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Ability of Different Chemically Modified Heparins To Potentiate the Biological Activity of Heparin-Binding Growth Factor 1: Lack of Correlation with Growth Factor Binding

David A. Belford, Ian A. Hendry,* and Christopher R. Parish

Divisions of Neuroscience and Cell Biology, John Curtin School of Medical Research, Australian National University, Canberra ACT 2601, Australia

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ABSTRACT: A range of chemically modified heparins was examined for their ability to bind heparin-binding growth factor 1 (HBGF-1; acidic fibroblast growth factor) and potentiate the in vitro mitogenic and neurotrophic activity of HBGF-1. It was found that carboxyl-reduced heparin bound HBGF-1 as effectively as the native heparin molecule. Totally desulfated heparin and N-desulfated heparin lack HBGF-1-binding capacity, and substitution of the exposed amino group with acetyl or acetoacetyl groups only partially restored binding capacity, indicating that *N*-sulfates only play a limited role in growth factor binding. However, the failure of totally desulfated, N-resulfated heparin to interact with HBGF-1 demonstrated that *N*-sulfates alone are insufficient and ester sulfates are absolutely essential for HBGF-1 binding. In contrast, the ability of the modified heparins to potentiate the mitogenic activity of HBGF-1 correlated only to a limited extent with their affinity for HBGF-1. Thus, the carboxyl-reduced molecule which displayed similar affinity for HBGF-1 as native heparin was consistently less potent in augmenting mitogenesis. Similarly, the N-acetylated and the N-acetoacetylated species, which had much lower affinity for HBGF-1 than the carboxyl-reduced molecule, conferred similar biological activity to HBGF-1 whereas N-desulfated heparin, which was unable to bind growth factor, potentiated the mitogenic activity of HBGF-1 for both 3T3 and HUVE cells. In contrast, the neurotrophic activity of HBGF-1 was potentiated by modified heparin species which failed to bind HBGF-1 and were without activity in the mitogenic assays. In fact, native heparin was much less effective at potentiating the neurotrophic activity of HBGF-1 than several of the modified heparins. Thus, heparin exerts its effects not only by binding of HBGF-1 but also by mechanisms independent of its binding activity probably via cell-surface heparin receptors.

Heparin and heparan sulfate proteoglycans (HSPG)[1] are involved in a range of biological functions, including cell–cell (Cole et al., 1986) and cell–substratum (Culp et al., 1980) adhesion, cellular proliferation and differentiation (Fritze et al., 1985), neurite outgrowth (Hantaz-Ambroise et al., 1987), synaptic function (Anderson & Fambrough, 1983), myelination (Carey et al., 1987), matrix assembly (Laurie et al., 1986), in vivo coagulation (Marcum & Rosenberg, 1989), and capillary permeability (Farquhar, 1981).

Heparin will bind to—and in many cases alter the biological activity of—a number of protein and glycoprotein ligands. Heparin is now known to potentiate the mitotic (Schreiber et al., 1985), chemotactic (Terranova et al., 1985), neurotrophic (Unsicker et al., 1987), and angiogenic (Lobb et al., 1985) properties of the pure acidic mitogen heparin-binding growth factor 1 (HBGF-1; also known as acidic fibroblast growth factor or aFGF), and heparin affinity chromatography forms the basis of the purification of HBGF-1 (Maciag et al., 1984).

The binding of heparin to HBGF-1 acts to potentiate its biological activity (Burgess & Maciag, 1989). Indirect evidence for such a mechanism has been derived from experiments that show heparin protects HBGF-1 against proteolytic attack (Lobb, 1988), as well as acid and heat denaturation

* Address correspondence to this author at the Division of Neuroscience.

[1] Abbreviations: DMEM, Dulbecco's modified Eagle's medium; HBGF-1, heparin-binding growth factor 1; HSPG, heparin and heparan sulfate proteoglycan(s); HUVE, human umbilical vein endothelial cell(s).

