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THE EFFECT OF ORAL CONTRACEPTIVES ON RAT PLATELET MEMBRANE GLYCOPROTEINS

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Female rats were administered oral contraceptives and the levels of sialic acid on platelet membrane and granule glycoproteins were compared to controls using a sialic acid assay and a fluorescein-conjugated wheat germ agglutinin binding assay and also by measuring the binding of ^{125}I-labelled wheat germ agglutinin to glycoprotein bands from platelets separated by polyacrylamide electrophoresis. The contraceptive-treated rats showed increased levels of glycoprotein sialylation which may partly explain the altered physiological function of the platelets.

Introduction

Oral contraceptives have been implicated in the increased incidence of thromboembolism observed in women, ranging from thrombophlebitis to fatal pulmonary and coronary embolism [1]. Blood platelets, obtained from women or female rats taking oral contraceptives, show enhanced in vitro coagulation and aggregation activities [2,3]. The mechanisms by which platelet physiological functions are altered by oral contraceptives are as yet poorly understood. However, recent studies performed on cells of different organs from a number of species have indicated that estrogen increases glycoprotein synthesis in female genital organs, in rabbit uterus and rat tracheal epithelium [4,5] and has an enhancing effect on the glycosyltransferase activities in rat endometrial tissue [6].

In human platelets, membrane glycoproteins are thought to play a major role in the formation of platelet aggregates. Platelets of patients with hereditary bleeding disorders show defects in adhesion and aggregation which can be correlated with lack of certain membrane glycoproteins [7]. In addition alterations in platelet physiological functions have been associated with quantitative changes in platelet membrane glycoproteins occurring in certain acquired diseases [8]. In vitro experiments have indicated that ethinylestradiol induces an increase in the surface levels of sialic acid on platelets [9] and platelets from patients with prostatic cancer who had been treated with estrogen showed an increased negative surface charge due to increased levels of sialic acid [10].

This paper deals with the effect of oral contraceptives (ethinylestradiol + lynestrenol) on rat platelet membrane glycoproteins.

Methods and Materials

Chemicals used for the washing and preparation of platelets for gel electrophoresis were obtained as previously described [11]. Wheat germ agglutinin and fluorescein-conjugated wheat germ agglutinin were from Miles Laboratories Inc. (Elkhart, IN, U.S.A.). Carrier-free Na^{125}I (17

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Ci/mg) was from New England Nuclear (Heidelberg, F.R.G.). Ethinylestradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yn-3,17-diol) was from Nutritional Biochemical Corp. (Cleveland, OH, U.S.A.). Lynestrenol (19-nor-17α-pregna-4-en-20-yn-17-ol) was a gift from Organon Co. (Oss, The Netherlands).

Six adult, female, Sprague-Dawley rats (180–250 g) were stomach fed with 20 μg ethinylestradiol and 500 μg lynestrenol dissolved in 0.5 ml olive oil per 100 g body weight. The control rats (six) were given 0.5 ml olive oil per 100 g body weight. Both groups were treated for eight days and were fasted overnight before the experiments.

Blood was drawn from the rats by the technique of Renaud and Lecompte [12]. The platelets were isolated and washed by the method of Massini and Lüscher [13] with the addition of 10 mM EDTA to the solutions. The total sialic acid content of the platelets was determined by the method of Aminoff [14]. The protein content of the samples was measured by the method of Lowry et al. [15].

The number of binding sites (n) and the association constant of fluorescein conjugated wheat germ agglutinin on washed platelets from contraceptive-treated female rats or controls were determined using the method of Monsigny et al. [16]. Different concentrations of lectin (5–50 μg) were incubated with $0.25 \cdot 10^9$/ml washed platelets for 1 h at 4°C. The platelets were washed twice in phosphate buffered saline containing 0.35% bovine serum albumin and then incubated in phosphate buffered saline containing 0.3 M GlcNAc for 1 h at 4°C. After centrifugation the content of fluorescein conjugated wheat germ agglutinin in the supernatant was measured on a Perkin-Elmer MPF-3 fluorometer. The data obtained were analysed using a Lineweaver-Burk plot [17].

