Reaction Components Influencing CAMP Factor Induced Lysis

By B. STERZIK AND F. J. FEHRENbach*

Robert Koch-Institut des Bundesgesundheitsamtes, Nordufer 20, 1000 Berlin 65, FRG

(Received 16 November 1984)

The reaction components and conditions affecting CAMP factor (Streptococcus agalactiae) induced lysis of target cells have been investigated. Both the amount of sphingomyelinase used and the time of preincubation with sphingomyelinase directly affected the rate of haemolysis by CAMP factor. The CAMP factor induced lysis was temperature dependent between 15 and 30 °C and was proportional to the amount of CAMP factor added.

INTRODUCTION

The CAMP factor is released by most strains of Streptococcus agalactiae and was described first by Christie et al. (1944). CAMP factor causes lysis of red blood cells (RBC) that contain at least 45 mol% of sphingomyelin in the cell membrane and which have been pretreated with Staphylococcus aureus β-toxin (sphingomyelinase). Thus, cow and sheep RBC are sensitive to CAMP factor lysis whereas RBC from human, horse, rabbit and guinea-pig are not lysed (Christie et al., 1944). The factor has been characterized as an extracellular polypeptide, to which molecular weight values of 15000 (Esseveld et al., 1975), 23500 (Bernheimer et al., 1979) and 33000 (Brown et al., 1972) have been attributed.

The role of sphingomyelinase in sensitizing target RBC is to hydrolyse sphingomyelin, releasing phosphorylcholine and N-acylsphingosine (ceramide). The hydrolysis products were identified by Doery et al. (1963) and shown by Colley et al. (1973) to be generated from the cell membrane without causing lysis. However, lysis can then be initiated by the addition of CAMP factor or by chilling pretreated cells - 'hot-cold lysis' (Rogolski, 1979). Although several aspects of the CAMP reaction have been studied in the past (Esseveld et al., 1958, 1975; Brown et al., 1974; Bernheimer et al., 1979) a detailed analysis of the interaction of the individual components of the CAMP reaction has not been published. This investigation was undertaken to study the conditions for the CAMP reaction in more detail.

METHODS

CAMP factor production. Streptococcus agalactiae was grown in a fermenter as described by Huser et al. (1983) and the CAMP factor purified by the method of Juergens et al. (1984). CAMP factor activity was determined as previously described (Huser et al., 1983).

Sphingomyelinase production. Sphingomyelinase (EC 3.1.4.12) was prepared from Staphylococcus aureus (T19) cultures as described by Brown et al. (1974). In addition, purified sphingomyelinase (specific activity 800 units mg⁻¹) from Bacillus cereus, purchased from Boehringer/Mannheim, was used as a standard. Sphingomyelinase activity was determined according to Krug et al. (1979).

RESULTS AND DISCUSSION

Dependence of the CAMP reaction on sphingomyelinase. Dependence of the CAMP reaction on the amount of sphingomyelinase was determined with sheep RBC (50 mol% sphingomyelin) as a target. The rate of haemolysis increased directly with an increase in sphingomyelinase

Abbreviation: RBC, red blood cells.

0001-2330 © 1985 SGM
Fig. 1. Effect of CAMP factor concentration on the rate of lysis of sheep RBC preincubated with sphingomyelinase. The RBC (1.5 \times 10^6 cells ml^{-1}) were incubated in 0.01 M-Tris/HCl buffer containing 0.14 M-NaCl and 0.01 M-MgCl_2, pH 7.4, with sphingomyelinase (0.6 units ml^{-1}) for 5 min at 30 °C. Haemolysis was started by adding CAMP factor and the rate was measured as \Delta OD_{546} min^{-1}. The bars indicate the standard error calculated from three experiments.

concentration in the range 0.12–0.42 units ml^{-1}. An excess of sphingomyelinase over 0.5 units ml^{-1} did not further increase the rate of lysis. Although the sphingomyelin content of cow and goat RBC (45 mol%) is slightly lower than that of sheep RBC, the former proved to be more sensitive to sphingomyelinase treatment. Complete sensitization of cow and goat RBC was achieved with 0.12 units ml^{-1} whereas sheep RBC needed 0.43 units ml^{-1}. The influence of the time of preincubation of sheep RBC with sphingomyelinase on the CAMP reaction was also investigated. The CAMP factor induced onset of lysis was measured as a function of the preincubation time of sheep RBC at low sphingomyelinase concentration (0.6 units ml^{-1}). The time to onset of lysis decreased from 200 to 10 s as the preincubation time with sphingomyelinase was increased from 0.3 to 6 min (the lower limit for time measurement by this assay was 10 s). Hydrolysis of the accessible membrane sphingomyelin in target cells thus appeared to occur rapidly and to exhibit saturation kinetics. Thus hydrolysis of membrane sphingomyelin in target cells may be achieved either rapidly with an excess of sphingomyelinase, or on prolonged incubation with small amounts of the enzyme. Sphingomyelinases from both *Staphylococcus aureus* and *Bacillus cereus* were equally active in sensitizing target cells in the CAMP reaction. Since, according to the purification protocol of Ikezawa *et al.* (1978), the commercial *B. cereus* sphingomyelinase used in this study contained no protease, lipase or phospholipase activity, the possibility of an interaction of other ‘membrane active’ components present in crude *S. aureus* sphingomyelinase preparations is excluded.

**Effect of temperature, pH and CAMP factor concentration.** When the temperature was raised, the rate of CAMP factor induced lysis increased linearly within the range 15–30 °C. The pH optimum for the reaction was between pH 7 and 8, the rate of lysis decreasing both below and above this range. The influence of the concentration of CAMP factor on the rate of haemolysis was studied using sheep RBC. The rate of haemolysis increased linearly as the concentration of CAMP factor was increased from 5 to 40 units ml^{-1} (Fig. 1).

**Effect of sphingomyelin content of target cells.** Wiseman & Caird (1967) and Bernheimer *et al.* (1974) reported that RBC of different species differed in their sensitivity to *Staphylococcus aureus* \(\beta\)-toxin (sphingomyelinase) as measured in the ‘hot-cold lysis’ test. These authors found a correlation between the sphingomyelin content of target cells and their sensitivity to ‘hot-cold lysis’. It was therefore of interest to compare the rates of CAMP factor lysis of different RBC species in relation to their sphingomyelin content. The relative rates of lysis determined for RBC
Properties of the CAMP reaction


This work was supported by the Deutsche Forschungsgemeinschaft, Sfb 9, Technische Universität Berlin, Projekt D2.

We thank Professor John P. Arbuthnott for valuable suggestions.

REFERENCES


