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CHANGES IN BIOCHEMICAL PARAMETERS OF CARBOHYDRATE METABOLISM FOLLOWING FENFLURAMINE ADMINISTRATION IN RATS

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ABSTRACT

Fenfluramine, an anorexigenic agent, is widely used in the treatment of obesity. Besides its anorectic effect, it may also have some effects on general metabolism with the consequence of weight loss. In this study, the effect of fenfluramine on the concentrations of some parameters related to carbohydrate metabolism was investigated. It was shown that the serum insulin level was reduced by 41%, four hours after fenfluramine administration, which was accompanied by the elevation of serum glucose levels by 26%. The liver glycogen content showed a transient reduction, but reached the control level four hours post-treatment. Corticosterone levels were elevated immediately, followed by a 20% reduction after four hours. The short-term effects of fenfluramine on thyroid hormones were not statistically significant.

Administration of fenfluramine for two weeks did not change the glycogen content of the liver significantly, but 64% and 103% increases were observed after four and six weeks of drug treatment, respectively. Insulin levels showed a gradual increase so that by the end of six weeks, 153% increase in insulin level was observed. No significant changes in serum glucose levels were seen during the period of treatment. Corticosterone concentration remained unchanged up to four weeks of treatment but a 32% reduction was seen after six weeks. The levels of T_4 and T_3 showed a transient increase, followed by a significant decrease after six weeks of treatment. It is concluded that fenfluramine has some important metabolic effect that is related to its action in decreasing food intake and weight loss.
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INTRODUCTION

Fenfluramine, an amphetamine derivative, was introduced originally as an anorectic drug and it is frequently used in the treatment of obesity.[1] Its action is thought to involve direct effects on serotoninergic systems at the level of hypothalamus,[2] leading to anorexia and weight loss. It is also evident that this drug may have some additional peripheral actions that may be unrelated to its action in decreasing food intake which would be of value in the treatment of obesity.[3] In this regard the effect of fenfluramine on plasma and liver levels of lipid fractions and on plasma and brain levels of tryptophan has already been investigated in our laboratory.[4,5] There is also evidence that this agent improves insulin action independent of its effects on food intake,[4] which in turn may affect carbohydrate metabolism. The present study was undertaken to investigate changes in the concentrations of some biochemical parameters

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Table I. The short-term effect of fenfluramine on some biochemical parameters in rats. Each value indicates the mean ± SEM of five experiments performed in duplicate. Values in parenthesis show percent increase or decrease from the control level. For more details see text.

parameter / Time	Glucose (mg/dl)	Glycogen (mg/g Prot.)	Insulin µIU/dl	Corticosterone (µg/dl)	T_4 (µg/dl)	T_3 (ng/dl)
Control	85.4 ± 5.7	206 ± 21.4	37.4 ± 4.4	37.7 ± 3.2	1.9 ± 0.2	65 ± 6.8
0.5 hr	96.5 ± 6.4 (13%)	169.6 ± 7.6 (-18%)	33.4 ± 4.7 (-10%)	47.2 ± 3.0 (+25%)	1.6 ± 0.2 (-20%)	50.8 ± 1.6 (-21%)
2 hr	109 ± 6.8 (+27%)	98.7 ± 9.7 (-52%)	31.6 ± 3.9 (-15%)	39.0 ± 3.1 (+3%)	2.1 ± 0.3 (+5%)	81 ± 5.1 (+24%)
4 hr	108 ± 4.6 (+26%)	201 ± 14.5 (-2%)	22 ± 3.5 (-41%)	29.9 ± 1.2 (-20%)	1.9 ± 0.4 ----	72.4 ± 10 (+11%)

related to carbohydrate metabolism following fenfluramine administration. The observed changes may be a secondary effect due to the alteration in hormonal balance of the animal.