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of sodium dodecyl sulphate-solubilized washed platelets from contraceptive-treated and control rats and binding of ^{125}I-labelled lectins to the separated glycoproteins were performed as previously described [11]. Equal amounts of protein (80 μg), reduced with dithiothreitol (1%), from contraceptive-treated and control rat platelets, were separated on 7% polyacrylamide gels. Lactoperoxidase-catalyzed surface-labelling of platelets with ^{125}I and indirect autoradiography with Kodak X-Omat films were carried out as previously described [18]. Densitometry of the autoradiograms was made with a Vernon PH I-6 microdensitometer. Peaks from the densitometer tracings were quantitated using a planimeter. The data obtained for the sialic acid concentration, the values of n and K, and the binding of ^{125}I-labelled wheat germ agglutinin to the glycoprotein bands were analysed statistically using the paired t-test.

Results

Platelets from female rats administered oral contraceptives had significantly higher amounts of total sialic acid present (> 130%) compared to platelets from control rats as measured by a sialic acid assay (Table I) and a fluorescent lectin (fluorescein-conjugated wheat germ agglutinin, sialic acid and GlcNAc specific) binding assay (Table II). The association constant, K, of fluorescein-conjugated wheat germ agglutinin to contraceptive-treated and control rat platelets did not differ significantly (Table II). Washed contraceptive-treated or control rat platelets were also solubilized in sodium dodecyl sulphate and electrophoresed on sodium dodecyl sulphate-polyacrylamide gels under reducing conditions. When

TABLE I

TOTAL SIALIC ACID CONTENT OF PLATELETS FROM FEMALE RATS TREATED WITH ORAL CONTRACEPTIVES AND THEIR CONTROLS

Six determinations per group. Paired t-test *, $P < 0.05$.

Total sialic acid	Contraceptive group		Control group	
	μg/10^9 platelets	μg/mg protein	μg/10^9 platelets	μg/mg protein
Mean ± S.E.	6.09 ± 0.19 *	7.72 ± 0.62 *	4.32 ± 0.85	5.85 ± 0.61

TABLE II

THE NUMBER OF BINDING SITES AND THE ASSOCIATION CONSTANT OF FITC-WGA TO PLATELETS FROM CONTRACEPTIVE-TREATED RATS AND THEIR CONTROLS

Six determinations per group. n, number of binding sites; K, association constant; FITC, fluorescein isothiocyanate; WGA, wheat germ agglutinin. Paired t-test, $P < 0.05$.

	Contraceptive group		Control group	
	$n (\times 10^5)$	$K (\times 10^7)$	$n (\times 10^5)$	$K (\times 10^7)$
Mean ± S.E.	2.90 ± 0.51 **	0.11 ± 0.03	1.59 ± 0.28 **	0.16 ± 0.03

TABLE III

AMOUNT OF ^{125}I-LABELLED WHEAT GERM AGGLUTININ BINDING TO FOUR OF THE MAJOR GLYCOPROTEIN BANDS FROM CONTRACEPTIVE-TREATED RAT PLATELETS AND THEIR CONTROLS

Peaks on densitometric tracings of autoradiograms were quantitated using a planimeter, GP, glycoprotein; CT, contraceptive-treated group (rats fed 20 μg ethinyl estradiol + 500 μg lynestrol per 100 g body wt.). C, control group (rats fed olive oil 0.5 mg/10 g body wt.). Six determinations per group. Paired t-test, $*P < 0.02$, $**P < 0.05$.

	Height of peaks (mm)							
GP bands	170 kDa		130 kDa		120 kDa		95 kDa	
	CT	C	CT	C	CT	C	CT	C
Mean ± S.E.	103.67 ± 4.14 **	87.67 ± 4.00	76.34 ± 3.67 **	52.67 ± 3.72	27.34 ± 2.69 *	19.67 ± 1.79	22.50 ± 2.53 *	15.67 ± 1.17