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(Gospodarowicz & Cheng, 1986), and increases the in vitro biological half-life of HBGF-1 from 7 to 36 h (Damon et al., 1989). The increased in vitro activity of HBGF-1 in the presence of heparin can be reproduced by the more frequent addition of HBGF-1 alone (Rosengart et al., 1988). This, together with data showing the binding of heparin induces a conformational change in HBGF-1 (Schreiber et al., 1985), suggests heparin confers structural stability and/or physically covers the sites of proteolytic attack.

Several studies have more directly linked the potentiating action of heparin to its ability to bind HBGF-1. Gordon et al. (1989) found that 40% of HSPG extracted from the ECM of cultured endothelial cells bound to an HBGF-1 affinity column and this fraction was some 100-fold more active in potentiating the mitogenic action of HBGF-1 than heparin. The flow-through fraction inhibited HBGF-1-induced mitogenesis of endothelial cells. The two fractions were found to differ in size of both their core proteins and their GAG chains. Other studies have shown that decreasing the affinity of HBGF-1 for heparin by proteolytic or chemical modification produces a corresponding decrease in the affinity for the HBGF-1 receptor and a decrease in mitotic activity (Lobb, 1988). Further, Schrieber et al. (1985) have provided compelling evidence that the modulation of HBGF-1 activity by heparin also occurs at the receptor level. Heparin enhanced the binding of HBGF-1 to LEII cells, decreasing the apparent K_d some 2.5-fold. This corresponded to a 5-fold decrease in the EC_{50} for the stimulation of thymidine uptake.

A previous report emphasized the importance of both the polysaccharide chain length and the degree of sulfation on the ability of heparin to potentiate HGBF-1 action (Sudhalter et al., 1989). Highly sulfated oligosaccharides were found to potentiate HBGF-1-induced mitogenesis to a degree proportional to chain length; oligosaccharides (up to tetradecasaccharides) of low sulfate content were inactive. Despite these earlier studies, there has been no systematic analysis of the structural features of heparin required to potentiate HBGF-1 action. In this study, we have attempted to correct this deficiency by examining the ability of a range of chemically modified heparins to (i) bind HBGF-1, (ii) potentiate the mitogenic action of HBGF-1 on both BALB/c 3T3 and HUVE cells, and (iii) potentiate the neurotrophic action of HBGF-1 on dissociated ciliary neurons of the E8 chick.

Materials and Methods

Reagents. Beef heart-derived HBGF-1 was purified as previously described (Watters & Hendry, 1987) except the heparin–agarose column was washed and eluted with a gradient of NH_4HCO_3, allowing immediate lyophilization and storage of the pure factor. Preparations were routinely checked for purity by SDS–PAGE.

Native bovine lung heparin was obtained from Sigma Chemical Co., St Louis, MO. A number of chemically modified heparins were synthesized, namely, (a) N-desulfated, (b) N-desulfated, N-acetylated, (c) N-desulfated, N-acetoacetylated, (d) totally desulfated, (e) totally desulfated, N-acetoacetylated, (f) totally desulfated, N-resulfated, and (g) carboxyl-reduced. Heparin was carboxyl-reduced, N-desulfated, and N-acetylated according to previously published methods (Nagasawa & Inoue, 1980a,b; Irimura et al., 1986; Taylor et al., 1976). Heparin was N-acetoacetylated with acetoacetic anhydride under identical reaction conditions used for acetylation (Irimura et al., 1986). However, totally desulfated heparin was prepared by a modified procedure which resulted in more effective desulfation than previous methods (Nagasawa & Inoue, 1980a,b). Initially, N-desulfated, N-acetoacetylated heparin was converted to its pyridinium salt and totally desulfated in DMSO–10% methanol as reported earlier (Nagasawa & Inoue, 1980a,b). Acetoacetyl groups were removed by the addition of hydroxylamine (50 mg/mL), adjusting the pH of the solution to 7.5 with 5 N NaOH and incubating the mixture at room temperature for 45–60 min. The totally desulfated heparin was exhaustively dialyzed, lyophilized, and, if necessary, N-resulfated under alkaline conditions with pyridine–sulfur trioxide (Irimura et al., 1986).

