injections into the mesenteric artery of either 1 μg of TTX, 100 to 250 μg of C_6 or 50 μg of morphine, but the rise in ileal tone preceded by the fast contraction was not affected by these blocking agents. The blockade of the fast contractile response to histamine lasted for about 30 min, after which the contraction appeared again. C_6, within the dose range used, had almost no effect on the ileum or caused a slight decrease in the resting ileal tone, whereas TTX and morphine transiently (1 to 5 min) and slightly (1 to 3 cmH_2O) raised the tone. Single intra-arterial injections of atropine in doses of 3 to 10 μg, which were sufficient to abolish for over 30 min the contractile response to 1 μg of carbachol, failed to block significantly the fast contractile response to 30 μg of histamine. When single injections of 50 to 100 μg of mepyramine were made into the mesenteric artery, they did not affect the fast contraction caused by 1 μg of 5-HT injected intra-arterially. In contrast, the same doses of mepyramine abolished not only the fast contraction but also the rise in ileal tone (suggesting a direct action on H_1-muscular receptor in the ileum) caused by 30 μg of histamine (Figure 3). The blockade of the response to histamine wore off within 30 min.

Discussion

The present study has shown that histamine injected into the superior mesenteric artery of the rat caused a monophasic fast contraction of the ileum. The fast contraction was blocked completely by TTX or morphine. This indicates that the contractile response to histamine is due entirely to excitation of neural elements in the ileal region.

The fast contraction was also abolished by mepyramine, a potent H_1-receptor antagonist (Ash & Schild, 1966). Mepyramine antagonizes histamine effects at neuronal histamine receptors (Trendelenburg, 1954; 1956; Lewis & Reit, 1965) as well as at muscular histamine receptors (Gaddum, 1956; Ash & Schild, 1966). The blockade by C_6 of the response to histamine excludes the possibility that exogenous histamine induces the fast contraction of the ileum

by direct acting on muscular histamine receptors and instead supports the conclusion that the contractile response to histamine results exclusively from excitation of neural elements in the ileal region.

On the basis of the finding that C_6, a typical nicotinic receptor antagonist, prevented the histamine-induced contraction, the question arises whether histamine might stimulate nicotinic receptors on neural elements or whether the blocking action of C_6 might not be specific in the present preparation. Histamine is known to have a C_6-resistant stimulant action on the superior cervical ganglion of the cat (Trendelenburg, 1954). Thus, it is unlikely that in the present preparation histamine had a direct stimulant action on nicotinic receptors or the blocking action of C_6 was non-specific. Alternatively, the problem would be settled if it were assumed that histamine stimulated those neurones in the myenteric neural plexus which are cholinergic and that C_6 blocked transmission via nicotinic receptors on cholinoceptor neurones synapsing cholinergic neurones stimulated by histamine.

It should be noted that the response to histamine was resistant to the blocking action of atropine or methysergide (not shown). The absence of any blocking effects on the response to histamine of these substances suggests that neurones functioning as the final common pathway to the smooth muscle cells of the ileal region may not be cholinergic or tryptaminergic. Additionally, catecholamines induce primarily only an ileal relaxation in the same sort of preparation (Sakai *et al.*, 1979).

The present results clearly demonstrate that histamine when injected into the isolated, blood-perfused intestinal preparation of the rat has a powerful stimulant action on neural elements in the ileal region, leading to the fast contraction. This finding contrasts with the well-known fact that in rat isolated ileal strips bathed in physiological salt solutions, histamine has little contractile effect.

Gratitude is due to Mr M. Akima for his skilful technical assistance.

References

ASH, A.S.F. & SCHILD, H.O. (1966). Receptors mediating some actions of histamine. *Br. J. Pharmac. Chemother.*, **27**, 427–439.

BROWNLEE, G. & JOHNSON, E.S. (1963). The site of the 5-hydroxytryptamine receptor on the intramural nervous plexus of the guinea-pig isolated ileum. *Br. J. Pharmac. Chemother.*, **21**, 306–322.

DAY, M. & VANE, J.R. (1963). An analysis of the direct and indirect actions of drugs on the isolated guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **20**, 150–170.

GADDUM, J.H. (1953). Tryptamine receptors. *J. Physiol.*, **119**, 363–368.

LEWIS, G.P. & REIT, E. (1965). The action of angiotensin and bradykinin on the superior cervical ganglion of the cat. *J. Physiol.*, **179**, 538–553.

ROCHA E SILVA, M., VALLE, J.R. & PICARELLI, Z.P. (1953). A pharmacological analysis of the mode of action of serotonin (5-hydroxytryptamine) upon the guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **8**, 378–388.

SAKAI, K., AKIMA, M. & SHIRAKI, Y. (1979). Comparative studies with 5-hydroxytryptamine and its derivatives in isolated, blood-perfused small intestine and ileum strip of the rat. *Jap. J. Pharmac.*, **29**, 223–233.

SAKAI, K., SHIRAKI, Y., TATSUMI, T. & TSUJI, K. (1979). The actions of 5-hydroxytryptamine and histamine on the isolated ileum of the tree shrew (*Tupaia glis*). *Br. J. Pharmac.*, **66**, 405–408.

TRENDELENBURG, U. (1954). The action of histamine and pilocarpine on the superior cervical ganglion and the adrenal glands of the cat. *Br. J. Pharmac. Chemother.*, **9**, 481–487.

TRENDELENBURG, U. (1956). The action of 5-hydroxytryptamine on the nictitating membrane and on the superior cervical ganglion of the cat. *Br. J. Pharmac. Chemother.*, **11**, 74–80.

ULRICH, K. (1965). A direct and an indirect action of 5-hydroxytryptamine on the distal part of the isolated colon of the rat. *J. Pharm. Pharmac.*, **17**, 710–720.

(*Received February 5, 1979.*
Revised March 1, 1979.)