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Inhibition of amino sugar formation by several nonsteroid anti-inflammatory agents

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THERE are many reports on the mechanisms of action of anti-inflammatory agents,[1,2] and some studies are concerned with amino sugar metabolism.[3–7] The over-production of mucopolysaccharides is often observed during the course of inflammation.[8]

The initial step in the biosynthesis of amino sugar moiety in mucopolysaccharides is the formation of glucosamine 6-phosphate. UDP-N-acetyl D-glucosamine, which derives from glucosamine 6-phosphate, is converted to UDP-N-acetyl D-galactosamine. These UDP-acetyl amino sugars are intermediates which participate in the synthesis of mucopolysaccharides. Thus, inhibitory effects on the activity of glucosamine 6-phosphate synthetase (L-glutamine: D-fructose 6-phosphate aminotransferase EC 2.6.1.16) might result in significant changes in mucopolysaccharides metabolism.

The present communication deals with the effect of several nonsteroid anti-inflammatory agents described below on the partially purified glucosamine 6-phosphate synthetase in rat liver.

(1) Sodium salicylate. (2) Antipyrine, 1,5-Dimethyl-2-phenyl-3-pyrazolone. (3) Benzydamine, 1-Benzyl-3-(3-(dimethyl amino) propoxy)-1 H-indazole (Angelini Francesco). (4) Ibufenac, (p-Isobutyl phenyl) acetic acid (Boots Pure Drug). (5) Bucolome, Paramidine, 5-Butyl-1-cyclohexyl barbituric acid (Takeda). (6) PAT, Fenamole, 1-Phenyl-5-aminotetrazole (Bristol).

The preparation of the enzyme from rat liver was already reported by Akamatsu *et al.*[9] $(NH_4)_2SO_4$ fraction of 105,000 *g* supernatant was subjected to gel filtration through Sephadex G-25 prior to use. Activity of the enzyme was assayed as follows. The reaction mixture was composed of 100 μmoles of Tris-HCl buffer (pH 7·5), 20 μmoles of D-fructose 6-phosphate, 20 μmoles of L-glutamine, 1 μmole of dithiothreitol, 1 μmole of EDTA, and the enzyme in a total volume of 1·0 ml. Incubation was carried out at 37°. D-Glucosamine 6-phosphate formed was determined by Elson–Morgan's color reaction.[9,10] The anti-inflammatory agents at the range of the concentration used in this experiment did not show any effects on the color production.

The rate of D-glucosamine 6-phosphate formation was proportional to enzyme concentration in the range employed and was linear for 2 hr both in the absence and in the presence of the inhibitors.

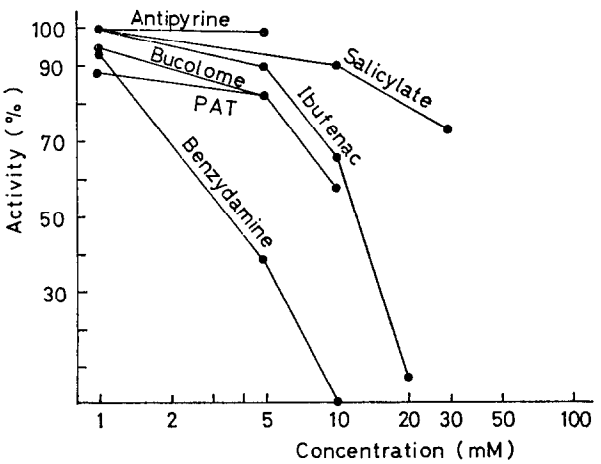

FIG. 1. Effect of several anti-inflammatory agents upon glucosamine 6-P synthetase activity in rat liver. The activity of the enzyme (protein 2·3 mg) during 2 hr incubation was determined in duplicate as described in the text except that various amounts of anti-inflammatory agents were added. The activity was expressed as per cent of control.

As was shown in Fig. 1, the most effective agent was benzydamine and the extent of inhibition was about the same as was reported by Schönhöfer on mefenamic acid, flufenamic acid, phenyl butazone and indomethacin.[5] PAT, bucolome, ibufenac and salicylate were less effective. Antipyrine exerted no inhibitory effect.