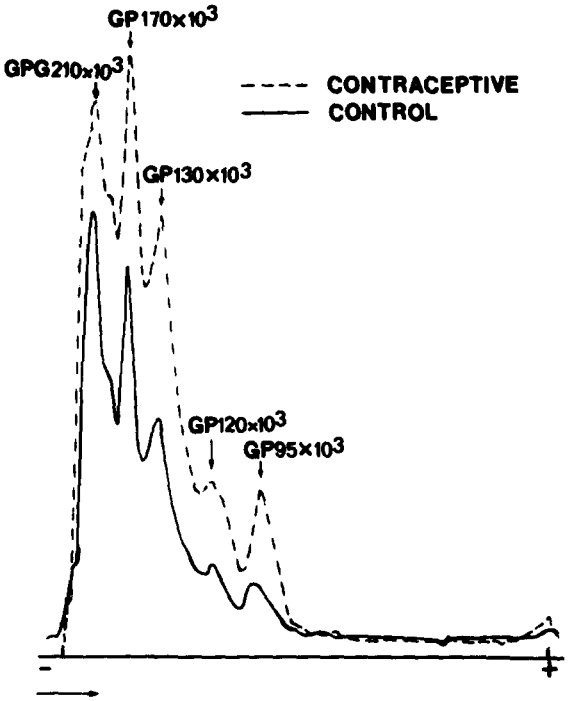

Fig. 1. Densitogram of autoradiogram of polyacrylamide gel electrophoresis of contraceptive-treated and control rat platelets after binding of ^{125}I-labelled wheat germ agglutinin to the glycoprotein (GP) bands.

these polyacrylamide gels were incubated with ^{125}I-labelled wheat germ agglutinin and extensively washed, the platelet membrane glycoprotein bands and an α-granule glycoprotein band (thrombospondin) from contraceptive-treated rats bound significantly more lectin than glycoprotein bands from control platelets (Table III, Fig. 1). When ^{125}I-labelled concanavalin A or *Lens culinaris* lectin were used, no difference was observed in the binding of these lectins to the glycoproteins of contraceptive-treated or control platelets (results not shown). No differences were seen in the intensity of staining of the glycoprotein bands with Coomassie blue G250 between platelets from contraceptive-treated rats and controls. When intact platelets were surface-labelled using ^{125}I, solubilized, separated by gel electrophoresis and the gels autoradiographed, no difference in the labelling of the surface proteins of contraceptive-treated and control platelets was observed (results not shown).

Discussion

These results clearly show that contraceptives increase significantly the level of sialic acid on

platelets of female rats. This finding is in close agreement with results which indicate that contraceptives enhance cellular glycoprotein synthesis and/or glycosyl transferase activities in organs of animals of different species [4–6,19]. In addition, recent evidence has shown that a precursor of cholesterol, lanosterol, is present in significantly increased amounts in platelets which have been administered contraceptives [20]. Hemming and co-workers [21] have demonstrated that cholesterol is a component of the lipid intermediate pathway involved in protein glycosylation and that rats fed a high cholesterol diet have a higher rate of hepatic *N*-glycosylation (22). The observed alteration in platelet sialic acid may therefore be the consequence of the contraceptives stimulating glycoprotein synthesis and/or glycosyltransferase activities in rat megakaryocytes. This hypothesis is supported by the fact that the contraceptive effect is not immediate but requires administration for periods of 4–5 days, which corresponds to the average life span of circulating rat platelets [23]. It is probable that this time is required for the contraceptive to affect the levels of sialic acid on the megakaryocytes significantly before shedding of platelets occurs.

The role of surface sialic acid in platelet aggregation is controversial. Its removal by neuraminidase treatment has been reported to lower [24], have no effect on [25], or slightly enhance [26,27] platelet activity in different species. It is, however, firmly established that platelets are removed from circulation after cleavage of as little as 8–10% of the surface sialic acid [27]. Mester et al. [28] increased the surface sialic acid levels of platelets by using a crude preparation of rat liver sialyltransferase and observed a reduction in platelet aggregation induced by ADP. Intravenous injection of high concentrations of sialic acid has been shown to inhibit platelet thrombus formation after laser injury in various animal species [29]. Most of these observations are from in vitro experiments and are therefore difficult to relate to the situation in vivo. In the case of platelets from contraceptive-treated rats, the increased sialic acid content and the hyperaggregability observed are not necessarily directly linked. Contraceptives alter the cholesterol: phospholipid ratio and therefore increase the microviscosity of the membrane [19]. In lymphocytes and fibroblasts an increase in the microviscosity of the membrane leads to the exposure of additional receptor sites on the cell surface [30]. In platelets an increase in the availability of surface receptors leading to an increased binding of physiological activators could explain the increase in platelet aggregation. The increased levels of sialic acid may be merely coincidental and may play no active role except to increase the negative surface charge of the platelets. On the other hand it has been shown that young platelets are more active and have higher levels of sialic acid [31,32]. The hyperactivity of the platelets from contraceptive-treated rats to various activators could also be due to an increased proportion of young platelets because of the megakaryocytes being stimulated into increased platelet production by the contraceptive. However, the contraceptive-treated rats did not show an elevated platelet count compared to the control rats.

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