Br. J. Pharmac. (1975), 54, 507-509

DISPLACEMENT BY METIAMIDE OF THE DOSE-RESPONSE CURVES TO PENTAGASTRIN AND METHACHOLINE IN THE CONSCIOUS RAT

L. LUNDELL
Institute of Physiology, University of Lund, Sweden

1 The effect of a specific histamine H_2-receptor antagonist, metiamide, on the acid dose-response curves for pentagastrin or methacholine was studied in rats provided with Heidenhain pouches.

2 Metiamide induced a shift to the right of the dose-response curve to pentagastrin and lowered the maximal secretion.

3 In contrast, metiamide only increased the ED_{50} for methacholine but did not alter the calculated maximal response.

4 The effect of metiamide on pentagastrin and methacholine-induced secretion is in agreement with the previously suggested difference in the part played by mucosal histamine in the mode of action of the two stimulatory agents.

Introduction

The recently discovered histamine H_2-receptor antagonists (Black, Duncan, Durant, Ganellin & Parsons, 1972; Black, Duncan, Emmett, Ganellin, Hesselbo, Parsons & Wyllie, 1973) have become important in attempts to determine the part played by gastric mucosal histamine in the process of exciting the parietal cell. From experiments mainly in the rat it has been proposed that gastrin evokes acid secretion by means of mobilizing mucosal histamine (see Kahlson, Rosengren & Svensson, 1973). The stable choline esters are considered to activate the parietal cells directly (Rosengren & Svensson, 1969); yet their secretory effectiveness appears dependent on small amounts of histamine continuously reaching the parietal cells (Johansson, Lundell, Rosengren & Svensson, 1972; Lundell, (1975a). In the present study an attempt was made to characterize the inhibition produced by a histamine H_2-receptor antagonist on pentagastrin or methacholine-induced acid secretion in order to obtain further information on the involvement of mucosal histamine in the mode of action of these stimuli.

Methods

Female rats of the Sprague-Dawley strain, weighing about 250 g, were used. They were fed a standard pellet diet and tap water and when provided with pouches had the choice of Tyrode solution or tap water. Heidenhain pouches were prepared in the main according to Alphin & Lin (1959) in fasted rats, anaesthetized with ether. A postoperative period of two months was allowed. Before each experiment, the rats were fasted for 18 h and they were then kept restrained in Bollman cages. Gastric juice was collected in 30 min samples by a perfusion technique (Svensson, 1970) and analysed for HCl by titration against 0.1 N NaOH with phenol red as an indicator. The secretory stimulants and metiamide (N-methyl-N'[2[(5-methyl imidazol-4yl)methyl thio] ethyl] thiourea) were infused via a polyethylene tube inserted in a tail vein, the tube being connected to a motor-driven syringe. In establishing dose-response curves for pentagastrin (Peptavlon ICI 50.123) and methacholine (methacholine chloride, Fluka AG) each dose was infused for 90 min in stepwise increasing doses. The acid output was calculated from the mean of the last two 30 min periods at each dose level. The infusion of metiamide started 1 h before the lowest dose of the stimulatory compound was added. The experiments with pentagastrin or methacholine alone or combined with metiamide were performed in a randomized order.

Results

The effect of metiamide on the dose-response curves for pentagastrin and methacholine was investigated in six rats provided with Heidenhain