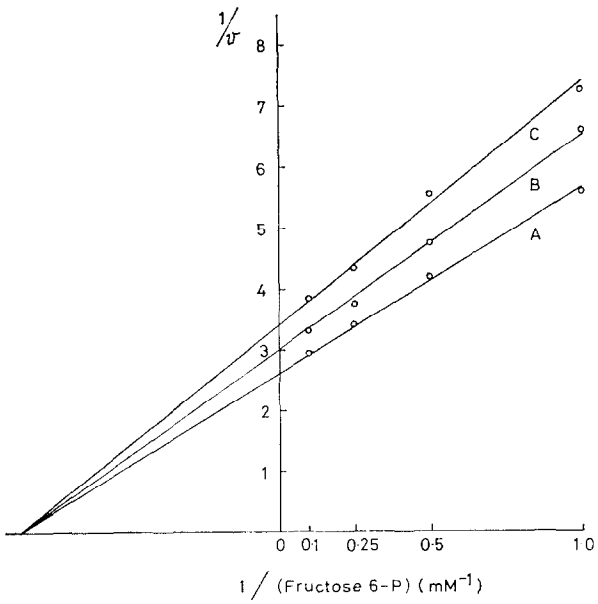

FIG. 2. Effect of fructose 6-P concentration on rat liver glucosamine 6-P synthetase activity in the presence of benzydamine. v is expressed as μmole of glucosamine 6-P formed in 1·5 hr. Reaction mixture contained in 1·0 ml: 100 μmoles Tris-HCl buffer (pH 7·5), 20 μmoles of glutamine, 1 μmole of dithiothreitol, 1 μmole of EDTA, 1·8 mg of enzyme protein, fructose 6-P as shown, and benzydamine: A, none; B, 2 mM; C, 4 mM.

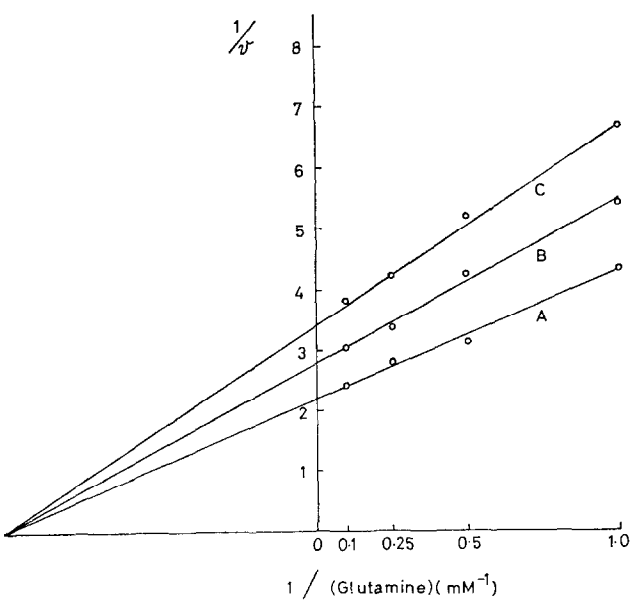

FIG. 3. Effect of benzydamine on the reaction rate as a function of glutamine concentration. v is expressed as μmoles of glucosamine 6-P formed in 1·5 hr. Reaction mixture contained in 1·0 ml: 100 μmoles of Tris-HCl buffer (pH 7·5), 20 μmoles of fructose 6-P, 1 μmole of dithiothreitol, 1 μmole of EDTA, 1·9 mg enzyme protein, glutamine as shown, and benzydamine: A, none; B, 2 mM; C, 4 mM.

The enzyme activity decreased in the mixture of enzyme and benzydamine, but it recovered by gel filtration. This shows that the benzydamine was removed and that the inhibition by benzydamine was reversible.

The kinetics of the inhibition by benzydamine were of non-competitive type with respect to fructose 6-phosphate and glutamine, respectively (Figs. 2 and 3). K_m values were 1·2 mM for fructose 6-phosphate and 1·0 mM for glutamine. Kornfeld[11] reported that K_m value for fructose 6-phosphate was 0·22 mM, and the higher value obtained in this study may be due to the amount of phosphoglucose isomerase (D-glucose 6-phosphate ketol-isomerase EC 5.3.1.9) in the crude enzyme as reported by Kornfeld *et al.*[12]

Although there are many testing methods of nonsteroid anti-inflammatory agents,[1,13] the present method can be used as one of the screenings of the agents for chronic inflammation such as rheumatic arthritis. This method is suitable for routine test because the enzyme of $(NH_4)_2SO_4$ fraction can be stored at − 20° at least 4 weeks without loss of activity, and even after 3 months it still retains 80 per cent of original activity.

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Amantadine and catecholamine uptake

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SEVERAL recent developments have created interest in the interaction of amantadine with biogenic amines, dopamine in particular. Amantadine has been shown to be effective in relieving the symptoms of Parkinsonism,[1,2] as has L-dopa,[3,4] the precursor of dopamine. Anti-Parkinson activity has been shown to be correlated for some drugs with the blockade of dopamine uptake in the striatum.[5] These findings have led several investigators to suggest that amantadine may act by blocking the uptake of dopamine.[6]