Br. J. Pharmac. (1978), **62**, 333–337

H_1- AND H_2-RECEPTORS IN THE SMOOTH MUSCLE OF THE RUMINANT STOMACH

A. OHGA & T. TANEIKE*

Department of Pharmacology, Faculty of Veterinary Medicine,
Hokkaido University, Sapporo 060, & *Department of Veterinary Pharmacology, The College of Dairying,
Rakuno Gakuen, Ebetsu 069-01, Japan

1 The effects of histamine on the longitudinal and circular smooth muscle from the rumen and reticulum of the bovine stomach have been analyzed by the use of H_1- and H_2-receptor antagonists.

2 Histamine caused three different types of response of the smooth muscle preparations: a contraction, contraction followed by relaxation, and a relaxation. These responses were resistant to the effect of tetrodotoxin, atropine, combined treatment with α- and β- adrenoceptor blocking agents, hexamethonium and also to guanethidine.

3 Mepyramine antagonized the contractile responses and metiamide antagonized the relaxation responses in a highly selective and competitive manner.

4 It is concluded that histamine-induced excitatory and inhibitory responses of the smooth muscles from the bovine forestomach are mediated by histamine H_1- and H_2-receptors, respectively.

Introduction

There is general agreement that histamine induces contraction by both direct and indirect actions on the smooth muscles of the simple stomach (Daniel, 1968). In sharp contrast, in the ruminants, it has been reported that intravenous administration of histamine causes paralysis of rumino-reticular motility and inhibition of eructation (Dougherty, 1942a; Clark, 1950; Duncan, 1954). Furthermore, histamine produces a relaxation of the isolated smooth muscle from the rumen and abomasum but does not cause any apparent contraction (Duncan, 1954; Sanford, 1961). There is also some evidence (Dougherty, 1942b; Dain, Neal & Dougherty, 1955) to support the idea that histamine is one of the causative substances of bloat and other intestinal disturbances of the ruminant. However, the mechanism of action and role of histamine on the ruminant stomach has not so far been studied systematically.

In the present experiments, we found that histamine caused contraction and relaxation of various smooth muscles isolated from the rumen and reticulum of the bovine stomach. These responses have been analyzed in terms of the receptors involved.

Methods

A part of the stomach wall (5 × 8 cm) was dissected from the dorsal sac of the rumen and the fundus of the reticulum of adult bovines (Holstein-Friesian, Jersey and Japanese Black) of either sex, immediately after slaughtering at a local abattoir. Pieces of the stomach wall were then immersed in cold saline (10.9% w/v NaCl solution) and transported to the laboratory with a delay of approximately 60 minutes. The serosal and mucosal layers were detached, and longitudinal (LM) or circular (CM) smooth muscle cut to give a strip about 2 cm in length and 0.2 cm in width. This strip was then suspended in a 5 ml bath containing Krebs solution of the following composition (mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 11.5 (pH 7.3–7.4), bubbled with 5% CO_2 in O_2, and maintained at 37°C. The preparation was allowed to equilibrate for 60 min, under a resting tension of 2 grams. Movements of the muscles were recorded isometrically with a mechano-electronic transducer on a pen recorder as described previously (Ohga & Taneike, 1977).

Drugs were injected into the bath in a volume of 0.05 ml and left in contact for 60 s with 20 min intervals between doses. Antagonists were added 3 to 4 min before the histamine. Final bath drug concentrations are expressed as molar concentration (M). The pA_2 values for mepyramine (in the presence of metiamide) and metiamide (in the presence of mepyramine) against histamine on the LM of the rumen were estimated by the method of Schild (1947). The following drugs were used: acetylcholine chloride (ACh, Tokyo Kasei), adrenaline bitartrate (Tokyo Kasei), atropine sulphate (Wako), guanethidine monosulphate (Regis Chemical), hexamethonium chloride (Tokyo Kasei), histamine dihydrochloride (Wako), mepyramine maleate (May & Baker), metiamide (Smith, Kline & French), phenoxybenzamine hydrochloride (Tokyo Kasei), 5-(3-tert-butylamino-2-hydroxy)propoxy-3,4-dihydrocarbostyril hydrochloride (OPC-1085, Otsuka), and tetrodotoxin citrate (Sankyo).

