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EFFECT OF ANTITHROMBIN III, α_1 -PROTEINASE INHIBITOR AND HEPARIN ON AMIDOLYTIC ACTIVITY OF NERVE GROWTH FACTOR (7S-NGF)

William F. Long and Frank B. Williamson

Dept. of Biochemistry, University of Aberdeen, Aberdeen AB9 1AS, U.K.

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Amidolysis catalysed by nerve growth factor (7S-NGF) and by a glandular kallikrein, unlike that catalysed by thrombin, was not inhibited by antithrombin III, either in the presence or absence of heparin. Inhibition by α_1 -proteinase inhibitor of thrombin-, but not of 7S-NGF- or kallikrein-catalysed amidolysis was alleviated by incubation of enzyme and heparin before addition of inhibitor. These data are discussed in terms of a possible control of growth factor activity by antiproteinases and glycosaminoglycans.

Nerve growth factor multipolypeptide complexes, including a species called 7S-NGF, contain a γ-subunit possessing arginyl-esteropeptidase activity (1). Mitogenic activity of peptide growth factors may involve promotion of proteinase binding to cell surface proteins; such proteinases may include activities which, like the 7S-NGF γ-subunit, are associated with growth factors themselves (2). It has been suggested that pericellular heparans might affect cell proliferation-associated proteinases by modulating the action of proteinase inhibitors (3). In this communication we report the effects on 7S-NGF-catalysed amidolysis of antithrombin III, α_1 -proteinase inhibitor and heparin.

MATERIALS AND METHODS

Materials: Bovine thrombin (EC 3.4.21.5; Lot No. YE 584; 270 NIH units.mg^{-1}) was from Parke Davis and Co., Pontypool, U.K. Murine submaxillary gland 7S-NGF (Lot No. 92F00561), porcine pancreatic kallikrein (EC3.4.21.8; Lot No. 60F0183), porcine intestinal heparin (Lot No. 46C0035; 170 USP units.mg^{-1}), α_1 -proteinase inhibitor (Lot No. 51F9345) and Polybrene were from Sigma Chemical Co., Poole, U.K. Human antithrombin III (Lot No. 57030) and tripeptide chromogenic substrates (see manufacturers' literature for structures corresponding to code-names quoted) were from KabiVitrum, London, U.K.
Methods: Reactions were carried out in buffer (0.11 mol.dm^{-3} NaCl, 0.05mol.dm^{-3} Tris/HCl, pH 7.7 at 37° C). 20mm^3 enzyme (5.6 µg.cm^{-3} 7S-NGF; 27.8 µg.cm^{-3} kallikrein or 0.56 units.cm^{-3} thrombin) were incubated for 6 min at 37° C with 20mm^3 heparin (concentrations in Figures) or buffer. 20mm^3 antithrombin III, α_1 -proteinase inhibitor (concentrations in Figures and Results text) or buffer were added and the mixture incubated for 6 min (antithrombin III experiments) or 180 min (α_1 -proteinase inhibitor experiments). 30 mm^3 of a mixture of substrate (0.17 mg.cm^{-3}) and Polybrene (0.17 mg.cm^{-3}) were added. Initial reaction rates

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were estimated by measuring p-nitroaniline released from substrates spectrophotometrically. Substrate S-2238 was used for thrombin experiments; unless stated, substrate S-2302 was used for 7S-NGF and kallikrein experiments. Rates are quoted in the Results section as percentages of control rates, ie. rates observed when enzymes were incubated with substrate only. Control amidolytic rates ($\Delta A_{1cm,405} \cdot min^{-1} \times 10^2$) of S-2302, S-2288, S-2251, S-2238, S-2266, S-2222 and S-2444 catalysed by 7S-NGF were 3.2, 2.9, 2.3, 2.3, 0.6, 0.2, <0.2 respectively; and catalysed by kallikrein were 0.88, 0.18, 0.10, 0.05, 0.93, 0.08, <0.02 respectively; control amidolytic rate ($\Delta A_{1cm,405} \cdot min^{-1} \times 10^2$) of S-2238 catalysed by thrombin was 3.8. Concentrations quoted throughout are final concentrations after addition of substrate-Polybrene mixture. Polybrene was included to prevent heparin-substrate interaction (4).