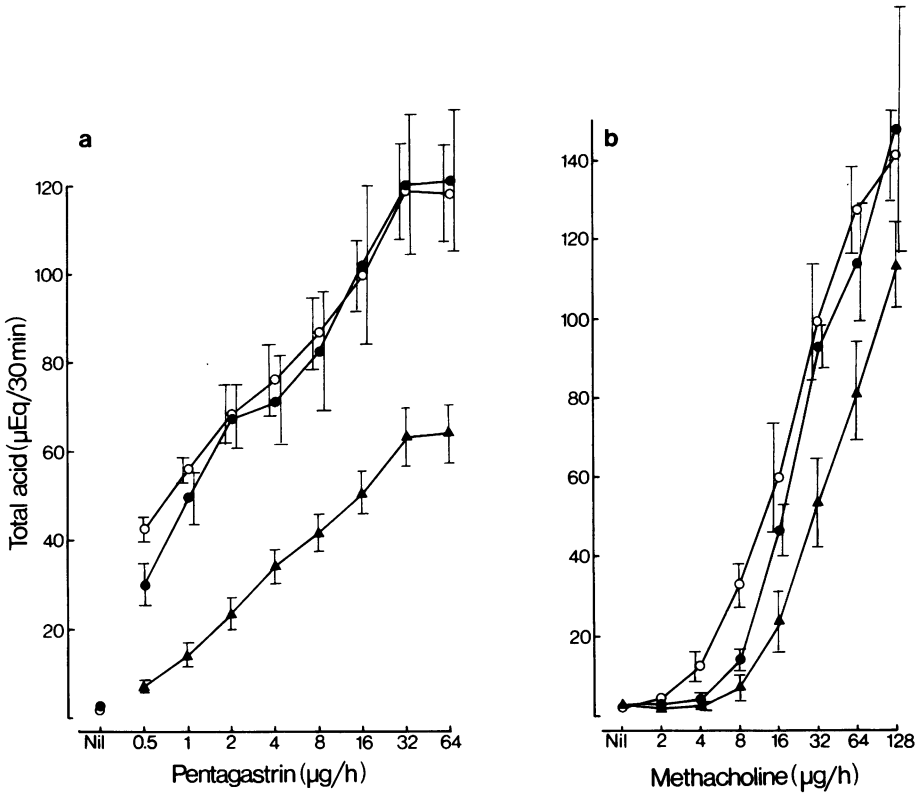

Figure 1 Acid secretion (μEq/30 min) in response to stepwise increasing doses of (a) pentagastrin or (b) methacholine alone (○) or one of the two compounds in combination with 50 μg/h (●) or 500 μg/h of metiamide (▲). Nil indicates interdigestive secretion. The mean and the s.e. mean are calculated from one determination in each of six Heidenhain pouch rats.

pouches. Interdigestive secretion was 2.4 ± 0.32 (s.e. mean) μEq/30 minutes. Infusion of stepwise increasing doses of pentagastrin induced graded secretory responses with an observed maximum of 119.3 ± 10.79 μEq/30 min on infusing 32 μg/h (Figure 1a). By application of Michaelis-Menten equations on the observed responses analogous to the equations used for enzyme reactions (Dowd & Riggs, 1965) the theoretical maximal responses were obtained as well as the ED_{50} values. The calculated maximal response for pentagastrin alone was 118.7 ± 11.38 μEq/30 min which did not significantly differ from the observed response. Metiamide in a dose of 500 μg/h reduced the observed and calculated maximal response to 64.1 ± 6.50 and 66.4 ± 7.40 μEq/30 min respectively.

The effect of metiamide on methacholine-induced acid secretion differed from that on pentagastrin (Figure 1b). Maximal responses could not be obtained in the present study since infusion of higher doses of methacholine than 128 μg/h was followed by adverse side effects. The ED_{50} value and calculated maximal response for methacholine alone were 24.1 ± 9.99 μg/h and 175.8 ± 24.53 μEq/30 min respectively. Neither 50 nor 500 μg/h of metiamide altered the calculated maximal response for methacholine but 50 μg/h metiamide inhibited ($P < 0.05$) the secretory response to the lower doses (2-8 μg/h) of methacholine and 500 μg/h increased the ED_{50} for methacholine from 24.1 ± 9.99 to 90.8 ± 23.3 μg/hour.

Discussion

Metiamide has been characterized as a competitive inhibitor of histamine-induced acid secretion in the rat and dog (Parsons, 1973). In the rat, infusion of increasing doses of pentagastrin mobilizes increasing amounts of histamine from the gastric mucosa until a maximum is reached. The dose of pentagastrin which evokes maximal acid secretion corresponds to that which mobilizes mucosal histamine at a maximal rate (Lundell, 1974; Lundell, 1975b). In the present study,

metiamide in a dose of 500 μg/h shifted the dose-response curve for pentagastrin to the right with a reduction of the maximal response. These results are compatible with the hypothesis that pentagastrin is not a direct stimulant of the parietal cell but depends on an intermediary mechanism, namely, the mobilization of histamine.

