Effects of phenobarbital and cimetidine on the blood glucose concentration and hepatic enzyme activities of congenitally obese mice

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ABSTRACT Blood glucose levels of congenitally obese mice, which can be used to represent an animal model of type II diabetes mellitus, were decreased significantly by phenobarbital treatment but not altered by acute cimetidine. Liver/body weight ratio, microsomal protein content, NADPH cytochrome C reductase as well as glucose-6-phosphatase activity in the liver homogenate were significantly increased by phenobarbital but not changed by cimetidine. These results suggest that the glucose-lowering effect of phenobarbital may be mediated through activation of glucose processing enzymes.

KEY WORDS enzyme induction; enzyme inhibitors; phenobarbital; cimetidine; glucose tolerance test; obese mice

Noninsulin-dependent diabetes mellitus (NIDDM or type II diabetes) is usually treated with diet and/or drugs like biguanides or sulfonylureas. It has been observed, however, that after a short period of treatment these drugs have a tendency to lose their efficacy, although the blood insulin levels remain high. This condition is called insulin resistance, and there is a possibility that oral antidiabetic drugs cause inhibition of the glucose processing system[1].

It is not clear how much the enzymes regulating glucose metabolism affect the blood glucose levels in NIDDM patients. In congenitally obese mice, which can be used to represent NIDDM in animals[2], the activities of both glycolytic and gluconeogenetic enzymes as well as the insulin levels are higher than those in their lean littermates[3]. This is a controversial situation since insulin is known to depress gluconeogenesis[4]. Obese mice have, however, hyperplastic adrenal cortices[5], and therefore an enhanced glucocorticoid secretion may be responsible for their increased gluconeogenesis[6].

It is possible that some of the enzymes involved in the metabolism of glucose are induced or inhibited by drugs. Hepatic drug metabolism is known to be worsened in alloxan-diabetic male rabbits[7] and in streptozotocin-diabetic male rats[8]. Moreover, it was recently demonstrated in healthy volunteers that the insulin mediated glucose disposal rate could be altered by drugs affecting hepatic microsomal enzyme activity[9]. Therefore it is of interest to study whether phenobarbital, a well-known inducer of microsomal enzymes[10] and cimetidine, an inhibitor of oxidative metabolism[11] would affect the blood glucose levels in congenitally obese mice. Moreover, the activities of microsomal glucose-6-phosphatase, NADPH cytochrome C reductase and UDP glucuronyltransferase were measured after the drug treatments.

MATERIALS AND METHODS

Animals C 57 BL/6 J-obob/Ola obese mice of both sexes at the age between 2 and 3 months were used in the experiment. The mice were kept in a room, where a temperature of 20 ± 1℃ was maintained by a thermostat, and where the relative humidity was kept at 50 ±10 %. The dark room was artificially illuminated from 06:00 to 20:00. The mice were given Tuohilampi

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rat–mouse feed *ad libitum* (Tuohilampi Farm and Animal Centre, Vihti, Finland).

Procedure The mice were divided into 3 groups with 6 ♂ and 6 ♀ in each. One group was injected ip phenobarbital (60 mg/kg) for 4 d, and another group received cimetidine (50 mg/kg) in a corresponding manner. The third group served as an untreated control unit. Blood samples were taken from orbital plexus just before the treatment and 24 h after the last injection. The blood glucose concentration (mmol glucose/L blood) was determined by the hexokinase method (Test-Combination Gluco-Quant^{R}, Boehringer Mannheim GmbH). Immediately after the last blood sample had been taken the mice were killed by cervical dislocation. The livers were quickly removed, weighed and frozen.

Preparation of microsomes Livers were cut up, weighed, and homogenized in a Potter glass homogenizer with 5 volumes of Tris 0.1 mol/L, $MgCl_2$ 5 mmol/L and 0.2 % Triton X-100 at a pH of 7.4 for transferase assays and with 5 volumes of sucrose 0.25 mol/L for phosphatase and reductase assays. Homogenates were centrifuged first at 600 × *g* for 10 min, then at 9000 × *g* for 15 min, and further at 100 000 × *g* for 60 min to isolate the microsomal fraction from the last sediment. Microsomes were resuspended with a concentration of 1 ml buffer/g fresh liver in Tris 0.1 mol/L and $MgCl_2$ 5 mmol/L, pH 7.4 for transferase and in Tris 50 mmol/L, pH 7.5 for phosphatase and reductase determinations. Microsomal protein was determined with a Bio-Rad protein assay kit.