The sulfate content of the chemically modified heparins was determined by turbidimetry of inorganic sulfate released by acid hydrolysis (Chandrasekaran & BeMiller, 1980), and the content of amino groups was determined fluorometrically using fluorescamine (Weigele et al., 1972) with glucosamine as the standard. The anticoagulant activity of the heparin preparations was estimated by the activated partial thromboplastin time and thrombin time tests as previously described (Parish et al., 1987).

Binding Assay of HBGF-1 to Modified Heparins. The relative affinities of the modified heparins for HBGF-1 were determined using a binding assay based on competition for heparin–agarose (Bio-Rad, Richmond, CA). Each modified heparin was added at various concentrations (up to 500 μg/mL) to a microfuge tube containing 4 μg of HBGF-1 in PBS. Each tube then received 40 μL of a 50% (v/v) suspension of heparin–agarose in PBS to make a final volume of 200 μL. After 1-h agitation at 4 °C, each heparin–agarose pellet was washed 3 times in PBS followed by the addition of 25 μL of SDS–PAGE sample buffer containing 15 mg/mL DTT. Samples were boiled for 3 min, then applied to a 14 cm × 10 cm × 0.75 mm polyacrylamide gel, and electrophoresed at a constant current of 16 mA for 3 h. Gels were stained with Coomassie Blue and HBGF-1 bands quantified by two-dimensional laser densitometry (Ultrascan XL, LKB, Bromma, Sweden). Each band was outlined and its total density integrated using Gel Scan XL version 1.2. For each gel, HBGF-1 bands were normalized to the average of four standards (4 μg of HBGF-1) included on the gel and expressed as a percentage of the total 4 μg added remaining bound to the heparin–agarose beads. Thus, 100% represents 4 μg of HBGF-1 bound to the beads, and 0% is total inhibition of binding. Concentrations giving 50% inhibition of binding were determined from a linear regression formula.

Mitogenic Assays. Stock cultures of BALB/c3T3 cells (Commonwealth Serum Laboratories, Parkville, Victoria, Australia) were maintained in Dulbecco's modified Eagle's medium (DMEM; Flow Labs, McLean, VA)/HCO_3/10% fetal calf serum (FCS; Commonwealth Serum Laboratories) at 37 °C (5% CO_2 incubator) in 60-mL flasks (Corning, NY). Prior to confluency, cultures were lightly trypsinized (0.025%; Worthington Biochemical Corp., Freehold, NJ), resuspended at 15000 cells/mL, and replated. Experimental cultures were seeded into 96-well plates (Nunc, Copenhagen, Denmark) and allowed to reach confluency (4–5 days), at which time the medium was replaced by serum-free DMEM/HCO_3. After 24-h serum deprivation, either HBGF-1 was added at the concentration shown in the presence of a 50 μg/mL sample of the indicated modified heparin or dose responses to the modified heparins were set up in the presence of 10 ng/mL HBGF-1. Cultures were incubated for a further 24 h at 37 °C (5% CO_2) before the addition of [^{3}H]thymidine (Amersham, U.K.). After a further 24-h incubation, the cultures were frozen and thawed twice and harvested onto glass fiber filters (Enzo Biochemicals Inc., NY) using a Titertek 530 cell harvester (Flow Labs), and the radioactivity was counted.

Pooled data from four separate duplicate experiments were analyzed.

HUVE cells (passages 5–12) were isolated and maintained in medium 199 (Flow Labs), 16% fetal calf serum, 25 μg/mL endothelial cell growth supplement (Sigma), and 50 μg/mL heparin. Mitogenic assays were carried out on quiescent cells in the presence of 16% fetal calf serum. Dose responses to the modified heparins were set up in the presence of 10 ng/mL HBGF-1, and [^{3}H]thymidine incorporation was determined as described above.

