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STIMULATION OF RAT GASTRIC ADENYLATE CYCLASE BY HISTAMINE AND HISTAMINE ANALOGUES AND BLOCKADE BY BURIMAMIDE

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Histamine, 4-methylhistamine, 3-(β-aminoethyl)-1,2,4 triazole and betazole, in that order, stimulated adenylate cyclase prepared from rat gastric tissue in a dose-dependent manner. Burimamide, an H_2-receptor blocking agent, in concentrations of $1\text{-}5 \times 10^{-6}$ M antagonized this effect. The data lend some support to the hypothesis that elevated levels of cyclic adenosine 3',5'-monophosphate may be involved in histamine-stimulated gastric secretions and that H_2-receptors are associated with adenylate cyclase.

The responses of many hormones and drugs may be related to their ability to activate adenylate cyclase in the target cell resulting in an intracellular increase in cyclic 3',5'-adenosine monophosphate (cyclic AMP) (Sutherland, Robison & Butcher, 1968). Histamine has been shown to activate adenylate cyclase and increase cyclic AMP in the guinea-pig heart (McNeill & Muschek, 1972; McNeill & Verma, 1974a). The increase in cyclic AMP has been shown to precede the positive inotropic effect of histamine and the events may be related. All effects of histamine in the heart are blocked in a competitive manner by burimamide, an H_2-receptor blocking agent (Black, Duncan, Durant, Ganellin & Parsons, 1972; McNeill & Verma, 1974a) but are poorly blocked by classical H_1-receptor antagonists such as tripelennamine, diphenhydramine or promethazine (McNeill & Muschek, 1972; McNeill & Verma, 1974b). Many of these studies suggested a relationship between adenylate cyclase and the H_2-receptor and McNeill & Muschek (1972) suggested that the cardiac histamine receptor could, in fact, be an active site on the adenylate cyclase molecule. H_2-receptors may also be found in the rat uterus and the gastric mucosa of several species (Black *et al.*, 1972). There have been several studies linking an increase in cyclic AMP with drug-induced acid secretion in the stomach (e.g. Alonso & Harris, 1965; Levine & Wilson, 1971 and many others) although the suggested link does not occur with all drugs, particularly papaverine (Mao, Jacobson & Shanbour, 1973). Burimamide will antagonize both the histamine-induced increase in gastric acid secretion and the increase in gastric cyclic AMP (Wyllie, Hesselbo & Black, 1972; Karppanen & Westermann, 1973) again indicating that H_2-receptors are involved in both processes.

The purpose of the present study was to investigate the effects of histamine and certain histamine analogues [betazole, 4-methylhistamine and 3-(β-aminoethyl)-1,2,4 triazole] on rat gastric adenylate cyclase and to ascertain the interaction of these drugs with burimamide.

Methods Stomach tissue homogenate for adenylate cyclase estimation was prepared by the method of Drummond & Duncan (1970) with minor modifications, from rats weighing 250-300 grams. A washed particulate preparation was prepared and the particles were suspended in 2 volumes of Tris buffer based on the original weight of the tissue. The assay system a total volume of 150 μl, consists of tris-(hydroxymethyl)amino-methane HCl 0.3 M, theophylline 0.05 M, $MgSO_4$ 0.225 M, KCl 0.083 M, phosphoenol pyruvate 0.3 M, pyruvate kinase (1 : 5 dilution) and adenosine 5'-triphosphate 5 mM. After addition of all components except enzyme the assay tubes were preincubated for 4 min and the reaction was started by adding 50 μl of the enzyme preparation. Incubation was carried out for 10 min at 37° C. The reaction was terminated by placing the tubes in boiling water. Denatured protein were removed by centrifugation at 10,000 x *g* for 5 minutes. A 50 μl portion of the clear supernatant was diluted eleven fold with 50 mM sodium acetate buffer and a 50 μl aliquot of the diluted sample was used for the determination of cyclic AMP by the method of Gilman (1970). Enzyme activity is expressed as pmol cyclic AMP formed per mg protein and per minute. Protein was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951).

Drugs used were histamine dihydrochloride (Sigma), burimamide and 4-methyl-histamine (Smith, Kline and French Lab., England), 3-(β-amino-ethyl)-1,2,4 triazole and betazole hydrochloride (Eli Lilly Co.).

Table 1 The effect of histamine and histamine analogues on rat gastric adenylate cyclase activity

Drug treatment	Dose of agonist (M)			Dose of agonist (plus burimamide)		
	10^{-5}	10^{-4}	10^{-3}	10^{-5}	10^{-4}	10^{-3}
Histamine	36.9 ± 1.3	44.6 ± 1.7	54.9 ± 2.5	23.2 ± 0.4	26.1 ± 1.3	40.0 ± 1.2
4-Methylhistamine	25.2 ± 0.9	38.1 ± 1.1	40.2 ± 2.8	14.5 ± 1.0	20.5 ± 0.6	26.1 ± 0.7
3-(β-Aminoethyl)-1,2,4 triazole	28.3 ± 0.9	34.6 ± 2.6	39.6 ± 2.2	15.5 ± 1.0	21.4 ± 1.0	26.8 ± 0.9
Betazole	20.3 ± 0.9	26.6 ± 0.8	35.0 ± 0.6	11.0 ± 0.6	16.5 ± 1.0	21.0 ± 0.8