Results

Not all smooth muscle strips showed spontaneous movement even after they had been set up for 60 minutes. Sensitivity of the muscles to added drugs was also subject to wide variations. It was observed that spontaneously active preparations usually gave a good response to ACh. Therefore, only those preparations showing some spontaneous activity or sensitive enough to respond to ACh 0.55 to 2.75 μM with tension increases of about 5 to 10 g were used in the experiments.

Under the conditions of the present experiments, histamine (0.54 to 540 μM) caused three different responses in the LM of the rumen: contraction alone (5 out of 17 preparations, type I), relaxation followed by contraction (8/17 preparations, type II), and pure relaxation (4/17 preparations, type III) (Figure 1). In a few preparations, the biphasic response (type II) was preceded by a quite small brief contraction. In the LM of the reticulum only type I (3/6 preparations) and type II (3/6) responses were obtained. The contractile response of the reticular smooth muscle induced by histamine was much more rapid in the rising and falling phases than those of the ruminal one. In the CM of the rumen ($n = 5$), histamine produced only contractions within the same close range (Figure 2a). All the responses produced by histamine in these preparations increased in a dose-dependent manner. Usually the inhibitory responses were much more prominent in preparations with a high intrinsic tone and spontaneous activity. However, there was no correlation between the types of response and the dose used.

The responses induced by histamine were not affected by tetrodotoxin (1.6 μM, $n = 8$). Neither atropine (0.72 μM, $n = 8$) nor combined treatment ($n = 4$) with phenoxybenzamine (1.5 μM) and OPC-1085 (β-adrenoceptor blocking agent, 3 μM) in doses that blocked the responses to ACh (0.55 to 2.8 μM) or adrenaline (1.5 to 3 μM), inhibited the histamine responses. Hexamethonium (370 μM, $n = 4$) and guanethidine (17 μM, $n = 3$) were also ineffective. Therefore a neural component does not seem to contribute to the histamine response.

The H_1-receptor antagonist, mepyramine (0.25 to 2.5 μM, $n = 27$), completely and reversibly blocked the contractions or the contractile components of biphasic or triphasic responses in all the preparations, and converted the responses into relaxations alone (Figure 2b). Relaxations or the inhibitory components of multiphasic responses were potentiated by mepyramine but in the presence of the H_1-receptor antagonist they were greatly reduced or abolished, in a dose-dependent manner, by addition of the H_2-receptor antagonist, metiamide (4.1 to 82 μM, $n = 16$; Figure 2c). If the H_2-antagonist, metiamide was added first the relaxations or inhibitory components were almost completely blocked, and the contractile responses augmented. In this case further addition of mepyramine invariably abolished these potentiated excitatory responses. The excitatory or inhibitory responses produced by ACh or adrenaline were little affected, even after combined treatment with both antihistamines.

Dose-response curves for the contractile (Figure 3) and relaxant (Figure 4) effects were obtained in the presence of either metiamide (41 μM) or mepyramine (1.3 μM) on the LM of the rumen. Both curves were shifted to the right in parallel by a further addition of mepyramine or metiamide, respectively. In separate experiments, pA_2 values for the antihistamines against histamine on the preparation were calculated. The mean pA_2 values for mepyramine (in the presence of metiamide 41 μM) were 8.31 (7.69 to 8.74, $n = 7$) and for metiamide (in the presence of mepyramine 0.25 μM) 5.47 (5.12 to 5.69, $n = 5$).