Neuronal Bioassay. Cultures of E8 chick ciliary neurons were established in collagen-coated 96-well microtiter plates (Nunc) as previously described (Bonyhady et al., 1980, 1982). Briefly, ganglia were dissected aseptically, dissociated with trypsin (0.08%; Worthington Biochemical Corp.) in calcium- and magnesium-free Hank's balanced salt solution, and suspended in DMEM buffered to pH 7.4 with 0.5 M *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid (HEPES) and containing 1% horse serum (Commonwealth Serum Laboratories). Dissociates were routinely preplated to remove nonneuronal cells. A dose response to the modified heparins was performed in the presence of a constant concentration (25 ng/mL) of beef heart-derived HBGF-1. Heparin samples to be assayed were diluted in the growth medium and added to the top well in a final volume of 100 μL, and a dilution series was set up across the plate. Neuronal cell suspension (100 μL; approximately 2200 neurons) was added to each well, and duplicate cultures were incubated for 24 h at 37 °C in a humidified air incubator. Cytosine arabinoside (10^{-5} M) was incorporated into some cultures to suppress the proliferation of nonneuronal cells. Neuronal survival was assessed by phase-contrast microscopy (200× magnification), the number of phase-bright adherent cells in four fields (approximately 11% of the total area) across the diameter of each well being recorded.

Results

Chemical Modification of Heparins. Seven chemically modified variants of bovine lung heparin were prepared and assessed for their ability to bind HBGF-1 and potentiate the biological activity of the growth factor. The sulfate content, amino group content, and anticoagulant activity of each heparin preparation were assessed and compared with predicted values. It was found that the sulfate content of native heparin was 32.1%; N-desulfated heparin 23.4%; N-desulfated, N-acetylated 22.8%; N-desulfated, N-acetoacetylated 19.8%; totally desulfated <0.2%; totally desulfated, N-acetoacetylated <0.2%; totally desulfated, N-resulfated 12.3%; and carboxyl-reduced 31.5%. These results indicate that removal and addition of sulfate groups were virtually complete. Assessment of the amino group content of the different heparin preparations confirmed this conclusion. N-Desulfation resulted in approximately 80% exposure of the amino groups of glucosamine, >99% of the free amino groups were blocked by acetylation and acetoacetylation, and there was 85% N-resulfation of totally desulfated heparin. The anticoagulant activity of the seven chemically modified heparins was measured by the activated partial thromboplastin time and thrombin time tests and was found, as reported by others (Casu, 1985), to be in all cases <1% of native heparin.

Binding of HBGF-1 to Different Chemically Modified Heparins. Initial experiments compared the ability of native heparin and its chemically modified variants to compete for the binding of HBGF-1 to heparin–agarose. HBGF-1 bound to heparin–agarose was quantified by SDS–PAGE and densitometer scanning of stained gels. Figure 1 demonstrates that

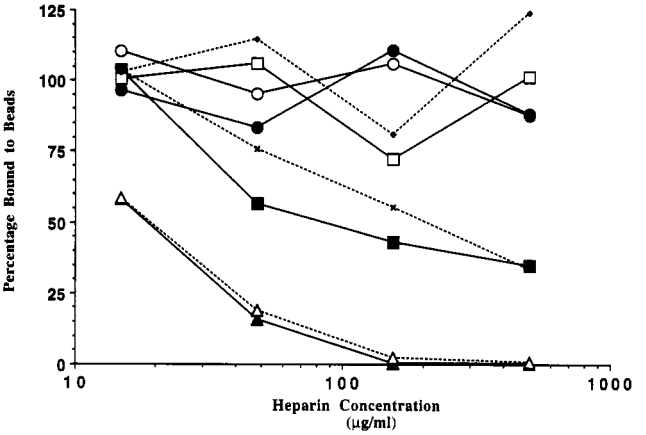

FIGURE 1: Inhibition of HBGF-1 binding to heparin–agarose by the indicated modified heparins as measured by SDS–PAGE and densitometer scanning of stained gels. Heparin (▲—▲) N-desulfated (○—○); N-desulfated, N-acetylated (×---×); N-desulfated, N-acetoacetylated (■—■); totally desulfated, N-acetoacetylated (●—●); totally desulfated (□—□); carboxyl-reduced (△---△); totally desulfated, N-resulfated (♦---♦). Values from each gel were normalized to the average of four HBGF-1 standards and are expressed as percentage of control binding. Each point represents the mean of duplicate determinations.

