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Short Report

Hormone-Induced Variation of Phosphatidate Phosphohydrolase Activity in Various Tissues of Pregnant Rats

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ABSTRACT

Variations in phosphatidate phosphohydrolase (PAP) activity and triacylglycerol concentration were measured in pregnant and hormone-treated non-pregnant female rats. PAP activity in adipose tissue was elevated by 61% during pregnancy. The increase in the enzyme activity was paralleled with a rise in serum triacylglycerol concentration (44%). Estradiol injecting into non-pregnant rats increased PAP activity of both adipose tissue (19.8%) and the liver (26%). Progesterone also elevated the enzyme activities of adipose tissue and liver by 10% and 55%, respectively. Both hormones increased serum triacylglycerol concentration (20-29%). The data demonstrated that hypertriglyceridemia observed during pregnancy was mediated through the hormonal effects on PAP activity, a key enzyme in glycerolipid metabolism. *Iran. Biomed. J. 5 (2 & 3): 103-106, 2001*

Keywords: Phosphohydrolase, Hyperlipidemia, Pregnancy

INTRODUCTION

Phosphadidate phosphohydrolase (phosphatidate phosphatase, EC 3.1.3.4) (PAP) catalyzes dephosphorylation of phosphatidic acid. Two distinct forms of PAP activity have been identified based on a differential inhibition by N-ethylmaleimide (NEM) [1, 2]. The NEM-insensitive and Mg^{2+}-independent PAP located in the plasma membrane of the cells is involved in signal transduction [3]. The second form, regarded as the metabolic form of PAP, presented in the cytosol and microsomes is sensitive to NEM and requires Mg^{2+} for its activity [4, 5]. The metabolic form of PAP is a regulatory enzyme in the biosynthesis of glycerolipids [4].

The activity of the enzyme in rat liver is affected by several hormones including glucocorticoids [6], adrenal hormones [7], growth hormone and insulin [8]. PAP, in adipose tissue, is regulated by fatty acids and norepinephrine [9], lipolytic agents [10] and corticotropin [11].

The change in the lipid metabolism including hyperlipidemia in pregnant women [12-14] is associated with increase in steroid hormones such as estrogens and progesterone [15]. The elevated levels of lipoproteins were also observed following consumption of estrogen containing contraceptives [12]. The administration of progesterone, testosterone, estradiol and dehydroepiandrosterone in rats alters hepatic PAP activity [16].

In the present study, variations of PAP activity in adipose tissue and liver of pregnant rats and of normal rats receiving progesterone and estradiol were investigated, and the involvement of this enzyme in hormone-induced hyperlipidemia was discussed.

MATERIALS AND METHODS

Chemicals. Phosphatidic acid (sodium salt) and dithiothreitol were obtained from Sigma Co. (U.S.A). Triacylglycerol enzymatic kit was taken from DarmanKave (Iran). All other chemicals were reagent grade.

Animals. Female Wistar rats (250-300 g) were obtained from the Pasteur Institute of Iran (Tehran). The rats were fed and maintained as described

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elsewhere [17]. The pregnant rats in the 3rd week of pregnancy were sacrificed for the tissue preparation. Non-pregnant animals were injected intraperitoneally with estradiol (2 µg/kg) or progesterone. (75 µg/kg) for 10 days before sacrifice. The solvent for both hormones was almond oil and the control animals received the same solvent.

Preparation of tissue homogenates. Immediately after decapitation of each rat, blood was collected and the liver was perfused with isotonic NaCl to eliminate blood using single passage perfusion system as explained before [17]. Then the liver was homogenized in 5 volume of 50 mM Tris-HCl buffer (pH 7.5) containing 1 mM EDTA and 0.225 M sucrose. The homogenate was centrifuged at 12,000 × g for 30 minutes and the supernatant was kept for the enzyme assay. The adipose tissue from mammary gland was removed, washed with 0.9% NaCl solution to remove inorganic phosphate and homogenized in 10 mMTris-HCl buffer pH 7.4 containing 0.25 M sucrose and 2 mM EGTA [18]. The homogenate was centrifuged at 1,000 × g for 1 minute. The lower layer was separated, centrifuged at 30,000 × g for 30 minutes and the upper layer kept for the enzyme assay.

