HEPARIN: A POTENT INHIBITOR OF OVARIAN LUTEINIZING HORMONE-SENSITIVE ADENYLATE CYCLASE

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1. Introduction

Sulfated mucopolysaccharides, such as heparin, heparan sulfate, chondroitin sulfates and dermatan sulfate, have been shown to be normal constituents of animal tissues and body fluids. Their biological functions are not yet known with certainty. A role as lubricants in the living organism was ascribed to these sulfated mucopolysaccharides due to their highly viscous and elastic properties in solution. Heparin displays anticoagulant and anti-lipaemic properties [1]. Direct effects on various enzymes have been described for polyelectrolytes including heparin and other sulfated glycosaminoglycans [2,3]. Wolff and Cook [4] have recently described the effects of polycations (RNAase A, spermin, spermidine and polylysine) and of polyanions (Dextran sulfate, Suramin and polyaspartic acid) on bovine thyroid-stimulating-hormone-sensitive adenylate cyclase.

Hyaluronic acid and chondroitin sulfuric acid were shown to be constituents of the follicular fluid in the bovine ovary [5]. We have recently shown that heparin-like substances as well as chondroitin-4-sulfate, chondroitin-6-sulfate and dermatan sulfate are synthesized and accumulate in the rat ovary [6,7]. In searching for a functional role for ovarian glycosaminoglycans we examined the possibility that these substances may modulate the activity of the LH-stimulated adenylate cyclase in the follicle, and thus participate in controlling ovulation-related events in the ovary. This hormone-sensitive enzyme is known to be stimulated equally well by both LH and hCG [8]. These hormones have also been shown to compete for the same receptor sites in this tissue [9].

In this study, we demonstrate that the sulfated glycosamineglycans, heparin, heparan sulfate and dermatan sulfate, are potent inhibitors of ovarian adenylate cyclase. Heparin was the most effective inhibitor, and was shown to inhibit LH/hCG stimulated ovarian adenylate cyclase but had only a small effect on enzyme activity when assayed in the absence of these hormones or when stimulated by NaF. The data presented here suggest a selective effect of heparin on the hormone receptor level or its coupling to adenylate cyclase, thus providing a new tool for the analysis of receptor enzyme interations.

Abbreviations: hCG, human chorionic gonadotropin; LH, luteinizing hormone; PMSG, present mare serum gonadotropin; Hep, heparin; DS, dermatan sulfate; HS, heparan sulfate; Dex S, dextran sulfate; Poly Glu, polyglutamic acid

2. Materials and methods

[α-^{32}P]ATP, adenosine 3′5′-cyclic monophosphate, dithiothreitol, GTP, creatine phosphate, creatine phosphokinase and dextran sulfate (mol wt ~ 500 000) were from Sigma Chemical Corp. Ovine LH (NIH-LH-S18) was kindly supplied by the US NIH Hormone Distribution Center. Pregnant mare serum gonadotropin (PMSG: Gestyl, NV) was purchased from Organon, Oss, Holland. Human chorionic gonadotropin 13 700 IU/mg was from Serono, Rome. Sodium heparin grade B 159 U/mg was from Calbiochem. Highly purified heparan sulfate, and highly purified dermatan sulfate were kindly supplied by Dr M. B. Marthews, University of Chicago, under a

contract (NO1-AM-5-2205) from the US NIH (NIAMD). Polyglutamic acid (mol wt ~ 10 000) was a gift from Dr N. Lotan, Weizmann Institute of Science.

Wistar-derived female rats 25 days old were given a single injection (15 IU) PMSG at 11.00–12.00 a.m. in order to increase the amount of ovarian LH-stimulatable adenylate cyclase activity [10]. Ovaries were excised 48 h later and plasma membranes were prepared by modification [11] of the method described by Neville [12]. Adenylate cyclase assays were performed as described [13]. Protein was determined according to Lowry et al. [14] using bovine serum albumin as standard.