Table I: ED_{50} of Modified Heparins on Survival of Ciliary Neurons and Proliferation of 3T3 and HUVE Cells[a]

heparin prepn	ED_{50} of heparin (μg/mL) HUVE	3T3	neurons
heparin	2	5	70
N-desulfated	>100	100	5
N-desulfated, N-acetylated	58	>100	12
N-desulfated, N-acetoacetylated	58	100	NA
totally desulfated, N-acetoacetylated	NA	NA	65
totally desulfated	NA	NA	25
carboxyl-reduced	60	37	9
totally desulfated, N-resulfated	NA	100	40

[a] Dose response curves were obtained for the modified heparins in the presence of a constant concentration (10 ng/mL) of beef heart HBGF-1. The dose required for 50% of the maximal stimulation observed was calculated by regression analysis of the curves. NA = not active.

the native and carboxyl-reduced heparins compete for binding of HBGF-1 with equal relative affinities; i.e., 50% inhibition was achieved at 15 and 17 μg/mL, respectively. N-Desulfated heparin did not inhibit binding of HBGF-1 at concentrations up to 500 μg/mL. Acetylation or acetoacetylation of the N-position of the glucosamine residues partially restored inhibitory capacity although these species were still approximately 10-fold less effective at competing with HBGF-1 binding to heparin–agarose than native heparin (50% inhibition at 170 μg/mL for N-acetylated and 140 μg/mL for N-acetoacetylated heparin). Totally desulfated; totally desulfated, N-acetoacetylated; and totally desulfated, N-resulfated heparins all failed to inhibit HBGF-1 binding over the concentration range tested.

Potentiation of Biological Activity of HBGF-1 by Chemically Modified Heparins. (A) Proliferation of HUVE Cells. The ability of the modified heparins to potentiate HBGF-1-induced proliferation of HUVE cells is shown in Figure 2. Native heparin is the most biologically active form of the molecule, potentiating the proliferative activity of HBGF-1 in log-linear fashion to 100 μg/mL with an ED_{50} of 2 μg/mL (Table I). Carboxyl-reduced heparin is less potent, 60 μg/mL being required to achieve the ED_{50} proliferation response of native heparin (Figure 2A). N-Desulfated heparin which had

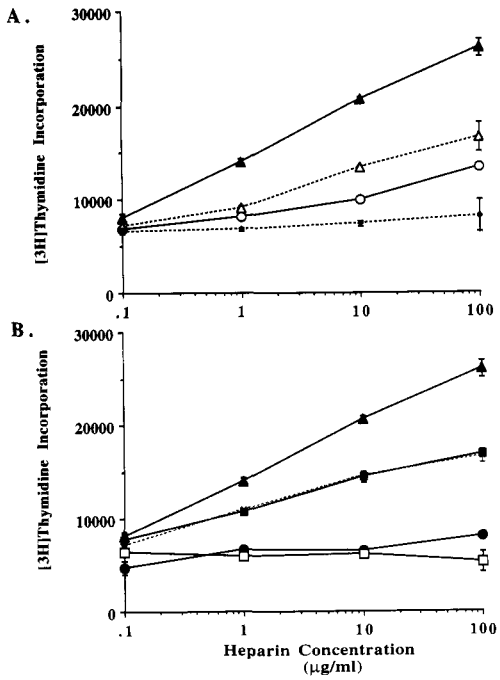

FIGURE 2: Effect of the modified heparins on HBGF-1-induced HUVE cell division. The dose response to the indicated modified heparin is shown in the presence of a constant concentration (10 ng/mL) of beef heart HBGF-1. [^{3}H]Thymidine incorporation is expressed as counts per minute. (A) Heparin (▲—▲); N-desulfated (○—○); carboxyl-reduced (△---△); totally desulfated, N-resulfated (♦---♦). (B) Heparin (▲—▲); N-desulfated, N-acetylated (×---×); N-desulfated, N-acetoacetylated (■—■); totally desulfated (□—□); totally desulfated, N-acetoacetylated (●—●). Values represent means and standard errors (n = 4) of one representative experiment. Identical results were obtained on three separate occasions.

