Mechanism of the stimulatory effect of 6-aminohexanoic acid on plasminogen activation by streptokinase or tissue plasminogen activator: The role of chloride

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SUMMARY

Studies were conducted on the mechanism of the stimulatory effect of 6-aminohexanoic acid (6-AH) during the in vitro activation of human glutamic plasminogen (Glu-Plg) by streptokinase or by tissue plasminogen activator (t-PA) and the possible role of the addition of physiological concentrations of NaCl to the buffer solution. Enhancement by 6-AH was investigated by measuring the rate of plasmin generation using chromogenic substrate H-D-glu-phe-lys-pNA (S-2403). Control studies using plasmin showed that the addition of 6-AH at concentrations below 20 mM did not significantly affect the initial rate of the amidolytic activity of plasmin with or without the addition of NaCl to 0.05 M Tris buffer (pH 7.4). On the other hand, addition of NaCl to the buffer slowed down the initial rate of activation of Glu-Plg by streptokinase or by t-PA while increasing the percent enhancement by 6-AH when compared with the controls. The ratios of the initial rates of plasmin generation in the presence or in the absence of 6-AH were plotted against the inverse of the volume fraction of Glu-Plg, streptokinase or t-PA after serial dilutions. The results showed that when the activation reactions were performed in 50 mM of Tris buffer (pH 7.4), the enhancements by 6-AH were related to its interaction with streptokinase or t-PA, while using the same Tris buffer containing 0.6 % NaCl, the enhancements by 6-AH were related to its interaction with both Glu-Plg and streptokinase or t-PA. However, upon increasing the NaCl to 0.9 %, the results showed that the enhancements by 6-AH of the activation of Glu-Plg by streptokinase or t-PA were related to its interaction with Glu-Plg. The results suggested that changes in the concentrations of NaCl play a regulatory role during the activation process.

INTRODUCTION

The discovery that 6-aminohexanoic acid (6-AH) inhibited fibrinolysis was first reported by Okamoto (1). Since then, the literature on this subject has been expanding and now includes the use of this substance in a variety of hemorrhagic and clotting disorders. Studies of the effects of binding of 6-AH to plasminogen on the various physical and functional properties of plasminogen were reported (2,3). Conformational changes of plasminogen induced during its activation to plasmin were reported to be similar to those occurring by the interaction of 6-AH with plasminogen (4,5). The inhibition by 6-AH of plasminogen to plasmin conversion by streptokinase was postulated as involving the formation of a plasminogen-6-AH complex that due to the altered conformation of plasminogen, was not acted on by streptokinase (6). A
double reciprocal plot of this reaction in the presence of 6-AH was reported (7) to show that the inhibition was noncompetitive and that $K_m$ was unaltered. In contrast to the above studies, the activation of glutamic plasminogen (Glu-Plg) by streptokinase showed an enhancement by 6-AH if the experiments were performed using a buffer containing the physiological level of NaCl (8). Buffers and chloride ions are normal constituents of plasma and may play a regulatory role in fibrinolysis. A detailed study was reported (9) on the effect of different concentrations of buffers, divalent cations and chloride on the inhibition by heparin of the activation of Glu-Plg by streptokinase. The results showed that increasing the concentration of the buffer or addition of NaCl slowed the reaction and also decreased the % inhibition by heparin (9). In the present report, studies were conducted on the effect of different concentrations of NaCl on the mechanism of enhancement by 6-AH of the in vitro activation of Glu-Plg by streptokinase or by tissue plasminogen activator.

MATERIALS AND METHODS

Materials

Plasmin substrate H-D-glu-phe-lys-pNA (S-2403) was purchased from DiaPharma Group Inc. (Westchester, Ohio, USA). Streptokinase, glutamic type plasminogen and human plasmin were purchased from American Diagnostica (Greenwich, Connecticut, USA). 6-Aminohexanoic acid and all other reagents were purchased from Sigma (St. Louis, Missouri, USA). Alteplase (t-PA) was obtained from Genentech Inc. (South San Francisco, California, USA). A model ELx 800 well counter (Bio-Tek Instruments, Winooski, Vermont, USA) was used at a wavelength of 405 nm for the rate study.

Effect of different levels of 6-AH on the rate of amidolytic action of plasmin

In all instances, the incubations were carried out at room temperature and plasmin action was measured using 0.36 mM chromogenic substrate, S-2403. The reactions were performed using a total volume of 700 µl in 0.05 M Tris (pH 7.4) and containing either no salt, 0.6 % NaCl or 0.9 % NaCl. Three hundred microliters of the reaction mixture was transferred to microplates and the absorbances were read at 405 nm using a Bio-Tek well counter (Model ELx, 800). Results on the graph are averages of three experiments run in duplicates. Details regarding the concentrations of the reagents used are described in the footnote under the figure. Percent enhancements were calculated from Δ O.D. at 20 minutes.

Effect of different levels of 6-AH on the activation of glutamic plasminogen by streptokinase or by tissue plasminogen activator.

The reactions were performed using 0.05 M Tris (pH 7.4) alone or containing 0.6 % or 0.9 % NaCl. The same procedures and the plasmin substrate S-2403 were used as reported above for plasmin except that the rates of plasmin generations from glutamic plasminogen by the action of streptokinase or t-PA were measured. The results were useful in determining the optimum level of 6-AH required for enhancement of the activation of glutamic plasminogen by streptokinase or by t-PA. The results are averages of three experiments run in duplicates. Details regarding the concentrations of the reagents used are described in the footnote under the figure. Percent enhancements were calculated from Δ O.D. at 10 minutes for 0.05 M Tris buffer (pH 7.4) and Δ O.D. at 30 minutes for Tris buffer containing 0.6 % and 0.9 % NaCl.

Nature of the interaction between 6-AH and streptokinase, or 6-AH and t-PA, or 6-AH and glutamic plasminogen or between 6-AH and both glutamic plasminogen and streptokinase or t-PA.

To determine whether the stimulatory effect of 6-AH was due to its interaction directed towards Glu-Plg or streptokinase or directed towards Glu-Plg or t-PA or both Glu-Plg and streptokinase or t-PA, experiments were performed at a fixed level of the cofactor and varying, in a serial fashion, the concentrations of either Glu-Plg, streptokinase or t-PA. In all instances, a control experiment was performed without either of the activators. The final volume of the reaction mixture and the procedure for measuring plasmin generation were the same as described for plasmin. The ratio’s of absorbance in the presence of 6-AH over the controls were plotted against the inverse of the volume fraction of Glu-Plg or streptokinase or t-PA following serial dilutions of each. The results are averages of three experiments. Details regarding the concentrations of the reagents used are described in the footnote under each figure. The initial rates of plasmin generation were obtained by measuring the Δ O.D. at 10 minutes for 0.05 M Tris buffer (pH 7.4) and at 30 minutes for Tris buffer containing 0.6 % or 0.9 % NaCl. Endpoint assays were used and the results were verified for linearity (8).