3. Results

Heparin (10 μg/ml) essentially abolished the activity of rat ovarian adenylate cyclase when stimulated by hCG (fig.1) or by LH (fig.2). Inhibition was dose-dependent over the range of 1–10 μg/ml, reaching 50% at a heparin-concentration of 2 μg/ml (fig.1). Hormone-stimulatable adenylate cyclase activity appeared to be more susceptible to the inhibitory action of heparin than was basal activity. At a concentration of 10 μg/ml heparin reduced hCG-sensitive adenylate cyclase activity to one-tenth and basal activity to one-third, and had only a marginal effect (15% inhibition) on NaF stimulated activity (fig.1), indicating a rather selective effect on the hormone-stimulatable adenylate cyclase activity. Other glycosaminoglycans when tested at concentrations of up to 100 μg/ml were also inhibitory, but to a lesser degree (fig.2). It was interesting to note that highly purified heparan sulfate which is structurally related to heparin [1], was much less effective and inhibited LH-dependent activity by only 66% at a concentration of 100 μg/ml, compared with 82% inhibition by heparin at a much lower concentration (10 μg/ml). Highly purified dermatan sulfate inhibited LH-sensitive enzyme activity by 38% at a concentration of 10 μg/ml and by 76% at 100 μg/ml. The sulfated mucopolysaccharides chondroitin-4-sulfate and chondroitin-6-sulfate, as well as the non-sulfated glycosaminoglycan hyaluronic acid were found to be without effect on adenylate cyclase even at concentrations of 100 μg/ml (data not shown). Two synthetic polyanions were tested for their effect on the LH-sensitive adenylate cyclase activity. Dextran sulfate at a concentration of

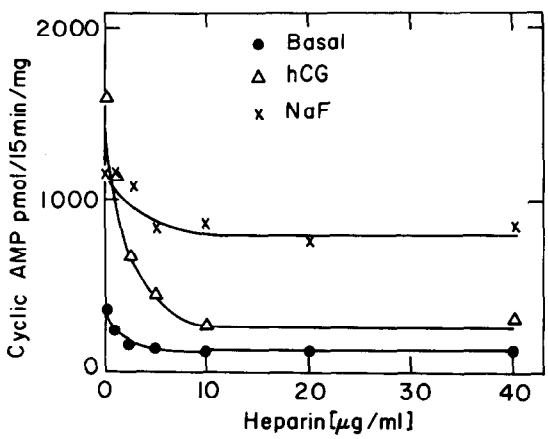

Fig.1. Inhibition of ovarian adenylate cyclase activity by heparin. The reaction mixture (final vol. 50 μl) contained: Tris acetate, pH 7.6, 25 mM: magnesium acetate, 5 mM; [α-^{32}P]ATP, 0.5 mM (2–6 × 10^6 cpm); adenosine 3′,5′-cyclic monophosphate, 0.05 mM; dithiothreitol, 1 mM; GTP, 0.01 mM, creatine phosphate, 5 mM; creatine phosphokinase, 50 U/ml and heparin at the concentration indicated. When added hCG was 5 i.u/ml and NaF 10 mM. The reaction was initiated by the addition of purified ovarian plasma membranes (3.7 μg protein/assay tube) and terminated after 15 min incubation at 30°C. Activity determined in the absence of LH is referred to as basal adenylate cyclase activity. Points represent mean values of duplicate assays; deviation within pair did not exceed 10%.

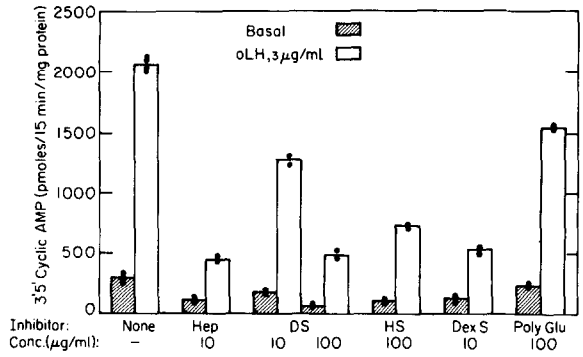

Fig.2. The inhibitory action of various polyanions on ovarian adenylate cyclase activity. The effect of heparin (Hep), dermatan sulfate (DS), heparan sulfate (HS), dextran sulfate (Dex S) and polyglutamic acid (Poly glu) on adenylate cyclase activity was determined as described in legend to fig.1 using 5.0 μg plasma membrane protein/assay tube.