been either acetylated or acetoacetylated exhibited similar biological activity to carboxyl-reduced heparin with 58 μg/mL required to potentiate HBGF-1 to a degree equivalent to the ED_{50} for heparin (Figure 2B). The N-desulfated heparin was much less active although significantly potentiated HBGF-1 activity at high concentrations (Figure 2B). In contrast, the totally desulfated; totally desulfated, N-acetoacetylated; and totally desulfated, N-resulfated forms were inactive.

(B) Proliferation of Balb/c 3T3 Cells. The ability of the modified heparins to potentiate HBGF-1-induced proliferation of 3T3 cells is shown in Table I. Native heparin is the most biologically active form of the molecule with an ED_{50} of 5 μg/mL, the carboxyl-reduced form was the next most potent with an ED_{50} of 37 μg/mL, and the remaining modified heparins were much less active. The potentiating effects of the modified heparins were confirmed by determining the dose response of 3T3 cells to HBGF-1 in the presence of a constant concentration (50 μg/mL) of modified heparin. Given that none of the heparins was inhibitory at this high concentration, it provided an accurate determination of the potentiating activity of the modified heparins. Dose response curves for HBGF-1 in the presence of the given modified heparin are shown in Figure 3A–D. In agreement with previous studies, addition of heparin resulted in a significant shift of the dose response curve to the left. A lesser degree of potentiation was seen with the addition of the carboxyl-reduced molecule (Figure 3A). N-Desulfation of the heparin chain diminished its capacity to augment HBGF-1-induced mitosis although this preparation increased the activity of HBGF-1 at every concentration tested (Figure 3B). Acetylation or acetoacetylation of the desulfated N-position in the heparin chain increased its potentiating ability although not to the level of activity

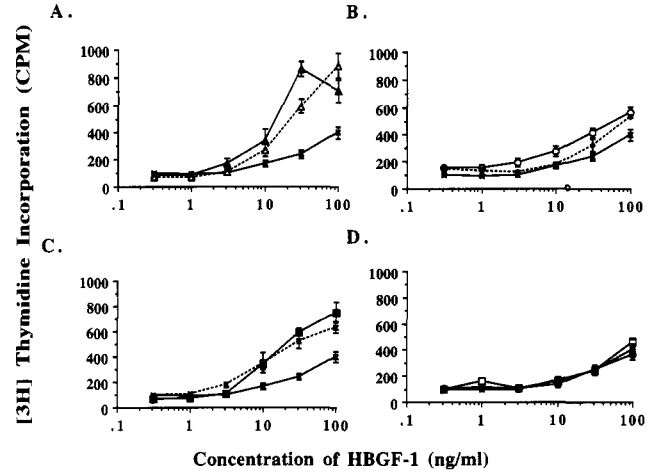

FIGURE 3: Effect of the modified heparins on HBGF-1-induced Balb/c3T3 cell division. A dose response to HBGF-1 is shown in the presence of a constant concentration (50 μg/mL) of the indicated modified heparin. [^{3}H]Thymidine incorporation is expressed as 10^{-2} cpm. (A) Control (×—×); heparin (▲—▲); carboxyl-reduced (△---△). (B) Control (×—×); N-desulfated (○—○); totally desulfated, N-resulfated (♦---♦). (C) Control (×—×); N-desulfated, N-acetylated (×---×); N-desulfated, N-acetoacetylated (■—■). (D) Control (×—×); totally desulfated (□—□); totally desulfated, N-acetoacetylated (●—●). Values represent means and standard errors of four experiments.