Enzyme activity is expressed as pmol cyclic AMP formed per mg protein per min (mean with s.e of 3 determinations). Activity in the absence of any drug was 11.6 ± 0.3 pmol mg protein^{-1} min^{-1} and was not affected by burimamide.
The concentration of burimamide for histamine experiments was 5×10^{-6} M and for all other experiments was 1×10^{-6} M.
All agonists produced a significant ($P < 0.05$) increase in gastric adenylate cyclase activity and burimamide significantly ($P < 0.05$) antagonized all drug-induced increases.

Results Histamine and its analogues stimulated rat gastric adenylate cyclase in a dose-dependent manner (Table 1). The order of potency was histamine > 4-methylhistamine = 3-(β-aminoethyl)-1,2,4 triazole > betazole. Burimamide antagonized the agonist response. Histamine (10^{-2} M) was able to overcome completely the blocking effect of burimamide 5×10^{-6} M since enzyme activity increased to 52.5 ± 1.6 pmol mg protein^{-1} min^{-1} in the presence of both agents. The maximum increase in enzyme activity was approximately three-fold in agreement with the work of Perrier & Laster (1970).

Discussion The relative order of potency of histamine and its analogues on rat gastric adenylate cyclase is similar to that found in investigations studying the actions of these drugs on cardiac adenylate cyclase (McNeill & Muschek, 1972; Verma & McNeill, 1974), cardiac cyclic AMP formation, phosphorylase activation and cardiac contractility (McNeill & Verma, 1974a). The rank order for the compounds is also similar to that noted when gastric acid secretion is measured. The findings are thus in accord with the hypothesis that histamine increases gastric secretion by elevating intracellular levels of cyclic AMP.

The data provide some evidence that H_2-receptors have similar properties in at least two tissues, gastric mucosa and heart, in stimulation by agonists and in blockade by burimamide. The data also provide further evidence for the association of the H_2-receptor with adenylate cyclase and the possibility that the H_2-receptor may be an active site on the adenylate cyclase molecule.

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References

ALONSO, D. & HARRIS, J.B. (1965). Effects of xanthines and histamine on ion transport and respiration by frog gastric mucosa. *Am. J. Physiol.*, **208**, 18-23.

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, New Biol.*, **236**, 385-390.

DRUMMOND, G.I. & DUNCAN, L. (1970). Adenyl cyclase in cardiac tissue. *J. biol. Chem.*, **245**, 979-983.

GILMAN, A.G. (1970). A protein binding assay for adenosine 3',5' cyclic monophosphate. *Proc. Natl. Acad. Sci.*, **67**, 305-312.

KARPPANEN, H.O. & WESTERMANN, E. (1973). Increased production of cyclic AMP in gastric tissue by stimulation of histamine (H_2) receptors. *Naunyn Schmiedeberg's Arch. Pharmac.*, **279**, 83-87.

LEVINE, R.A. & WILSON, D.E. (1971). The role of cyclic AMP in gastric secretion. *Ann. N.Y. Acad. Sci.*, **185**, 363-375.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with folin phenol reagent. *J. biol. Chem.*, **193**, 365-375.

MAO, C.C., JACOBSON, E.D. & SHANBOUR, L.L. (1973). Mucosal cyclic AMP and secretion in the dog stomach. *Am. J. Physiol.*, **225**, 893-896.

McNEILL, J.H. & MUSCHEK, L.D. (1972). Histamine effects on cardiac contractility, phosphorylase and adenyl cyclase. *J. mol. cell. Cardiol.*, **4**, 611-624.

McNEILL, J.H. & VERMA, S.C. (1974a). Blockade by burimamide of the effects of histamine and histamine

analogs on cardiac contractility, phosphorylase activation and cyclic AMP. *J. Pharmac. exp. Ther.*, **188**, 180-188.

McNEILL, J.H. & VERMA, S.C. (1974b). Blockade of cardiac histamine receptors by promethazine. *Can. J. Physiol. Pharmac.*, **52**, 23-27.

PERRIER, C. & LASTER, L. (1970). Adenyl cyclase activity of guinea pig gastric mucosa: stimulation of histamine and prostaglandins. *J. clin. Invest.*, **49**, 73.

SUTHERLAND, E.W., ROBISON, G.A. & BUTCHER, R.K. (1968). Some aspects of the biological role of cyclic 3′,5′ adenosine monophosphate (cyclic AMP). *Circulation*, **34**, 279-306.

VERMA, S.C. & McNEILL, J.H. (1974). Blockade by burimamide of the effects of histamine and histamine analogs on cardiac adenylate cyclase. *J. Pharm. Pharmac.*, **26**, 372-373.

WYLLIE, T.H., HESSELBO, T. & BLACK, J.W. (1972). Effects in man of histamine H_2 receptor blockade by burimamide. *Lancet*, **ii**, 1117-1120.

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