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THE EFFECTS OF HYDRAZINE ON THE PHOSPHATIDATE PHOSPHOHYDROLASE ACTIVITY IN RAT LIVER

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Abstract—Injection of hydrazine (0.7 mmole/kg) in the male fasting rats caused an increase in phosphatidate phosphohydrolase (PAP) activity in the soluble fraction of the liver. The increased PAP activity was parallel with a rise in hepatic triacylglycerol (TG) (3.5-fold) and in the catecholamine concentration (3.4-fold) in adrenal glands. Hydrazine also increased serum glucose. The hydrazine-induced increase in PAP activity and TG accumulation was completely prevented by adrenalectomy. The data suggest that increased PAP activity is at least partly responsible for hydrazine-induced fatty liver and that adrenal hormones may take part in the mechanism by which hydrazine exerts its effects on the liver.

Several studies have been done on hydrazine-induced fatty liver with different conclusions regarding the mechanism by which hydrazine increases fats in the liver [1–6]. We have previously shown that fat storage in rat liver upon hydrazine injection is partly due to decreased TG secretion from the liver and that hydrazine effects on liver lipids are abolished by adrenalectomy [7].

There are several reports which support the view that phosphatidate phosphohydrolase (PAP)* (EC 3.1.3.4.) is a regulatory enzyme in TG synthesis in the liver [8–12]. More recently, the regulatory role of this enzyme has been reviewed [13].

The present work was undertaken to investigate whether in hydrazine treated animals liver TG would also accumulate due to change in the activity of liver PAP, and to elucidate further the possible relationship between adrenal hormones and hydrazine-induced liver responses.

MATERIALS AND METHODS

Reagents

Phosphatidic acid (sodium salt), dithiothreitol and epinephrine were purchased from Sigma Co. (U.S.A.). Hydrazine hydrochloride was from Merck Co. (F.R.G.). Kit for enzymatic determination of triacylglycerol was obtained from Environmental Chemical Specialities Co. (U.S.A.). Pentobarbital was received from Abbott Laboratories (U.S.A.). All chemicals were reagent grade.

Hydrazine solution

Hydrazine solution containing 0.7 mmole/ml was prepared immediately before use and its pH adjusted to 7.4 with 1 N HCl or NaOH.

Treatment of rats and adrenalectomy of animals

Adult male Wistar rats were obtained from Pasteur Institute (Tehran). The rats were fed with a 41 B diet [12] before any experiment and treated as described before [7]. Normal and adrenalectomized rats were selected in groups of 10 weighing within 10–20 g of the mean of the group. All animals were put on fast for 24 hr before the experiments. For each group five rats were injected with hydrazine (0.7 mmole/kg body wt.) and five rats with saline as control. Rats of each group were decapitated at time intervals of 3, 12, 24 and 48 hr after injection. For adrenalectomized rats the time interval of 12 hr was omitted. Bilateral adrenalectomy was performed according to Zarrow *et al.* [14]. These rats were housed individually at 22° and fed with the diet 41 B but were given 0.15 M NaCl solution to drink instead of water to avoid electrolyte imbalance brought about by the loss of mineralocorticoids. After adrenalectomy the animals received ampicillin (250 mg/l of their drinking NaCl solution) for a week to prevent probable infection and then allowed an additional week to recover before the experiments.

Analytical methods

Preparation of liver soluble fraction. The method was that described by Savolainen [15].

Assay of phosphatidate phosphohydrolase activity. The activity was measured in the rat liver soluble fraction by determining the release of P_i from an aqueous dispersion of phosphatidate used as substrate [16]. Each assay contained in volume of 0.5 ml: 40 mM Tris–HCl pH 7.4, 2 mM $MgCl_2$, 1 mM dithiothreitol, 2 mM sodium phosphatidate and the enzyme solution (105,000 *g* supernatant). Reactions were started by adding phosphatidate emulsion and after 10 min incubation at 37° 1 ml of 10% trichloroacetic acid was added to stop the reaction, hence the concentration of inorganic phosphate was determined [17].

Catecholamine determination. Adrenal glands of each rat were removed immediately after decapitation and homogenized in 5 ml of 0.4 N $HClO_4$. Catecholamines were purified according to the method of Sullivan *et al.* [18] and measured as described by Lynch *et al.* [19].

