Blockade by burimamide of the effects of histamine and histamine analogues on cardiac adenylate cyclase

Histamine has been reported to increase cardiac contractility in isolated hearts obtained from cats, rabbits, guinea-pigs and frogs (Bartlett, 1963). Recently it has been shown that histamine, 3-(β-aminoethyl)-1,2,4-triazole (TD) and betazole increased cardiac cyclic AMP before producing a positive inotropic effect (McNeil & Verma, 1974). Both effects were blocked in a competitive manner by burimamide, an H_2-histamine receptor blocking agent. Histamine is also capable of stimulating guinea-pig cardiac adenylate cyclase (McNeill & Muschek, 1972). Thus a logical sequence of events would appear to be: (1) interaction of histamine with adenylate cyclase, (2) elevation of cyclic AMP and (3) positive inotropic and phosphorylase activating effect. The effect of histamine on adenylate cyclase is not blocked by propranolol and is poorly blocked by classical antihistamines such as tripelennamine and diphenhydramine (McNeill & Muschek, 1972). Therefore it was of interest to determine the interaction between histamine and certain histamine analogues and burimamide on cardiac adenylate cyclase.

Materials. Tris (SigmapH 7–9), theophylline, pyruvate kinase, ATP (disodium) and histamine dihydrochloride (all from Sigma Chem. Co.); 2-phosphoenol pyruvic acid (Calbiochem Co.); $MgSO_4$, analytical (Mallenckrodt); KCl, A.C.S. (Fischer); burimamide and 4-methylhistamine (SKF); 3-(β-aminoethyl) 1,2,4-triazole and betazole HCl (Eli Lilly).

Methods. A washed particulate preparation containing adenylate cyclase was freshly prepared from guinea-pig heart according to Drummond & Duncan (1970). Each assay tube (in a total volume of 150 μl) consisted of tris HCl 0·3 M, theophylline 0·06 M, $MgSO_4$ 0·225 M, KCl 0·083 M, phosphoenol pyruvate 0·3 M, pyruvate kinase (1 : 5 dilution) and ATP 5 mM. After addition of all the components except enzyme the assay tubes were preincubated for 4 min and the reaction was started by adding 50 μl of the enzyme preparation. Incubations were for 10 min at 37°. The reaction was terminated by placing the tubes in a boiling water bath for 3 min. Denatured protein was removed by centrifugation at 10 000 *g* for 5 min. A portion of the clear supernatant (50 μl) was diluted 11-fold with 50 mM sodium acetate buffer and a 50 μl aliquot of the diluted sample was used for the determination of cyclic AMP (Gilman, 1970). Enzyme activity is expressed as pmol cyclic AMP produced mg^{-1} protein min^{-1}. Protein was determined according to Lowry, Rosebrough & others (1951).

The order of potency of the compounds for stimulating cardiac adenylate cyclase was histamine$>$ 4-methyl-histamine$\geq$TD$>$betazole (Fig. 1a). Stimulation by the agonists was blocked, in an apparently competitive manner, by burimamide (Fig. 1b, c). Burimamide (5×10^{-6} and 1×10^{-5} M) produced progressive shifts in the histamine dose-response curve (Fig. 1b). Burimamide (1×10^{-6} M) shifted the dose-response curves to 4-methylhistamine, TD and betazole to the right (Fig. 1b, c).

The data presented indicate that the stimulatory effect of histamine and histamine analogues on guinea-pig cardiac adenylate cyclase is antagonized by burimamide. The antagonism appears to be competitive and is specific in that previous studies had indicated that propranolol, α-adrenoceptor blocking agents (phentolamine and tolazoline) and H_1-receptor blocking agents (tripelennamine and diphenhydramine) do not antagonize the histamine response competitively (McNeill & Muschek, 1972). The interaction between burimamide and the agonists was similar to that noted when the effects of these agents on cardiac cyclic AMP, contractility, and phosphorylase

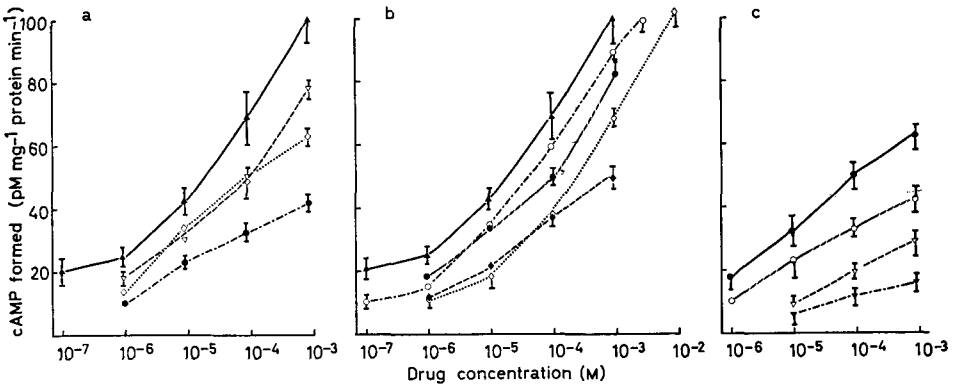

FIG. 1a. The effect of various doses of histamine, 4-methylhistamine, TD and betazole on the activity of guinea-pig cardiac adenylate cyclase. — ▲ Histamine. – – ▽ 4-Methylhistamine. · · · ◇ TD. – · – ● Betazole.

b. The effect of burimamide (5 to 10 × 10^{-6} M) on the stimulation of guinea-pig cardiac adenylate cyclase by various doses of histamine and 4-methylhistamine. — ▲ Histamine. – · – ○ Histamine + burimamide (5 × 10^{-6}M). · · · ◇ Histamine + burimamide (1 × 10^{-5}M). ● 4-Methylhistamine. – – ◆ 4-Methylhistamine + burimamide (1 × 10^{-6}M).

c. The effect of burimamide (1 × 10^{-6} M) on the stimulation of guinea-pig cardiac adenylate cylcase by various doses of betazole and TD. Each point represents the mean ± s.e. of 4 determinations. — ● TD. ○ Betazole. – – ▽ TD + burimamide (1 × 10^{-6} M). – · – ▼ Betazole + burimamide (1 × 10^{-6} M).

were studied (McNeill & Verma, 1974). The data are thus consistent with the hypothesis that histamine and its analogues produce their positive inotropic effect by occupying a histamine receptor, stimulating adenylate cyclase and elevating cyclic AMP (McNeill & Muschek, 1972; McNeill & Verma, 1974).

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