RESULTS

Effect of antithrombin III on 7S-NGF-catalysed amidolysis: Antithrombin III inhibited thrombin activity but did not inhibit amidolysis of S-2302 catalysed by 7S-NGF or kallikrein (Fig. 1a). Higher concentrations of antithrombin III than shown in the Figure increase amidolytic rates catalysed by 7S-NGF and kallikrein: at an antithrombin III concentration of 1.11 units.cm^{-3}, rates were 115% and 116% respectively of control rates. No amidolysis occurred when antithrombin III alone was incubated with substrate. Similar effects were observed on 7S-NGF-catalysed amidolysis of S-2288, S-2251 and S-2238, and on kallikrein-catalysed amidolysis of S-2266.

Heparin potentiated the inhibitory effect of antithrombin III on thrombin activity (Fig. 1b); under similar conditions antithrombin III in the absence

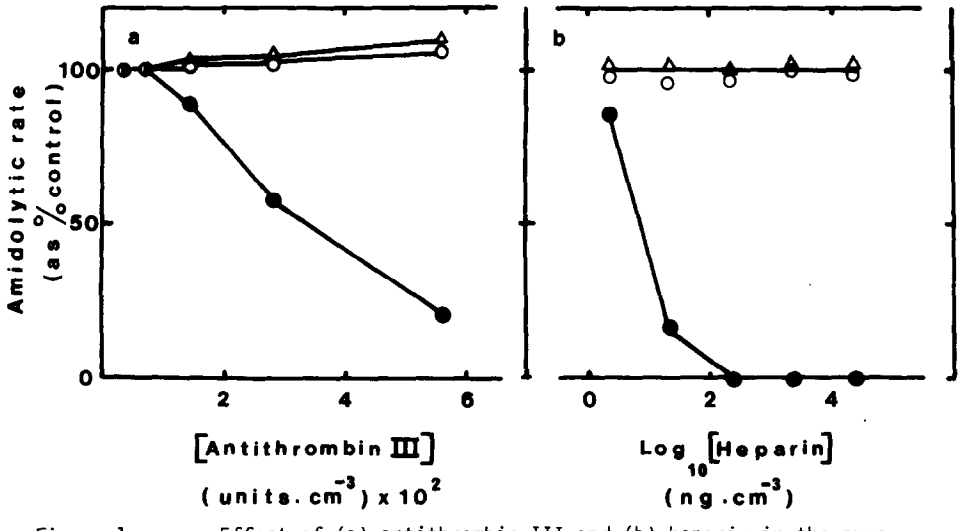

Figure 1. Effect of (a) antithrombin III and (b) heparin in the presence of antithrombin III on amidolysis catalysed by (●) thrombin, (○) glandular kallikrein and (△) 7S-NGF. Details of experiments are given in the text.

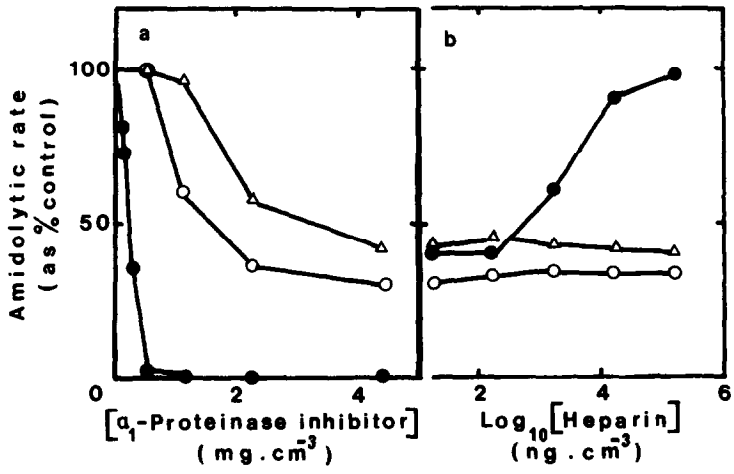

Figure 2. Effect of (a) α_1 - proteinase inhibitor and (b) α_1 - proteinase inhibitor in the presence of heparin on amidolysis catalysed by (●) thrombin, (○) glandular kallikrein and (△) 7S-NGF. Details of experiments are given in the text.

of heparin decreased thrombin activity to 85% of that observed when thrombin only was incubated with substrate. Heparin, in the presence of the same concentration of antithrombin III, did not affect amidolysis of S-2302 catalysed by 7S-NGF or kallikrein (Fig. 1b). Heparin, over the same concentration range, also failed to affect the amidolytic rate when antithrombin III (1.11 units.cm^{-3}) was incubated with 7S-NGF or kallikrein. Similarly, over this concentration range, heparin alone did not affect thrombin, 7S-NGF or kallikrein activities. No amidolysis occurred when heparin, with or without antithrombin III, was incubated with substrate in absence of enzyme. In parallel experiments, heparin with or without antithrombin III did not affect 7S-NGF-catalysed amidolysis of S-2288, S-2251 and S-2238, or kallikrein-catalysed amidolysis of S-2266.

