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THE EFFECTS OF GLUCAGON, INSULIN AND STEROID HORMONES ON PHOSPHATIDATE PHOSPHOHYDROLASE ACTIVITY IN RAT LIVERS

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ABSTRACT

The effects of steroid hormones, glucagon and insulin on rat liver phosphatidate phosphohydrolase (PAP) activity were studied both *in vitro* and *in vivo*. Incubation of rat hepatocytes with each hormone showed that dehydroepiandrosterone (DHEA), progesterone and testosterone increase PAP activity by 44.6, 37 and 36.9%, respectively. Estradiol, however, decreased enzyme activity by 13.6% under the same conditions. Similar results were obtained when these hormones were injected in rats, in which PAP activity increased by DHEA (19.7%), testosterone (17%) and progesterone (88%) and decreased by estradiol (38.8%). Incubation of the hepatocytes with insulin however, did not change PAP activity significantly even at 144 μM concentrations, whereas glucagon progressively stimulated the enzyme activity, reaching 71% at 50 μM concentration under the same conditions. In rats injected with glucagon, PAP activity also rose up to 79% after 30 min, after which time it declined but remained above the control level at 45 min. The data imply the role of PAP activity in the mechanism by which androgens and progesterone increase serum triacylglycerols and decrease serum HDL-c and their possible regulation by these hormones.

Keywords: Phosphatidate phosphohydrolase, steroid hormone, insulin, glucagon

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INTRODUCTION

Phosphatidate phosphohydrolase (PAP) (EC. 3.1.3.4) is a key enzyme in triacylglycerol biosynthesis.[1,2] Studies concerning regulation of PAP activity have shown that epinephrine, both *in vitro*[3] and *in vivo*,[4] glucocorticoids[5] and cortisol[6] affect the activity of this enzyme in the liver. The stimulatory effects of cortisol and corticosterone result from an increase in protein synthesis.[7] Insulin alone does not alter PAP activity and antagonizes the stimulatory effect of corticosterone and growth hormone in isolated rat hepatocytes.[8,9]

Other studies have shown that oleate promotes translocation of PAP from the cytosol to the membrane-associated compartment, resulting in an increase in enzyme activity.[10] This may be associated with the increase in triacylglycerol biosynthesis in the liver and its release into the blood stream brought about by conditions that alter free fatty acid (FFA) concentrations such as stress and diabetes.[11]

In the present work, the *in vitro* and *in vivo* effects of selected hormones on rat hepatic PAP activity were examined and the involvement of this enzyme in the mechanism by which androgens increase serum lipids[12] and estrogen exert the opposite effect[13] are discussed. In this study the effects of glucagon and insulin on hepatic PAP activity were also studied to further clarify the relationship between increased triacylglycerol biosynthesis and the role of these hormones in diabetic conditions.

MATERIALS AND METHODS

Chemicals

Sodium phosphatidate, dithiothreitol, insulin and glucagon were obtained from Sigma Co. (U.S.A.). Dehydroepiandrosterone, estradiol, testosterone and progesterone were purchased from Merck Co. (Germany). All other chemicals were reagent grade.

Animals

Wistar rats (210-240g) were obtained from Pasteur Institute (Tehran). The animals, having free access to food and water, were maintained as described before.[4] For *in vivo* studies the rats were deprived of food 24 h prior to the experiments.

Hepatocyte isolation and incubations

Hepatocytes were isolated from the liver perfused *in situ* using Ca^{++}-free Hank's solution containing citrate as described by Suzangar[8] and Dickson.[14] Cell viability was assessed with trypan blue staining, generally exceeding 90%. Incubation was performed at 37°C in Krebs-Hensleit bicarbonate buffer (KH), pH 7.5[15] under an atmosphere of 95% O_2 and 5% CO_2 (vol/vol) for 1h for steroids and 2h for insulin and glucagon with shaking (90 cycle/min). The incubation mixture contained 1.2×10^7 cells/mL and the indicated amount of each hormone (see Figure legends) in a total volume of 2 mL. The solvents used for hormones were dimethylsulfoxide (DMSO) for DHEA, testosterone, estradiol and progesterone and water for glucagon and insulin. Incubations were terminated by separating the cells from the medium by centrifugation at 150 g for 1 minute. The cells were washed 3 times with KH and resuspended in 5.5 volume of 50 mM Tris-HCl buffer, pH 7.5, containing 225 mM sucrose and 1 mM EDTA, homogenized and centrifuged at 12000 g for 30 min. PAP activity was measured in the supernatant fluid.