Discussion

The present experiments indicate that histamine has direct excitatory and inhibitory effects on the bovine ruminal and reticular smooth muscle. The contractions and relaxations were selectively and competitively blocked by the H_1 and H_2-receptor antagonists, mepyramine (Ash & Schild, 1966) and metiamide (Black, Duncan, Emmett, Ganellin, Hesselbo, Parsons & Wyllie, 1973). The pA_2 value for mepyramine (8.31) against histamine on the LM of the rumen was close to that reported in guinea-pig ileum ($pA_2 = 8.71$, Schild, 1947). However, the value for metiamide (5.47) was slightly smaller than those reported in other tissues. Black *et al.* (1973) have reported the K_d values (dissociation constant) for metiamide on guinea-pig atria and rat uterus. These correspond to pA_2 of 6.03 (5.93–6.13) and 6.12 (5.86–6.39), respectively. The difference in the pA_2 values between ours and others may result from different experimental conditions. We determined the pA_2 value by the method of Schild (2 min contact; Schild, 1947). Furthermore, the values may be complicated by the fact that we could determine the pA_2 value for mepyramine only when the H_2-receptor was already blocked by metiamide and the pA_2 value for

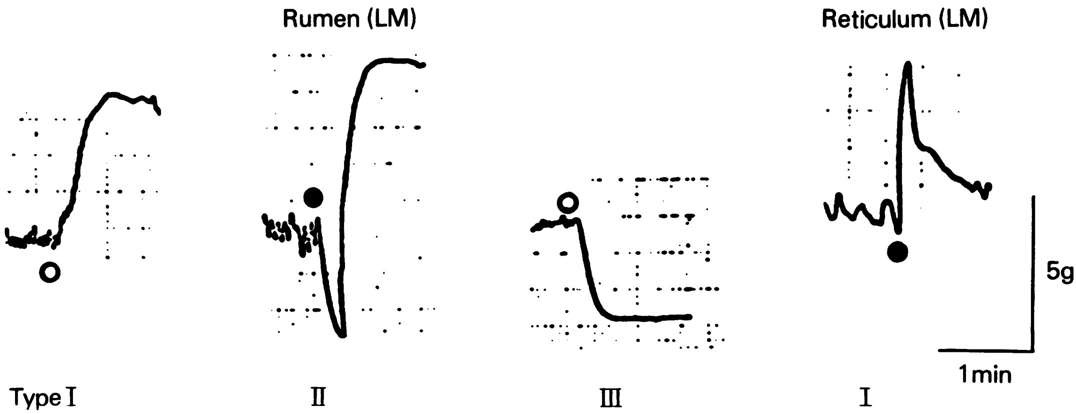

Figure 1 Effect of histamine on the longitudinal smooth muscle isolated from the rumen and reticulum of the bovine stomach. The three different types of response produced by histamine, contraction alone (type I), relaxation followed by contraction (type II) and pure relaxation (type III) are shown. Symbols indicate addition of histamine in a concentration of 2.7 μM (O) or 27 μM (●). The horizontal bar shows the scale for 1 min and the vertical bar the scale for 5 grams.

