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Biochemical Pharmacology, Vol. 28, pp. 343–344.

0006-2952/79/0115-0343 $02.00/0

Inhibition of histamine-sensitive adenylate cyclase from the guinea pig gastric mucosa by nolinium bromide

(*Received* 15 *March* 1978; *accepted* 17 *May* 1978)

Recently discovered H_2-receptor antagonists [1, 2] not only have pharmacological properties which are distinct from those of classical antihistaminics (H_1-receptor antagonists) but also have a very characteristic chemical composition [2, 3]. H_2-histamine antagonists are basically structural analogs of histamine; these compounds all contain an imidazole ring with a polar, although uncharged, side chain [2, 3] (Fig. 1). On the other hand, H_1-antagonists are distinguished by the presence of aryl rings and resemble histamine only by the presence of an ethylene group with ammonium (or other similarly charged groups) at the termination of the side chain [2–4] (Fig. 1). The unique structural features of H_2-receptor antagonists are believed to be a prerequisite for their specific pharmacological effects, including their ability to block histamine-stimulated gastric acid secretion [3, 4].

There is considerable evidence indicating that in numerous vertebrate species [4, 5], including man [6], the HCl secreting action of histamine is elicited via mediation of cAMP as a second messenger. For example, in guinea pig, man, dog, rabbit and rat gastric mucosa [4–6] the stimulation of adenylate cyclase and/or the accumulation of cyclic 3′,5′-adenosine monophosphate (cAMP) in this tissue elicited by histamine is specifically blocked by H_2-receptor antagonists, thus supporting the notion that the fundic gastric mucosa adenylate cyclase is associated with H_2-receptors [4, 5].

Nolinium bromide [2-(3,4-dichlorophenylaminoanilino)-quinolizinium bromide] (Fig. 1), a recently synthesized compound with known antispasmodic properties [7, 8], was also found to be an effective inhibitor of gastric HCl secretion [7, 8]. Because of the potential importance of adenylate cyclase in the regulation of gastric HCl secretion, and because of considerable structural diversity between nolinium bromide (Nolinium Br) and classical H_2-receptor antagonists (Fig. 1) we addressed ourselves to the question of whether Nolinium Br may exert its HCl antisecretory effects through inhibition of histamine-stimulated adenylate cyclase. We examined the effects of Nolinium Br on basal adenylate cyclase activity and the activity stimulated by histamine, by prostaglandin PGE_2 and by the non-hormonal stimulatory agents such as 5′-guanyl-iminodiphosphate [Gpp(NH)p] and sodium fluoride (NaF).

Adenylate cyclase activity was measured in the crude membrane fraction for the guinea pig fundic mucosa, as described in detail in our previous communications [9, 10]. The protein content of our enzyme preparations was measured by the method of Lowry *et al.* [11]; the specific activity of adenylate cyclase was expressed in pmoles/min/mg of protein [9, 10]. Stimulation of adenylate cyclase was expressed as per cent increase ($\Delta\% \pm$ S.E.M.) over basal activity of the enzyme.

As in previous experiments, gastric fundic mucosa adenylate cyclase was stimulated markedly by 10^{-4} M histamine ($\Delta\% + 340 \pm 69$; n = 7), by 3×10^{-5} M PGE_2 ($\Delta\% + 149 \pm 44$, n = 4), by 10^{-4} M Gpp(NH)p ($\Delta\% + 444 \pm 53$, n = 4) and by sodium fluoride ($\Delta\% + 741 \pm 86$, n = 7). In the presence of 2×10^{-4} M Nolinium Br the stimulation of adenylate cyclase by 10^{-4} M histamine was markedly reduced to $\Delta\% + 94 \pm 27\%$ ($P < 0.05$, paired *t*-test; n = 7). On the other hand, Nolinium Br in the same concentration had no effect on the basal activity of adenylate cyclase or on the enzyme activity stimulated by PGE_2, Gpp(NH)p or by NaF.

Inhibition of histamine-stimulated adenylate cyclase activity with Nolinium Br was dose-dependent (Fig. 2, upper panel) over a range of concentrations similar to the inhibitory concentrations observed for metiamide [9, 12, 13]. The effects of Nolinium Br were also tested over varying concentrations of histamine. In the presence of Nolinium Br the slope of the dose–response curve of adenylate cyclase stimulation by histamine was displaced to the right, and the maximal stimulation by histamine was reduced (Fig. 2, lower panel).

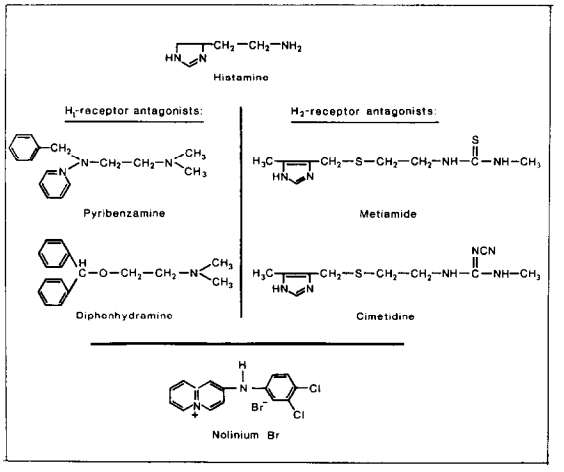

Fig. 1. Comparison of the chemical structures of histamine, typical H_2-antagonists (metiamide and cimetidine), typical H_1-antagonists (pyribenzamine and diphenhydramine) and nolinium bromide.