In vivo studies

Rats were selected in groups of four. For estradiol and progesterone, female and for testosterone, DHEA and glucagon, male rats were injected (i.v.) through the tail. Steroid hormones were dissolved in DMSO and glucagon and insulin in water. The injections consisted of 0.2 mL containing different concentrations of each hormone (see Figure legends). Control animals received corresponding solvents. For steroid hormones, after 24h, and for glucagon, at 15, 20 and 45 minute intervals, the rats were decapitated and each liver was perfused immediately with saline to remove inorganic phosphate in the extracellular fluid. The liver of each rat was homogenized in 4 volumes of 50 mM Tris-HCl buffer, pH 7.5, containing 225 mM sucrose and 1 mM EDTA using a Teflon homogenizer. The homogenate was centrifuged at 12000 g for 30 minutes and the supernatant fluid was used for determination of PAP activity.

Measurement of PAP activity

Enzyme activity was measured by determining the release of inorganic phosphate (Pi) from an aqueous dispersion of phosphatidate used as substrate.[16] Each assay contained 40 mM $MgCl_2$ and the enzyme solution in a total volume of 0.5 mL. The reaction was started by adding the substrate, and after 10 min of incubation at 37°C, 1.0 mL of 10% trichloroacetic acid was added to stop the reaction. Hence, the concentration of Pi was measured.[17] A control sample was taken containing the assay mixture without the substrate. Protein was measured by the method of Lowry et al.[18]

RESULTS

In vitro studies

Incubation of hepatocytes with different concentrations of each steroid hormone revealed that PAP activity was significantly affected by these hormones. The data presented in Table I showed that DHEA raised PAP activity (44.6%) at concentrations of up to 8 µg/mL of the incubation medium

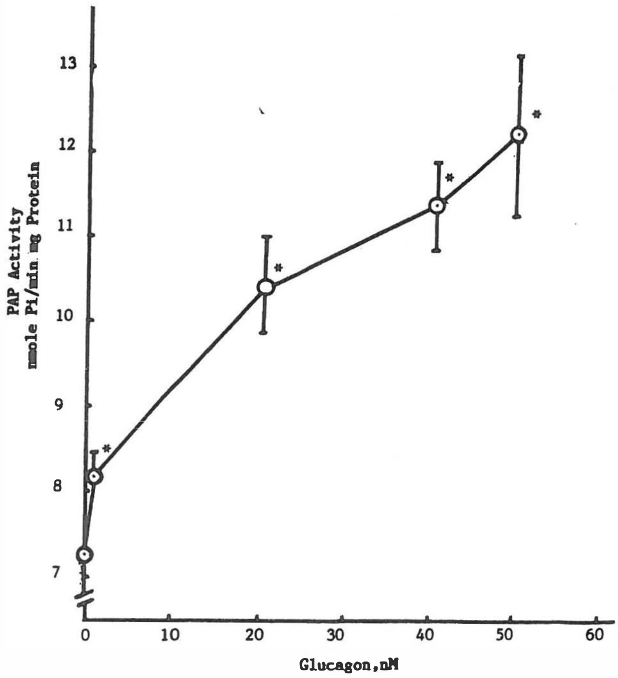

Fig. 1. The effect of glucagon on PAP activity of rat hepatocytes. Hepatocytes (1.2×10^7 cells/mL of incubation mixture) were incubated with different concentrations of glucagon for 2h and PAP activity measured as described in *Materials and Methods*. Values represent Mean±S.E. of 3 separate experiments.
* Significantly different from control (p<0.05).

