Short communications

Inhibition of streptolysin O-induced haemolysis by theobromine sodium salicylate

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Theobromine sodium salicylate exerts an inhibitory effect on lysis of rabbit red cells by streptolysin O. No similar action of related compounds has been demonstrated.

Streptolysin O has been implicated in the pathogenesis of rheumatic fever (Kirschner & Howie, 1952; Halbert, Bircher & Dahle, 1961). Several inhibitors of this toxin have hitherto been reported (Howard, Wallace & Payling Wright, 1953; Raskova, 1958; Thiele, Petersen, Nowak & Urbaschek, 1965).

By testing several drugs for anti-streptolysin O activity *in vitro* we found that theobromine sodium salicylate considerably inhibits streptolysin O-induced haemolysis. We extended our studies to other related and some unrelated compounds to determine whether they have a similar action.

Methods.—Fresh rabbit's blood was obtained by heart puncture and collected in bottles containing 3·8% sodium citrate solution. The red cells were washed three times in 0·9% sodium chloride and a 5% suspension in pH 6·5 phosphate buffer (0·425 g NaCl, 0·842 g $NaH_2PO_4 \cdot 2H_2O$, 0·895 g $Na_2HPO_4 \cdot 12H_2O$ per litre) was made.

Agents under investigation were dissolved in 0·9% sodium chloride and the pH adjusted to 6·5. Insoluble substances were initially dissolved in 2 ml 0·1 N HCl or 0·1 N NaOH, diluted to the appropriate volume with saline and the pH readjusted to 6·5.

Paired 3 ml aliquots of the drug solutions were added to 1 ml of the red blood cell suspension in test tubes. The liquids were mixed gently, 1 ml diluted streptolysin O (Wellcome) was added and the test tubes were placed in a water bath at 37° C for 45 minutes. The tubes were inverted at the beginning of the incubation period and 15 min later. After incubation the tubes were centrifuged at 3,000 r.p.m. for 15 min and the absorbance of the supernatant fluid was determined using an SP.500 Unicam spectrophotometer at 540 nm, using saline as the blank. With every set of drug-containing tubes two other pairs of tubes were set up: one containing 4 ml saline plus 1 ml erythrocyte suspension (saline sample) and another containing 3 ml buffer, 1 ml red cell suspension and 1 ml streptolysin O dilution (control sample). The necessary dilution of streptolysin was determined by preliminary experiment.

Percentage inhibition of haemolysis was calculated from the formula:

$$\% \text{ Inhibition} = 100 - \left[\left(\frac{\text{O.D. drug sample} - \text{O.D. saline sample}}{\text{O.D. control sample} - \text{O.D. saline sample}} \right) \times 100 \right]$$

TABLE 1. *Effect of xanthine derivatives and of sodium salicylate on streptolysin O-induced haemolysis of rabbit red cells*

Compound	1×10^{-3} M	5×10^{-4} M	1×10^{-4} M	5×10^{-5} M
Theobromine sodium salicylate	47·16±1·51* (14)	41·51±1·25* (14)	11·43±1·46* (14)	8·18±2·08* (13)
Theobromine	1·56±1·22 (10)	0·25±1·79 (10)	2·24±1·97 (10)	−0·50±0·94 (10)
Sodium salicylate	0·24±1·70 (10)	0·74±1·64 (10)	1·94±2·16 (10)	0·68±1·90 (10)
Caffeine	4·41±1·21† (10)	3·97±2·31 (10)	3·18±1·49 (9)	3·20±2·15 (10)
Theophylline	0·59±2·63 (7)	0·18±2·12 (7)	−0·54±1·91 (7)	−0·43±1·54 (7)

Values are mean percentage inhibition ±S.E. Numbers of experiments are given in parentheses. *$P<0.001$; †$P<0.01$ (Student's t test).

Results.—Table 1 shows that theobromine sodium salicylate inhibits streptolysin O-induced haemolysis at all concentrations tested. Of the xanthine derivatives which have been tested none has a similar action, with the sole exception of caffeine, which has a statistically significant effect at a concentration of 1×10^{-3} M. Neither theobromine base nor sodium salicylate alone had any inhibitory effect. Only the combination of the two substances as theobromine sodium salicylate was capable of producing it.

Other drugs tested included acetylsalicylic acid, aminophylline, aminopyrine, atropine sulphate, dextromethorphan hydrobromide (1,2-dihydroxy-3-propyl)-theophylline, l-ephedrine sulphate, flufenamic acid, hydrochlorothiazide, indomethacin, papaverine hydrochloride, pentazocine, phenobarbitone, phenylbutazone, pilocarpine nitrate, procaine hydrochloride, promethazine hydrochloride and hyoscine hydrobromide. None had antistreptolysin activity.

Discussion.—Not much can be deduced concerning the mechanism of the antistreptolysin action of theobromine sodium salicylate. It seems unlikely, however, that it is the same as that of cholesterol and related compounds (Howard *et al.*, 1953) where the inhibition of haemolysis is attributed to a complex formation between the lysin and the cholesterol.

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