MATERIAL

Male Wistar rats (100-150 gr) (obtained from Pasteur Institute, Tehran) were used for the experiments. They were kept at standard conditions in our animal labs. Fenfluramine hydrochloride (20 mg coated tablets) was purchased from Loghman Pharmaceutical, Tehran. Radioactive kits for the determination of hormones were obtained from Diagnostic Products Company, (DPC), Los Angeles, USA. All other chemicals used in this study were of reagent grade and obtained from Sigma Chemical Company.

METHODS

Animals were chosen in groups of five and kept in separate cages. In short-term study, animals were injected intraperitoneally with the acute dose of fenfluramine (100 mg/kg body weight). Treated animals were killed either 0.5, 2 and/or 4 hours post-injection. Animals used for long-term study received daily intraperitoneal doses of 10 mg/kg fenfluramine for either 1,2,4, or 6 weeks. Groups of animals were injected with normal saline in parallel with each experiment and considered as controls.

At the time of the experiment, the animals were killed by decapitation, their blood collected and allowed to coagulate. Their sera were then separated by centrifugation and used for the analysis. To avoid the probable diurnal changes in serum hormone levels, care was taken to perform the sampling at the same time each day. Livers were removed immediately for the determination of glycogen content, which was performed according to the method of Kemp, et al.[7] Serum glucose was determined by orthotoluidine method as reported by Caraway.[8] The serum concentration of hormones including insulin, corticosterone, thyroxine (T_4) and T_3 were determined by immunoradiometric technique.

RESULTS

Short term effects of fenfluramine on biochemical parameters are shown in Table I. It is shown that the concentration of glucose is elevated following fenfluramine administration, which is accompanied by a reduction in serum insulin level. The liver glycogen content showed a reduction, but it reached the control level four hours post-treatment. The level of corticosterone showed an increase (25 percent) 0.5 hr after fenfluramine administration, followed by a gradual decrease in this hormone level so that 20 percent reduction was observed after four hours. Changes observed in the levels of thyroid hormones were not statistically significant .

Long-term effects of fenfluramine were also studied, the results of which are shown in Table II. According to results, the concentration of insulin in serum tends to increase following the continous administration of

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Table II. The long-term effect of fenfluramine on the biochemical parameters in rats. Each figure shows the mean ± SEM of five experiments. In paranthesis the percent increase or decrease are shown. For details about the method of the experiments, see text.

parameter / Week	Glucose (mg/dl)	Glycogen (mg/g Prot.)	Insulin µIU/dl	Corticosterone (µg/dl)	T_4 (µg/dl)	T_3 (ng/dl)
Control	85.4 ± 5.7	206 ± 21	37.4 ± 4.4	37.7 ± 3.2	1.9 ± 0.2	65 ± 6.2
1	73.1 ± 2 (-14%)	176.6 ± 3.1 (-14%)	40.6 ± 10 (+9%)	30.4 ± 2.6 (-19%)	1.2 ± 0.1 (-36%)	73.4 ± 2.7 (+13%)
2	68.6 ± 2.5 (-19%)	202 ± 22 (-1.8%)	45 ± 10 (+20%)	38.9 ± 1.7 (+3%)	4.0 ± 0.5 (+101%)	80 ± 8 (±23%)
4	84.3 ± 3.4 ----	338 ± 41 (+64%)	44.5 ± 9 (+19%)	37 ± 4.5 ----	1.9 ± 0.3 ----	89.8 ± 2.8 (+38%)
6	105 ± 4 (+23%)	474 ± 40 (+103%)	94.8 ± 4 (+153%)	25.6 ± 1 (-32%)	1.1 ± 0.2 (-41%)	48 ± 6.4 (-26%)

fenfluramine, so that after six weeks of treatment, the hormone level reached 94.8 µIU/mL compared with the control level of 37.4 ($P<0.01$). The increase in insulin level was accompanied by the accumulation of glycogen in the liver. Thus, 103 percent increase in glycogen content of the liver was observed following six weeks of drug administration ($P<0.01$). Serum glucose level did not change significantly after four weeks of treatment, but the continuation of the treatment for six weeks increased serum glucose level by 23 percent. No significant changes were observed in corticosterone level after four weeks of fenfluramine administration, but a reduction of 32 percent was seen after six weeks. Thyroid hormones were first elevated due to the action of fenfluramine, whereas the continuation of the treatment led to a consequent reduction.