Table II: Effect of Modified Heparins on Survival of Ciliary Neurons[a]

heparin prepn	cell no. at heparin concn (μg/mL) of 10	100
native heparin	396 ± 34	385 ± 12
N-desulfated	517 ± 28	671 ± 17
N-desulfated, N-acetylated	418 ± 42	539 ± 58
N-desulfated, N-acetoacetylated	396 ± 35	396 ± 44
totally desulfated, N-acetoacetylated	319 ± 16	605 ± 8
totally desulfated	407 ± 46	594 ± 20
carboxyl-reduced	440 ± 23	583 ± 31
totally desulfated, N-resulfated	385 ± 46	473 ± 13
control (no heparin)	264 ± 19	

[a] Effect of the modified heparins on HBGF-1-promoted ciliary neuronal survival. The survival response to the modified heparin is shown in the presence of a constant concentration (25 ng/mL) of beef heart-derived HBGF-1. Pooled data from four to six experiments are expressed as the total number ± standard error of survival neurons per well.

observed with the native molecule (Figure 3C); i.e., HBGF-1 activity in the presence of the N-acetylated or N-acetoacetylated molecules is similar to that in the presence of the carboxyl-reduced heparin. The totally desulfated, N-acetoacetylated and the totally desulfated heparins possessed no growth augmenting capacity (Figure 3D). N-Resulfation of the desulfated molecule produced a heparin species that slightly potentiated HBGF-1 at all concentrations tested (Figure 3B).

(C) Survival of Ciliary Neurons. The ability of the modified heparins to potentiate the neurotrophic activity of HBGF-1 was, in general, less than their ability to augment mitogenic activity. Tables I and II show that the neurotrophic activity of HBGF-1 for ciliary neurons is potentiated by all heparin species, although the native molecule is less active in enhancing HBGF-1 activity for ciliary neurons than some of the modified derivatives. Most active were the heparin molecules that were N-desulfated, carboxyl-reduced, or totally denuded of sulfate groups. These species exhibited a dose-related potentiation of HBGF-1 activity peaking around (2–2.5) × control at 100 μg/mL. There was no neuronal

survival in cultures containing the heparin preparations without HBGF-1.

Discussion

Using a range of chemically modified heparins, this study aimed to establish the structural features of heparin which play a critical role in HBGF-1 binding and potentiation of HBGF-1 action. In terms of HBGF-1 binding, the observation that carboxyl-reduced heparin bound HBGF-1 as effectively as the native heparin molecule indicated that the carboxyl groups of the uronic acid residues play little or no role in HBGF-1 binding. In contrast, totally desulfated heparin failed to interact with HBGF-1, implying that sulfate groups are essential for HBGF-1 binding. N-Desulfated heparin also lacked HBGF-1-binding capacity, and substitution of the exposed amino group with acetyl or acetoacetyl groups only partially restored binding capacity, indicating that *N*-sulfates play some role in growth factor binding. However, the failure of totally desulfated, N-resulfated heparin to interact with HBGF-1 demonstrated that *N*-sulfates alone are insufficient and ester sulfates are absolutely essential for HBGF-1 binding although whether the positioning of the ester sulfates on the polysaccharide backbone is of critical importance remains to be determined. The structural requirements for HBGF-1 binding to heparin are similar to those reported for the inhibition by heparin of complement C3 convertase (Kazatchkine et al., 1981) and the inhibition of smooth muscle cell proliferation (Castellot et al., 1985).

The ability of heparin to enhance HBGF-1-induced mitosis is well documented (Gospodarowicz & Cheng, 1986). Our results using 3T3 and HUVE cells are in line with those previously reported. However, the ability of the modified heparins to potentiate the mitogenic activity of HBGF-1 correlates only to a limited extent with their affinity for HBGF-1. The carboxyl-reduced molecule displayed similar affinity for HBGF-1 compared to the native heparin, although was consistently less potent in augmenting mitogenesis. The N-acetylated and the N-acetoacetylated species, although possessing less affinity for HBGF-1 than the carboxyl-reduced molecule, conferred similar biological activity to HBGF-1 for both 3T3 and HUVE cells. Moveover, N-desulfated heparin, although exhibiting no binding activity, increased the mitogenic activity of HBGF-1 for both 3T3 and HUVE cells above that seen with HBGF-1 alone. To a lesser extent, the totally desulfated, N-resulfated heparin potentiated HBGF-1 activity over 3T3 cells, an effect not seen with HUVE cells, whereas this heparin species failed to compete for HBGF-1 binding at concentrations up to 500 μg/mL. The totally desulfated, N-acetoacetylated and the totally desulfated heparins neither competed for HBGF-1 binding nor potentiated HBGF-1 above control levels.