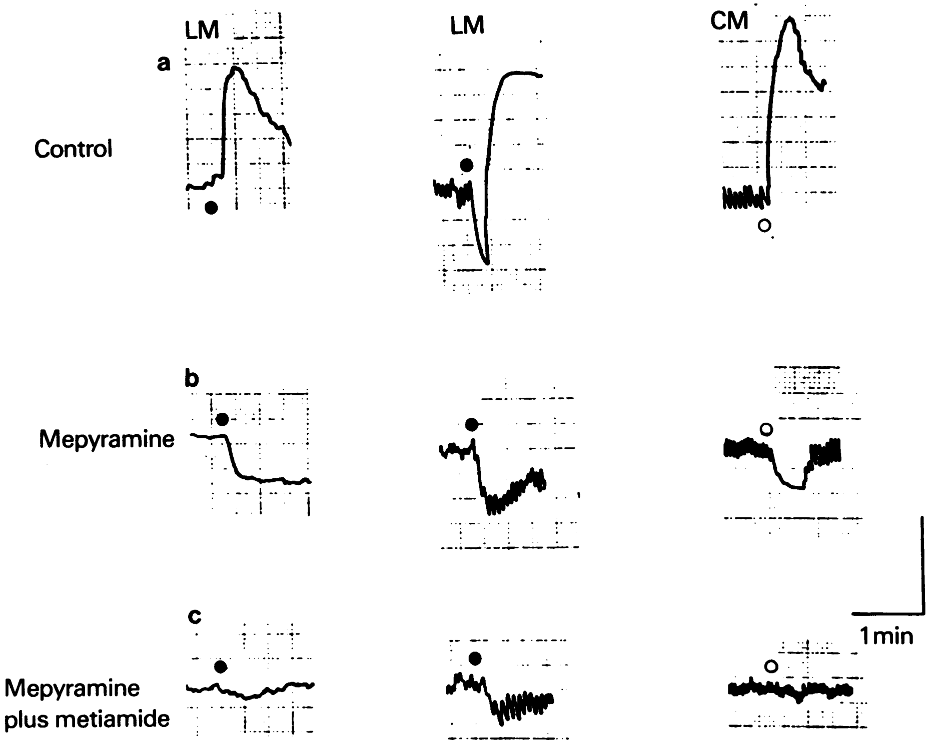

Figure 2 Effects of mepyramine and metiamide on the histamine-induced responses of the longitudinal (LM) and circular (CM) smooth muscle isolated from the rumen of the bovine stomach. (a) Control responses induced by histamine in dose of 11 μM (O) and 27 μM (●); (b) the responses in the presence of mepyramine 0.25 μM; (c) the responses in the presence of mepyramine 0.25 μM plus metiamide 41 μM. The horizontal bar shows the scale for 1 min and the vertical bar the scale for 5 grams.

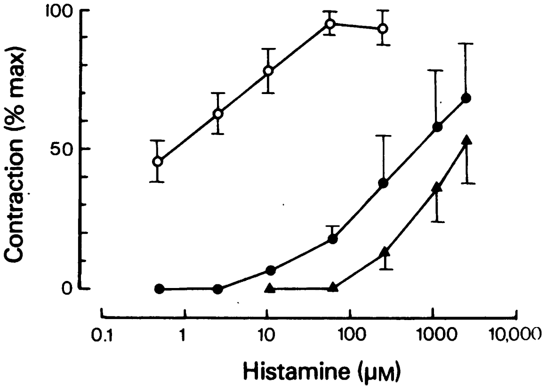

Figure 3 Effects of mepyramine on the histamine-induced contractions in the presence of metiamide of the longitudinal smooth muscle of the rumen: (O) histamine plus metiamide 41 μM ($n = 6$); (●) histamine plus metiamide 41 μM plus mepyramine 0.25 μM ($n = 4$); (▲) histamine plus metiamide 41 μM plus mepyramine 1.3 μM ($n = 5$). Ordinate scale: amplitude of contractions expressed as a percentage of the maximum response. Each point is the mean value and vertical bars show the s.e. mean. Abscissa scale: molar (M) dose of histamine on a logarithmic scale.

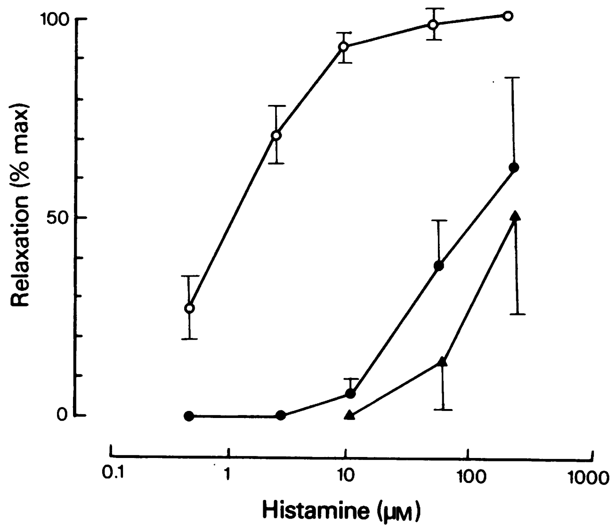

Figure 4 Effects of metiamide on the histamine-induced relaxations in the presence of mepyramine on the longitudinal smooth muscle of the rumen: (O) histamine plus mepyramine 1.3 μM ($n = 6$); (●) histamine plus mepyramine 1.3 μM plus metiamide 21 μM ($n = 4$); (▲) histamine plus mepyramine 1.3 μM plus metiamide 41 μM ($n = 4$). Ordinate scale: amplitude of relaxations expressed as a percentage of the maximum response. Each point is the mean value and vertical bars show the s.e. mean. Abscissa scale: molar (M) dose of histamine on a logarithmic scale.