The results thus show that Nolinium Br acts as a rather specific inhibitor of histamine-stimulated adenylate cyclase from the gastric mucosa. The specificity of this compound as an antagonist of histamine action is demonstrated by the fact that Nolinium Br did not influence the basal activity of adenylate cyclase and, perhaps more importantly, that it did not inhibit stimulation of adenylate cyclase by PGE_2, Gpp(NH)p or NaF. There is considerable evidence that histamine and prostaglandin stimulate two different adenylate cyclases, both present in gastric fundic mucosa [5, 10], and that only histamine-stimulated adenylate cyclase is inhibited by H_2-receptor antagonists [5, 14]; stimulation of fundic mucosal adenylate cyclase by NaF or Gpp(NH)p is not inhibited by H_2-receptor antagonists [13]. The inhibition of histamine-stimulated adenylate cyclase activity by Nolinium Br resembles the actions of other H_2-receptor antagonists. This inhibition also occurs over a similar range of concentration observed for metiamide an H_2-receptor antagonist [9, 12–14]; Nolinium Br appears to be, on a molar basis, a weaker inhibitor than cimetidine to date the most potent H_2-receptor antagonist [13]. The effect of Nolinium Br, however, appears not to be the same as the action of classical H_2-receptor antagonists [4, 5, 12–14], in that the inhibition is not completely competitive, but rather appears to have a noncompetitive component as well (Fig. 2).

The similarity of the effects of Nolinium Br and other H_2-receptor antagonists on the adenylate cyclase in cell-free systems from gastric mucosa suggests that this antispasmodic compound may inhibit gastric secretion through mechanisms similar to those proposed for H_2-receptor antagonists (blockage of histamine-stimulated adenylate cyclase at the H_2-receptor component).

The most interesting aspect of the present findings is the considerable similarity between the actions of Nolinium Br on H_2-receptor-associated adenylate cyclase and the action of typical H_2-antihistaminics [9, 12], and on the other hand, the striking dissimilarity between the chemical structures of these compounds (Fig. 1). Nolinium Br lacks both the imidazole ring as well as the side chain with the uncharged polar group. It should be noted that modifications of the imidazole ring of histamine analogs do not confer histamine antagonistic properties to such compounds [2]. Since Nolinium Br has aryl ring constituents as well as a positively charged ammonium group, it actually appears to be closer in structure to classical H_1-receptor antagonists [1–3], although it does not possess the ethylene backbone which is the equivalent of histamine's side chain in H_1-receptor antagonists [3, 4]. Our findings thus suggest that H_2-receptors in gastric mucosa may be specifically blocked by a compound which lacks the major structural features considered to be necessary for H_2-receptor antagonism [2, 3]. The present findings resemble the results of recent studies performed on central nervous system tissue in which tricyclic antidepressants [15] or derivatives of D-lysergic acid [16], all compounds different in structure from H_2-receptor antagonists, also inhibited H_2-receptor-associated central nervous system adenylate cyclase activity.

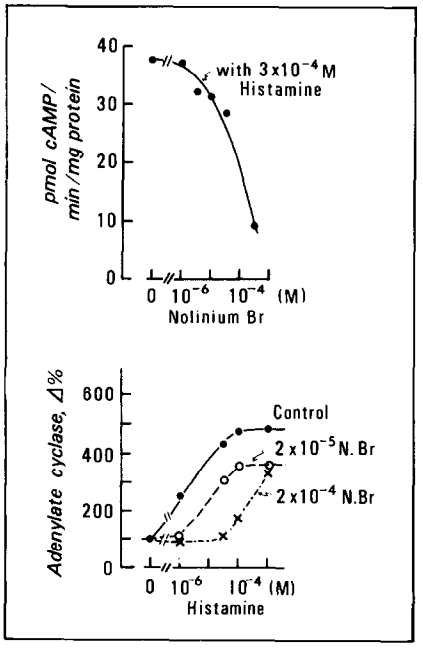

Fig. 2. Upper panel: Inhibition of the adenylate cyclase activity stimulated by 3×10^{-4} M histamine (ordinate) by the increasing levels of Nolinium Br (abscissa). Lower panel: Stimulation (Δ% increase over basal activity) of the adenylate cyclase by increasing concentrations of histamine in the absence (control) or in the presence of Nolinium Br (N. Br).

Acknowledgements—This study was supported by the United States Public Health Service Research Grant AM-16105 from the National Institute of Arthritis, Metabolic and Digestive Diseases and by the Mayo Foundation. Dr. T. P. Dousa is an Established Investigator of the American Heart Association. We thank Mrs. Ardith Walker for her excellent secretarial assistance.

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