Thus the increase in T_3 level was 13, 23 and 38 percent following treatment for one two and four weeks respectively, but levels decreased by 26 percent if the treatment was continued for six weeks. Serum T_4 level was also increased by about 100 percent after two weeks of fenfluramine administration, whereas a reduction of 41 percent was observed after six weeks.

DISCUSSION

Fenfluramine, an anti-obesity drug, is structurally but not functionally related to amphetamine and is reported to have some effects on biomolecular metabolism,[3,6] hormonal balance of obese subjects.[9]

We have already reported that fenfluramine affects the metabolism of lipids, the body. The action of this drug on the metabolism of biomolecules may be secondary to its effect on the hormonal status of the body. In this study it was shown that the levels of insulin, corticosterone and thyroid hormones were affected by fenfluramine, the consequence of which are changes in carbohydrate metabolism. It has also been reported that fenfluramine treatment improved glycemic control in obese glucose-intolerant humans and in non-insulin dependent diabetes mellitus independent of its effects on food intake and body weight.[11] Our results indicate that following the administration of acute doses of fenfluramine, the glycogen of the liver was immediately reduced which may be due to either increased rate of glycogenolysis or decreased rate of glycogenesis. This reduction in liver glycogen content is accompanied by an increase in serum glucose and a decrease in serum insulin levels.

It is probable that the reduction in liver glycogen level is due to the inhibition of insulin synthesis or release from the pancreatic cells. There are reports indicating that fenfluramine inhibited the release of insulin from the perfused rat pancreas.[12,13] Alternatively fenfluramine may affect the liver enzymes involved in glycogen metabolism directly, the consequence of which is the reduction in the concentration of this carbohydrate storage compound.

The reduction in liver glycogenesis due to the inhibition of liver phophoglucomutase by fenfluramine has already been reported.[14] However the reduction in liver glycogen level is transient, so that it reaches the control level four

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hours after fenfluramine injection. As the insulin level at this time is still below control, the increase in liver glycogen level may be mediated through some other mechanisms involving hormones such as corticosterone and thyroid hormones. It has been reported that the administration of fenfluramine immediately increased the serum corticosterone level[15,16] which is in agreement with the results obtained in this study. Fenfluramine did not have any significant effect on thyroid hormones in short-term, which is in agreement with the report that this drug had no effect on TSH release.[13]

In long-term however, fenfluramine had different effects on serum insulin and liver glycogen levels. The administration of fenfluramine for six weeks led to 153 percent increase in insulin level, followed by 103 percent increase in liver glycogen level. This is consistent with the findings that long term administration of fenfluramine increased insulin secretion[18] and also inhibits glycogenolysis.[19]

Our result showed that long term administration of fenfluramine led to a decrease in corticosterone level which may be partly responsible for the observed metabolic changes.

Administration of fenfluramine for two weeks increased the serum concentration of T_3 and T_4 which in turn may increase the rate of basal metabolism leading to weight loss. However continuation of the treatment for up to six weeks led to the reduction of these hormone levels which is in good agreement with the well-documented transient effect of this anorectic agent.[20,21]

The reduction in thyroid hormone levels after six weeks of treatment may be mediated through some unknown mechanism responsible for setting up the metabolic balance in the body.

The slight increase in the concentration of serum glucose despite the elevated level of insulin could be explained by the insulin resistance and reduced glucose tolerance which might have occurred in these conditions.

It is concluded that although the mechanism by which fenfluramine exerts its effects in short term may be different from its long-term effects, this anorectic drug affects the hormonal status of the body, the outcome of which could be changes in the metabolism of fuels in the body.

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