Effect of α_1 - proteinase inhibitor on 7S-NGF-catalysed amidolysis: α_1-Proteinase inhibitor inhibited thrombin-, 7S-NGF- and kallikrein activity (Fig. 2a). In parallel experiments, similar effects were observed on 7S-NGF-catalysed amidolysis of S-2288, S-2251 and S-2238, and on kallikrein-catalysed amidolysis of S-2266; with these substrates, amidolytic rates in the presence of α_1 - proteinase inhibitor (4.44 mg.cm^{-3}) were, respectively, 20%, 27%, 70% and 50% of control rates.

α_1 - Proteinase inhibitor concentrations (0.28, 4.44 and 4.44 mg.cm^{-3}) were chosen which produced inhibiton (to 37, 42 and 32% of control rates respectively) of thrombin-(S-2238), 7S-NGF-(S-2302) and kallikrein-(S-2302) catalysed amidolysis respectively. Heparin alleviated α_1-proteinase inhibition of thrombin, but did not affect inhibition of 7S-NGF or kallikrein (Fig. 2b). In parallel experiments, heparin did not affect inhibition by α_1 - proteinase inhibitor of 7S-NGF-catalysed amidolysis of S-2288, S-2251 and S-2238, or kallikrein-catalysed amidolysis of S-2266.

DISCUSSION

Proteinases at cell surfaces may be affected by antagonists similar, or in some cases perhaps identical, to those present in plasma. Heparins modulate plasma proteinase activities in several ways, many involving interactions with proteinase inhibitors. Heparans may similarly affect proteinases, perhaps while present on blood-vessel walls (summarised in 4). We have suggested that metabolically variable fine structures of pericellular heparans in tissues other than blood-vessels may affect the ability of these molecules to influence cell proliferation-associated surface proteinase activities (including those associated with peptide growth factors) (4).

7S-NGF activity was compared to that of a glandular kallikrein because of sequence similarities between kallikrein and 7S-NGF γ-subunit (5.6) and because of subunit kininogenase activity (1). Specificity of 7S-NGF activity towards tripeptide substrates was similar but not identical to the specificity range of kallikrein, which itself accorded with kallikrein specificity patterns reported elsewhere (7).

Because an antiproteinase immunologically related to plasma antithrombin III has been detected on cell membranes (8,9), and because leukocyte surface proteinase activity may be inhibited by antithrombin III (10), the effect of this inhibitor on 7S-NGF activity was examined. Even in the presence of heparin, which accelerates antithrombin III inhibition of thrombin (Fig. 1b; 11), no inhibition of 7S-NGF or kallikrein activity was detected.

Because an antiproteinase resembling plasma α_1 - proteinase inhibitor has been detected on cell surfaces, and because this plasma inhibitor inhibits lymphocyte surface proteinase activity and the mitogenic response of lymphocytes (summarised in 3), the effect of α_1 - proteinase inhibitor on 7S-NGF activity was examined. Some inhibition of 7S-NGF- and of kallikrein-catalysed amidolysis was observed; inhibition of kallikrein by this inhibitor has been reported previously (12). Inhibition of thrombin by α_1 - proteinase inhibitor can be prevented by pre-incubation of enzyme with heparin (Fig. 2b; 13-17), an effect depending on heparin-proteinase interaction (14,15,18). No such protection by heparin of 7S-NGF or kallikrein was demonstrated.

In summary these results suggest that heparin (or heparan) modulation of 7S-NGF activity through the action of antithrombin III or α_1 -proteinase inhibitor is unlikely. A nerve growth factor preparation is able to substitute for $\overline{C1}$ of the classical complement pathway, presumably through the action of the γ -subunit; this action is prevented by the plasma antiproteinase $\overline{C1s}$-inhibitor (19). As $\overline{C1s}$-inhibitor is produced by cultured cells, and may be modulated by heparin (summarised in 4), the action of $\overline{C1s}$-inhibitor and heparin-modulated $\overline{C1s}$-inhibitor on growth factor proteolytic activity warrants attention.

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