Potentiation of neurotrophic activity revealed further variation in the activity of the modified heparin species. Indeed, heparin species without activity in the mitogenic assays were effective in promoting the neurotrophic activity of HBGF-1. In fact, removal of all sulfate groups resulted in heparin preparations capable of potentiating neurotrophic activity. Taken together, these results suggest additional mechanisms of HBGF-1 activation apart from those based entirely on a heparin–HBGF-1 association.

The observed variation in the biological activity of the modified heparins may be the result of several factors. First, the assays are different as the neuronal survival bioassay is of shorter time course than the cell proliferation assays and depends on cell survival rather than induction of DNA synthesis. Second, heparin may potentiate HBGF-1 action by protecting it from proteolysis, an effect which may be cell-dependent. There is ample evidence that heparin–HBGF-1 complexes are resistant to proteolysis (Gospodarowicz & Cheng, 1986; Rosengart et al., 1988) but the modified heparins that do not bind HBGF-1 could not potentiate growth factor action by this mechanism. Third, HSPG are known to be involved in cell attachment and spreading (Culp et al., 1980). Differential effects of the modified heparins on this aspect of cell behavior may account for some of the observed differences in activity. Neurons must first attach to the tissue culture substratum in order to survive, and it is possible that such mechanical factors are influenced to different degrees by the modified heparins. Early reports indicated that heparan sulfate and heparin were nonpermissive substrates for neuronal attachment and neurite outgrowth (Manthorpe et al., 1983) although more recently it was reported that an HSPG increased neurite elongation by dissociated E14 rat spinal neurons (Hantaz-Ambroise et al., 1987). Finally, it seems likely that heparin and its active derivatives act directly on target cells at a site distinct from the HBGF-1 receptor (Gospodarowicz & Cheng, 1986; Neufeld et al., 1987). In this context, cell-surface-binding sites for heparin have been described on smooth muscle cells (Castellot et al., 1985), hepatocytes (Kjellen et al., 1977), endothelial cells (Glimelius et al., 1978), fibroblasts (Chong & Parish, 1986), and neurons (Vidovic et al., 1986) as well as on cells of the reticuloendothelial system including macrophages and monocytes (Chong & Parish, 1986) and lymphocytes (Parish & Snowden, 1985). Furthermore, heparin has been shown to act on smooth muscle cells via cell-surface receptors to inhibit division both in vivo (Clowes & Karnovsky, 1977) and in vitro (Reilly et al., 1986). The recent demonstration that cell-surface-associated HSPG are essential for basic FGF action supports this view (Yayon et al., 1991; Rapraeger et al., 1991) although whether a similar situation exists with HBGF-1 remains to be clarified. In fact, recent studies have shown that both heparin and carboxyl-reduced heparin have no detectable effect on the high-affinity binding of radiolabeled HBGF-1 to fibroblasts (K. Brown and C. Parish, unpublished results).

Several other studies have shown that the simple association of heparin with growth factors does not account for all the reported potentiating effects of heparin on growth factor activity. For example, heparin, heparan sulfate, and chondroitin sulfate have been reported to potentiate the neurotrophic effects of NGF on PC12 cells (Neufeld et al., 1987; Damon et al., 1988) although heparin does not bind NGF (Watters and Hendry, unpublished observation). Heparin has also been reported to have differential effects on adrenal chromaffin cells, potentiating the neurite outgrowth response to HBGF-1, but having no effect on HBGF-1-induced mitogenesis (Claude et al., 1988). The present study has shown that the ability of modified heparin species to potentiate the biological activity of HBGF-1 is not only a direct function of their ability to bind HBGF-1. It is proposed that some of the biological activity of heparin is due to the direct action of heparin on cells possibly via heparin-specific receptors on the cell surface.

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