Table I. The effects of steroids and insulin on PAP activity in isolated rat hepatocytes.

Substance Added	PAP Activity nmol Pi/min.mg Protein	% Change
None (control)	7.54±0.32	—
DHEA(mg/L):		
0.16	10.1±1.0*	34.0
8	10.9±1.1*	44.6
32	9.4±0.3*	24.7
Estradiol (μg/L):		
0.03	5.7±0.5*	24.4
3.0	6.0±0.30	20.4
2.2	6.5±0.31	13.6
Progesterone (μg/L):		
2	9.16±0.78*	21.5
20	10.23±0.76*	36.9
200	9.27±0.17*	22.1
Testosterone (μg/L):		
1	10.32±0.30*	36.9
10	9.42±0.47*	24.9
50	9.16±0.45*	21.5
Insulin (μmol/L):		
36	7.65±0.58	1.45
72	7.72±0.26	2.4
144	8.06±0.48	6.9

Hepatocytes (1.2×10^7 cells/mL of incubation mixture) were incubated in the presence of the indicated hormones, 2h for insulin and 1h for other hormones and PAP activity was measured as described in *Materials and Methods*. The data represent Mean±S.E. value of 3 separate experiments (2 tubes/incubation).
*Significantly different from control ($p<0.01$).

after which enzyme activity declined but remained above control levels (24.7%). Progesterone also exerted a similar pattern in which PAP activity rose up to about 37% at a hormone cencentration of 20 ng/mL but declined when the hormone concentration increased up to 200 ng/mL where the stimulatory effect was only 22%. Enzyme activity was stimulated by testosterone by 36.6% and 21.5% at hormone concentrations of 1 and 50 ng/mL, respectively. Estradiol, however, decreased PAP activity as its concentration was increased up to 2.2 ng/mL, at which the enzyme inhibition was 13.6%. Table I also shows that insulin did not significantly change PAP activity even at a concentration of 144 μM. The presence of glucagon at different concentrations in the incubation medium progressively stimulated PAP activity, reaching 71% at 50 μM (Fig. 1).

In vivo studies

The *in vivo* effects of steroid homones on hepatic PAP activity are shown in Table II. The results demonstrated that injection of DHEA, testosterone or progesterone stimulated PAP activity by 19,17, and 8.8% after 24 h, respectively. Estradiol, however, inhibited enzyme activity by 38.8% during the same period of time.

When rats were injected with glucagon, PAP activity rose up to 79% after 30 min, after which it declined but remained above the control level at 45 min (Fig. 2).

Table II. The effects of steroid hormones on PAP activity in rat liver.

Injected	PAP Activity	Change
None (control)	3.4±0.13	—
DHEA (8 μg/kg):	4.07±0.1*	19.7
Testosterone (50 ng/kg)	3.98±0.19*	17
Progesterone (100 ng/kg)	3.70±0.16*	8.8
Estradiol (1.5 ng/kg)	2.08±0.32*	38.8

Rats were injected (i.v.) with the indicated hormones and decapitated after 24h. PAP activity was measured in the liver as described in *Materials and Methods*. Values represent Mean±S.E. of 4 rats.
*Significantly different from control ($p<0.01$).