metiamide only in the presence of the H_1-receptor blocker, mepyramine. From the results presented here, it is most likely that the histamine-induced excitatory and inhibitory effects are mediated by two distinct histamine receptors (H_1 for contraction and H_2 for relaxation) on the smooth muscles.

No appreciable contractile responses to histamine have been reported previously for the isolated smooth muscle of the sheep stomach (Duncan, 1954; Sanford, 1961). The difference in the histamine-induced response between our results and those of the workers might be explained by differences in the preparation used (LM, CM or stomach wall), or a different distribution of H_1- and H_2-receptors on the smooth muscles.

It has been reported that mepyramine partially inhibited the relaxation of ruminal and abomasal strips produced by histamine (Sanford, 1961) and that other H_1-antagonists reversed the ruminal paralysis caused by histamine (Clark, 1950). However, in the former author's experiments, relatively higher concentrations of mepyramine (2.5 to 10 μM) were used and the results may be explained by non-specific effects of the antagonist. In the latter author's work, antagonism was only seen 6 to 10 min after administration of the antihistamines and this could follow reversal of the histamine-induced hypotension by the H_1-receptor blockers.

If histamine is involved in bloat and other digestive disturbances of the ruminant, H_2-antagonists may be of therapeutic value.

The authors wish to thank Dr Y. Nakazato for his useful suggestions and comments concerning this manuscript. We are also grateful to May & Baker Ltd. for supplying mepyramine maleate.

References

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

BLACK, J.W., DUNCAN, W.A.M., EMMETT, J.C., GANELLIN, C.R., HESSELBO, T., PARSONS, M.E. & WYLLIE, J.H. (1973). Metiamide—an orally active histamine H_2-receptor antagonist. *Agents and Actions*, **3**, 133–137.

CLARK, R. (1950). The paralytic action of histamine on the ruminal musculature. *J. S. Afr. Vet. med. Ass.*, **31**, 13–17.

DAIN, J.A., NEAL, A.L. & DOUGHERTY, R.W. (1955). The occurrence of histamine and tyramine in rumen ingesta of experimentally over-fed sheep. *J. Anim. Sci.*, **14**, 930–935.

DANIEL, E.E. (1968). Pharmacology of the gastrointestinal tract. In *Handbook of Physiology*, section 6; Alimentary Canal, vol., IV. ed. Code, C. F. pp. 2267–2324. Washington, D.C.: American Physiological Society.

DOUGHERTY, R.W. (1942a). A study of drugs affecting the motility of the bovine rumen. *Cornell Vet.*, **32**, 269–280.

DOUGHERTY, R.W. (1942b). Analyses of gas and ingesta of bloated cattle. *Am. J. Vet. Res.*, **3**, 401–402.

DUNCAN, D.L. (1954). Responses of the gastric musculature of the sheep to some humoral agents and related substances. *J. Physiol.*, **125**, 475–487.

OHGA, A. & TANEIKE, T. (1977). Dissimilarity between the responses to adenosine triphosphate or its related compounds and non-adrenergic inhibitory nerve stimulation in the longitudinal smooth muscle of pig stomach. *Br. J. Pharmac.*, **60**, 221–231.

SANFORD, J. (1961). Some responses of isolated muscle from the sheep stomach. *Q. Jl exp. Physiol.*, **46**, 167–174.

SCHILD, H.O. (1947). pA, a new scale for the measurement of drug antagonism. *Br. J. Pharmac. Chemother*, **2**, 189–206.

(*Received May 4, 1977.*
Revised August 8, 1977.)