Determination of PAP activity. PAP activity in the liver homogenate was measured in 0.1 M Tris-HCl buffer pH 7.4 containing 1 mM dithiothreitol, 1 mM phosphatidic acid (sodium salt), 2 mM $MgCl_2$ and an appropriate amount of the supernatant as described before [17]. Hence, the inorganic phosphate released was measured [18]. The enzyme activity in the adipose tissue and the supernatant was also measured by determining inorganic phosphate released in 60 mM Tris-HCl buffer pH 7.5 containing 0.5 mM phosphatidate, 2.5 mM $MgCl_2$ 1 mg/ml bovine serum albumin. According to Jamdar and Cao [19].

Other analytical methods. Triacylglycerol was determined in the serum using commercial enzymatic kit (DarmanKaveh, Iran). Protein was measured by the method of Lowry *et al.* [20].

Table 1. PAP activity and triacylglycerol concentration in pregnant and non-pregnant rats.

Animals	**PAP activity (nmole pi/min/mg protein)**		**Serum triacylglycerol (mg/dl)**
	Adipose tissue	**Liver**	
Non-pregnant	11.4 ± 0.23	4.3 ± 0.40	72.5 ± 9.1
Pregnant	18.3 ± 2.01	4.5 ± 0.36	104.0 ± 13.8*

The enzyme activity and triacylglycerol concentration were measured as described in M & M. The values represent mean ± SE of 8 rats. *Significantly different from non-pregnant animals (P<0.05).

Table 2. The effects of estradiol and progestrone on PAP activity in rats.

Injected	**PAP activity (nmole pi/min/mg protein)**		**Serum triacylglycerol (mg/dl)**
	Adipose tissue	**Liver**	
Non (control)	8.93 ± 0.37	2.1 ± 0.15	60.5 ± 2.8
Estradiol	10.70 ± 0.83*	2.65 ± 0.19*	72.9 ± 4.2*
Progestrone	9.85 ± 0.25*	3.26 ± 0.22*	78.1 ± 5.8*

Female rats were injected daily with estradiol (2 µg/kg) or progesterone (75 µg/kg) for 10 days. Controls rats received the hormone solvent (almond oil). Triacylglycerol concentration and PAP activity were measured as described in M & M. The values represent mean ± SE of 5 animals. *Significantly different from non-pregnant animals (P<0.05).

RESULTS

The variations in PAP activity and triacylglycerol concentration during pregnancy are shown in Table 1. PAP activity in the adipose tissue was elevated by 61% in pregnant animals. In the liver, however, only slight increase (6%) in the enzyme activity was observed. The increase in PAP activity was paralleled with a rise in the serum triacylglycerol concentration (44%). Table 2 demonstrates the effects of estradiol and progesterone on PAP activity and triacylglycerol concentration in non-pregnant female rats. Estradiol increased PAP activity of both adipose tissue (19.8%) and liver (26%).

Progesterone injection also elevated the enzyme activity of the adipose tissue and liver by 10% and 55%, respectively. Both hormones increased serum triacylglycerol concentration (20-29%).

DISCUSSION

Several reports concerning the possible regulation of PAP by certain hormones exist [6-11]. During pregnancy estrogens and progesterone levels are significantly elevated [16, 21]. The increase in PAP activity of adipose tissue during pregnancy (Table 1) is, therefore, likely to be associated with the rise in progesterone and/or estradiol.

Jamdar and Cao [22] have reported that glycerolipid biosynthesis is associated with the nutritional and hormonal status of the animals. Long-term administration of an oral contraceptive containing ethinyl estradiol and cyproterone acetate in women sharply increased triacylglycerol and other lipoprotein concentrations in the serum [23]. The only reported work concerning the effect of pregnancy on PAP activity has shown that membrane-associated PAP increases during the 4th week of pregnancy in rabbit lung [24]. The data in the present study demonstrated a close relationship between elevated hormone and triacylglycerol concentration and PAP activity, a key enzyme in triacylglycerol biosynthesis and hyper lipidemia. Several mechanisms may be involved in hormone-induced PAP activity. Estradiol stimulates cAMP concentration which can affect PAP activity [6]. The steroid effect on protein synthesis and degradation may also elevate the enzyme activity [25]. Silliman *et al.* [21] have shown that hypertriglyceridemia during late pregnancy is associated with high insulin level. Insulin increases adipose tissue PAP activity but does not affect the hepatic enzyme [26].

In short, it appears the mechanism by which serum triacylglycerol is accumulated during pregnancy involves, at least in part, the increase of PAP activity mediated through the hormonal effects. Whether these effects are direct or indirect has to be clarified.

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