Metiamide also inhibited the secretion evoked by methacholine but the inhibition differed from that seen with pentagastrin. No difference was noted between the calculated maximal responses obtained with methacholine alone and in combination with the two doses of metiamide, but a progressive increase in the ED_{50} for methacholine was recorded. These results should not be taken as evidence that methacholine activates the H_2-receptor on the parietal cell but rather to show that metiamide blocks the action of histamine mobilized during the interdigestive state, thus interrupting the facilitating relationship that already exists between small amounts of histamine and methacholine (Johansson *et al.*, 1972). The failure of metiamide to reduce the calculated maximal response to methacholine may be accounted for by an increased release of antral gastrin and a subsequent mobilization of mucosal histamine. It should be mentioned that enhanced plasma gastrin in response to a stable choline ester has been noted in the dog only with huge doses of the agent (Sjödin & Nilsson, 1973).

Metiamide was a generous gift from Professor J.W. Black, Smith, Kline and French Laboratories Ltd., England. The investigation was supported by grants from the Swedish Medical Research Council and the Medical Faculty of the University of Lund, Sweden.

References

ALPHIN, R.S. & LIN, T.M. (1959). Preparation of chronic denervated gastric pouches in the rat. *Am. J. Physiol.*, **197**, 257-259.

BLACK, J.W., DUNCAN, W.A.M., DURANT, C.J., GANELLIN, C.R. & PARSONS, E.M. (1972). Definition and antagonism of histamine H_2-receptors. *Nature, Lond.*, **236**, 385-390.

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.

DOWD, J.E. & RIGGS, D.S. (1965). A comparison of estimates of Michaelis-Menten kinetic constants from various linear transformations. *J. bio. Chem.*, **240**, 863-869.

JOHANSSON, I., LUNDELL, L., ROSENGREN, E. & SVENSSON, S.E. (1972). Gastric secretion and its facilitation as related to gastric mucosal histamine. *J. Physiol.*, **226**, 431-452.

KAHLSON, G., ROSENGREN, E. & SVENSSON, S.E. (1973). Histamine and gastric secretion with special reference to the rat. In *The Pharmacology of Gastrointestinal Motility and Secretion.* ed. Holton, P., section 39a (International Encyclopedia of Pharmacology and Therapeutics). Oxford: Pergamon Press.

LUNDELL, L. (1974). Histamine metabolism of the gastric mucosa following antrectomy. *J. Physiol.*, **241**, 437-451.

LUNDELL, L. (1975a). Elucidation by a H_2-receptor antagonist of the significance of mucosal histamine mobilization in exciting acid secretion. *J. Physiol.* (in press).

LUNDELL, L. (1975b). Alterations in secretory patterns following antrectomy in rats with Pavlov pouches. *J. Physiol.*, **234**, 703-721.

PARSONS, M.E. (1973). The evidence that inhibition of histamine-stimulated gastric secretion is a result of the blockade of histamine H_2-receptors. In *International Symposium on Histamine H_2-Receptor Antagonists*, ed. Wood, C.J. & Simkins, M. Alison, pp. 207-216. London: Smith, Kline & French.

ROSENGREN, E. & SVENSSON, S.E. (1969). The role of the antrum and the vagus nerve in the formation of gastric mucosal histamine. *J. Physiol.*, **205**, 275-288.

SJODIN, L. & NILSSON, G. (1973). Gastric acid secretion and plasma gastrin levels during urecholine infusion in the dog. *Acta physiol. scand.* suppl., **396**, 63.

SVENSSON, S.E. (1970). The secretory patterns of three stomach preparations in the rat. *J. Physiol.*, **207**, 329-350.

(Received December 16, 1974. Revised April 12, 1975.)