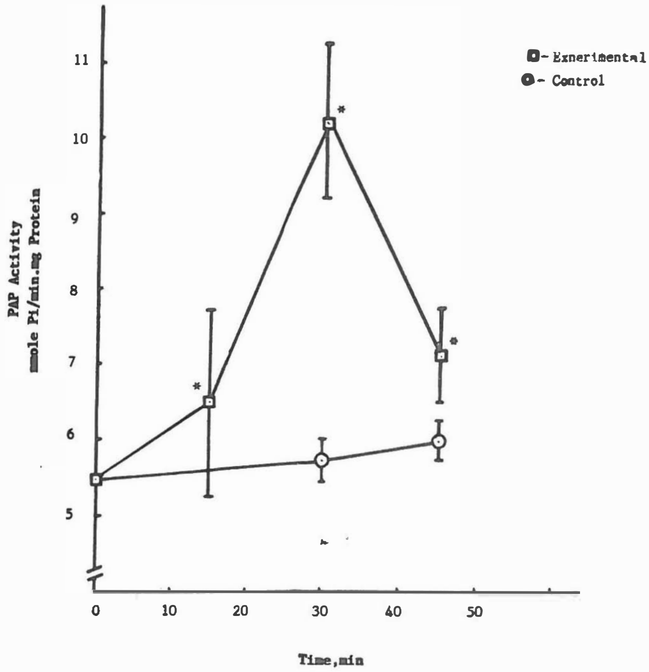

Fig. 2. Time dependent effect of glucagon on PAP activity of rat liver. Rats were injected (i.v.) with glucagon (0.4 mg/kg) and were decapitated after the indicated time intervals. PAP activity was measured in the liver as described in *Materials and Methods*. Values represent Mean±S.E. of 4 rats.
* Significantly different from control ($p<0.05$).

DISCUSSION

The results of this study demonstrated that DHEA, testosterone and progesterone stimulated hepatic PAP activity both *in vivo* and *in vitro*. Estradiol, however, inhibited enzyme activity under the same conditions. Other reports have shown that androgens and progesterone increase serum triacylglyceroland decrease serum HDL-c,[12] whereas transdermal estrogen therapy shows an opposite effect.[13] It is therefore quite possible that these hormones exert their effects on lipid metabolism through hepatic PAP activity, a regulatory enzyme in triacylglycerol biosynthesis.[2] The present data may also explain the mechanism by which coronary heart disease is associated with the levels of plasma lipids, including HDL-c.[19] Hyperlipidemia and low concentrations of plasma HDL-c are the cause of atherosclerosis; androgens, progesterone and high levels of triacylglycerol decrease HDL concentrations.[20] In fact, atherosclerosis starts from VLDL-c, in which the major lipid component is triacylglycerol.[21] During the past decade several studies have shown that estrogens decrease the risk of atherosclerosis and myocardial infarction;[22] this effect may be the result of a decrease in triacylglycerol and an increase in HDL-c concentration.[13,23,24] Studies performed on monkeys have shown that testosterone decreases plasma HDL-c concentrations which in turn stimulates coronary heart disease.[25]

Our previous studies have shown a circadian rhythm for VLDL-c glycerol secretion rate from the liver which coincided with those of liver PAP activity and plasma triacylglycerol concentration.[26] It is, therefore, probable that the change in hepatic PAP activity is related to the steroid hormone-induced alteration of lipid metabolism.

The stimulatory effects of androgens and progesterone and the inhibitory effect of estradiol on PAP activity may be the result of increased enzyme biosynthesis and/or its decreased degradation, both of which have been proven for glucose 6-phosphate dehydrogenase.[27-9]

The finding that glucagon stimulates PAP activity in isolated hepatocytes agrees with other reports in which cAMP was suggested to be involved in glucagon-induced increase of PAP activity.[7,30] The *in vivo* stimulatory effect of glucagon on hepatic PAP activity observed may be caused by the lipolytic effect of glucagon releasing FFA into the blood stream and subsequent increase in their liver uptake.

Cascales et al.[2] have reported that treatment of rat hepatocytes with oleic acid results in an increase in microsomal PAP activity with a concomitant decrease in cytosolic enzyme activity. It is suggested that intracellular translocation of PAP in response to a fatty acid load to the liver plays a regulatory role in triacylglycerol biosynthesis.[31] We have previously found that in adrenalectomized rats injected with epinephrine, which raises plasma FFA levels, there was a rise in both cytosolic and membrane-associated hepatic PAP activity with a simultaneous increase in triacylglycerol concentrations.[32]

The activity of PAP did not significantly change following insulin injection. Pittner et al. however, have reported that insulin antagonizes the stimulatory effect of glucagon in rats.[30] Perhaps in the absence of glucagon, the low concentration of FFA does not permit PAP translocation from the cytosol to membrane-associated compartments; consequently, no change in PAP activity is observed.

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