Lipid analysis. Liver TG was measured using a commercial enzymatic kit (see above). The standard

* Abbreviations used: PAP, phosphatidate phosphohydrolase; TG, triacylglycerole.

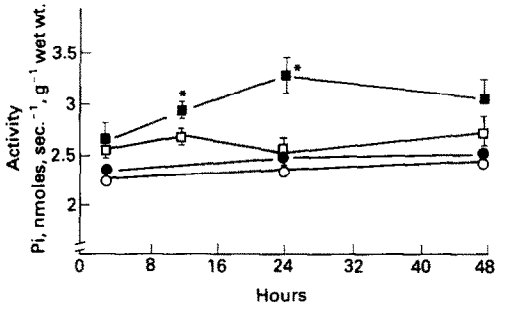

Fig. 1. Effect of a single dose of hydrazine on the activity of soluble phosphatidate phosphohydrolase in rat liver. Results are expressed as nmoles of P_i released per sec per g wet weight of liver. Each point represents mean ± SD of 5 rats: Intact rats: ■, hydrazine treated; □, control; adrenalectomized rats: ●, hydrazine treated; ○, control; *significantly different from controls ($P < 0.001$). For details see text.

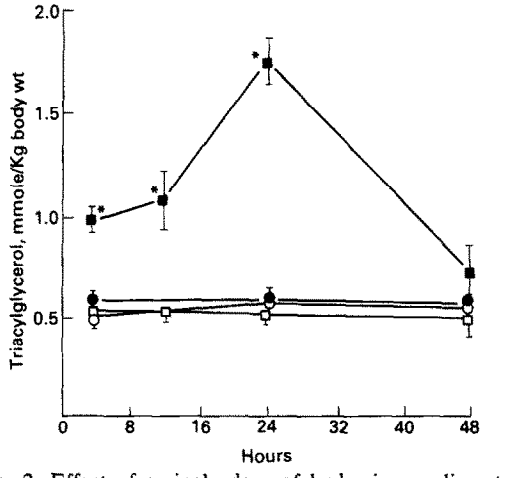

Fig. 2. Effect of a single dose of hydrazine on liver triacylglycerol concentration of intact and adrenalectomized rats. Symbols and experimental conditions as in Fig. 1.

method was used to determine serum glucose [20]. Student's *t*-test was used to analyze statistical differences between hydrazine-treated and control rats.

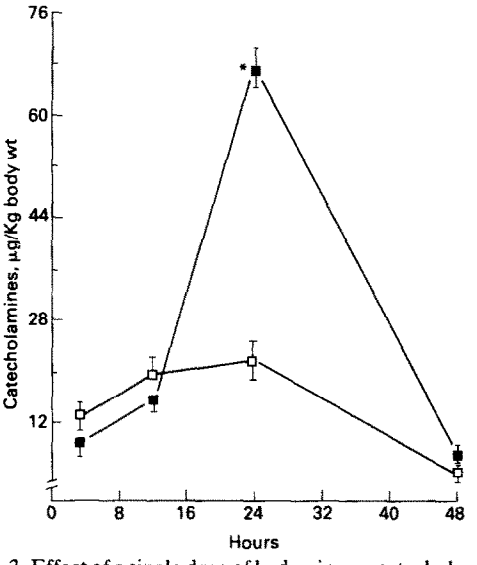

Fig. 3. Effect of a single dose of hydrazine on catecholamine concentration in adrenal glands. Symbols and experimental conditions as in Fig. 1 for intact rats.

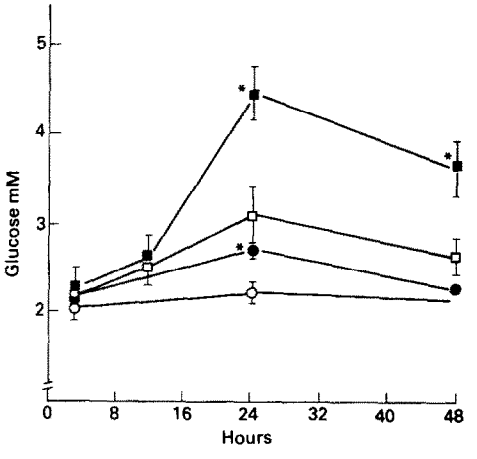

Fig. 4. Effect of a single dose of hydrazine on serum glucose of intact and adrenalectomized rats. Symbols and experimental conditions as in Fig. 1.