10 μg/ml inhibited 77% of the LH-simulatable activity, while polyglutamic acid exerted only a weak inhibitory action (25% loss of activity) at a concentration of 100 μg/ml (fig.2).

4. Discussion

This study demonstrates that heparin, and to a lesser extent, dermatan sulfate and heparan sulfate, inhibit the activity of LH-sensitive adenylate cyclase of the rat ovary. Other sulfated glycosaminoglycans such as chondroitin-4-sulfate and chondroitin-6-sulfate, and the non-sulfated substance hyaluronic acid were found to be without effect. This finding is suggestive of a selective effect which may be related to the degree of sulfation and the proximity of the sulfate groups to each other on the sugar backbone [1,15]. The inhibitory effect could not be attributed solely to the nonspecific action of negative charges, since polyglutamic acid, a negatively charged polypeptide, was only slightly inhibitory.

Hormone-dependent adenylate cyclase activity was more susceptible to the inhibitory action of heparin than was basal activity. In the presence of NaF, adenylate cyclase activity was hardly effected. The relative resistance of catalytic activity in the absence of hormone (i.e., basal and NaF) suggests that heparin acts mainly at the hormone receptor level. This is further supported by the finding that hormone binding to this membrane preparation was inhibited selectively by those glycosaminoglycans which exerted inhibitory action on adenylate cyclase activity (Salomon, Y. and Amsterdam, A., unpublished). However, the inhibitory action on basal enzyme activity, though more limited, suggests that heparin also exerts a direct action on adenylate cyclase.

Wolff and Cook [4] described an inhibitory action of dextran sulfate on thyroid-stimulating-hormone-sensitive adenylate cyclase of bovine thyroid gland. This indicates that the inhibitory action of sulfated polyanions is not restricted to ovarian adenylate cyclase. However, the inhibitory action of naturally occurring glycosaminoglycans on adenylate cyclase in other tissues has to be established. Heparin modified catalytic parameters of soluble monomeric enzymes, such as trypsin [3], amylase and ribonuclease [2]. As shown in this study, heparin influenced mainly regulatory properties of the membrane bound adenylate cyclase, an enzyme which is generally believed to be a multicomponent structure. Being a negatively charged polymer it is not unlikely that by adsorbing to opposite charges exposed on the surface of the plasma membrane, heparin may also restrict the lateral mobility of the components of adenylate cyclase, thus interfering with its normal function. Heparin therefore represents a new potential tool for the study of receptor enzyme interactions.

The occurrence of heparin-like substances and dermatan sulfate in the rat ovary was recently described in our laboratory [6,7]. It is therefore tempting to suggest that these substances might play a regulatory role in ovulation-related events by affecting the activity of adenylate cyclase in the ovary. Degradation of ovarian mucopolysaccharides at late stages of follicular maturation has been demonstrated [5,16]. Since the barrier between the follicular tissue and the vascular system is highly permeable [17,18], it is likely that the resulting breakdown products may be readily removed, thus permitting activation of LH-sensitive adenylate cyclase. Interestingly, polycystic ovaries, which fail to ovulate, are characterized by abnormally high levels of acid mucopolysaccharides [19].

To the best of our knowledge, this is the first description of a well-defined inhibitor of adenylate cyclase naturally occurring in body fluids. Whether heparin and related substances also participate in regulation of adenylate cyclase activity in other tissues in which they are abundant, e.g., liver, lung and arterial wall, is an issue which should be investigated.

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