RESULTS

Normal rats

Hydrazine enhanced soluble PAP activity in the liver up to 24 hr after which it declined (Fig. 1). Increased activity of this enzyme was parallel with a rise in liver TG which was also maximal at 24 hr (Fig. 2). Catecholamines in the adrenal glands, after hydrazine injection, did not change significantly up to 12 hr but rose markedly at 24 hr (more than 3-fold) and returned to control levels at 48 hr (Fig. 3). Hydrazine caused a progressive increase with time in serum glucose during the first 24 hr and remained above controls for 48 hr (Fig. 4).

Adrenalectomized rats

Adrenalectomized rats were also treated with the same dose of hydrazine as described for normal animals to study the possible involvement of adrenal hormones in the mechanism by which hydrazine exerts its effects on lipid and carbohydrate metabolism. In these animals, hydrazine did not produce significant changes in either PAP activity (Fig. 1) or in TG accumulation (Fig. 2) in the liver. The data suggest that catecholamines and/or corticoids may be responsible for hydrazine-induced increase in the hepatic PAP activity and TG storage. The effect of hydrazine on serum glucose in adrenalectomized rats was less pronounced than that in the intact animals (Fig. 4).

DISCUSSION

The mechanism of hydrazine-induced fatty liver is still a controversial subject. The accumulation of fats in the liver by hydrazine appears to be related to high activity of PAP (Figs. 1, 2). Lamb and Banks [5] reported that hydrazine-dependent increase in

liver TG measured *in vitro* correlates with a rise in PAP activity. Hydrazine also increases the concentration of nonesterified fatty acids in blood and their uptake by the liver [1, 6] and decreases TG secretion from the liver [7].

Since prior bilateral adrenalectomy prevents hydrazine effects on the liver (Figs. 1, 2) and because hydrazine changes circulating corticosterone concentration [7, 21], corticoid hormones could be responsible for hepatic responses observed upon hydrazine treatment. Although there is a significant rise in the concentration of catecholamines in the adrenal glands of hydrazine-injected rats (Fig. 3) which peaks at about the same time as the liver responses (Figs. 1, 2), one cannot tell yet whether increased catecholamine concentration is associated with the rise in either TG concentration or PAP activity. The elevated catecholamines in the adrenal glands may arise from an increase in the rate of synthesis or failure of release without altering the circulating concentrations of these hormones. However, the findings that epinephrine [22] and norepinephrine [23] produce fatty liver and that the activity of PAP is controlled by glucocortocoids and cAMP [24] suggest that the involvement of catecholamines in the mechanism by which hydrazine produces fatty liver is likely.

Cascales *et al.* [25] have reported that treatment of rat hepatocytes with oleic acid resulted in an increase in microsomal PAP activity with a concomitant decrease in cytosolic activity. It is suggested that intracellular translocation of PAP in response to a fatty acid load to the liver plays a regulatory role in TG synthesis [13]. The translocation of the enzyme between the two subcellular fractions was also affected by cAMP [26].

If hydrazine could increase circulating concentration of catecholamines, as it happened in the adrenal glands (Fig. 3), the elevated fatty acid induced by hydrazine [1] and consequently possible regulation of hepatic PAP activity could had been related to these hormones. We have now started to reproduce hepatic responses to hydrazine in adrenalectomized rats by exogenous catecholamines to prove the point. The slight fluctuations seen in the concentration of catecholamines in the adrenals of control animals (Fig. 3) may be due to the stress brought about by injection of saline.

Direct effect of corticotropin on liver PAP activity or TG concentration is unlikely, because following adrenalectomy that the release of corticotropin from pituitary gland must have been increased, no change in either parameters was observed. The hyperglycemia seen in intact rats (Fig. 4) is contradictory to the inhibitory effect of hydrazine on gluconeogenesis [27].

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