Enzyme assays For glucose-6-phosphatase (EC 3.1.3.9) assay, a fraction of microsomes was incubated at 37℃ with glucose-6-phosphate 40 mmol/L as substrate in sodium citrate 50 mmol/L buffer, pH 6.5[12]. The reaction was terminated after 15 min with 10% trichloroacetic acid and the proteins were removed by centrifugation. The phosphate released in the enzyme reaction was determined from the supernatant with a Boehringer-Mannheim phosphate assay kit.

NADPH cytochrome C reductase (EC 1.6.2.4) activity was studied as a 3-min change in absorbance at 550 nm in a Shimadzu UV-240 spectrophotometer[13]. The enzyme activity was calculated with a value of $E = 19.1\ mmol^{-1} \cdot cm^{-1}$.

UDP-glucuronyltransferase (EC 2.4.1.17) activity was tested with *p*-nitrophenol as substrate[14]. The enzyme was activated with 0.2% Triton x-100 in the homogenization buffer. The total volume of the reaction mixture was 100 µl including the enzyme, Tris 60 mmol/L, pH 7.4, $MgCl_2$ 3 mmol/L, UDP-glucuronic acid (UDPGA) 3 mmol/L and *p*-nitrophenol 0.8 mmol/L. UDPGA was omitted in the blanks. The reaction was brought to an end after 10 min with 10 µl of perchloric acid 4 mol/L. After centrifugation a fraction of the supernatant was analysed in a HPLC system consisting of a pump (Spectroflow 400, Kratos), an injector with a 20 µl sample loop (Rheodyne 7125) and a 4.6 × 150 mm column (Ultra Techsphere ODS, 5 µm) with a precolumn. The column was connected to a spectrophotometer (Spectroflow 770, Kratos). The mobile phase ($NaHPO_4$ 10 mmol/L in 20% methanol, pH 3.0) was pumped at a flow rate of 2.0 ml/min. In these conditions the retention times were 2.8 and 8.8 min for *p*-nitrophenolglucuronide and *p*-nitrophenol, respectively.

The enzyme activities are expressed in nmol product/(g liver·min).

Statistics Means and SD were calculated. The differences between the treatments were evaluated by one-way analyses of variances and by a Duncan's multiple comparison tests.

RESULTS

The *blood glucose* concentration was sig-

Tab 1. Hepatic changes in congenitally obese mice after ip phenobarbital 60 mg/kg or cimetidine 50 mg/kg for 4 d. n = 9 - 12, $\bar{x} \pm SD$. *p>0.05, ***p<0.01 *vs* untreated controls.

	Untreated controls	Phenobarbital-treated	Cimetidine-treated
Liver wt/body wt ratio, %	8.4±0.2	9.6±1.2*	8.1±1.0*
Microsomal protein, mg/g liver	11.5±6.6	24.1±10.3***	12.5±6.3*
Glucose-6-phosphatase, nmol/(g liver·min)	1830±753	3184±1121***	1939±1031*
NADPH cytochrome C reductase, nmol/(g liver·min)	477±234	1677±10***	511±260*
UDP glucuronyltransferase, nmol/(g liver·min)	592±387	647±254*	335±208*

nificantly decreased (from 12.2 ± 3.9 to 6.8 ±1.8 mmol/L, $p<0.01$) in the phenobarbital-treated group. Cimetidine caused no change in the blood glucose levels (13.5 ± 4.6 mmol/L before treatment, 13.5 ± 6.2 mmol/L after treatment). The difference between the blood glucose levels in the phenobarbital-treated and in the cimetidine-treated mice was significant ($p<0.01$). In control mice, blood glucose levels were not changed: 12.7±3.0 mmol/L before treatment, 10.4 ± 2.8 mmol/L after treatment.

After a four-day treatment with phenobarbital the *microsomal protein* content was significantly increased in comparison with that of the other groups ($p<0.01$). The activity of *NADPH cytochrome C reductase* was also significantly ($p<0.01$) elevated after the phenobarbital treatment. In comparison with the intact controls, neither phenobarbital nor cimetidine affected *UDP-glucuronyltransferase*. However, there was a significant ($p<0.05$) difference in the UDP-glucuronyltransferase activities between the phenobarbital-treated and the cimetidine-treated mice (Tab 1).

Glucose-6-phosphatase was also significantly induced ($p<0.01$) by phenobarbital, while cimetidine had no effect on glucose-6-phosphatase (Tab 1).

DISCUSSION

The present results clearly prove the ability of a phenobarbital treatment to decrease fasting blood glucose levels in obese mice, which are considered to be moderate animal models of NIDDM in man. Although phenobarbital is able to increase the liver blood flow[10] which as such may improve glucose utilization, this drug is primarily a broad-spectrum enzyme inducer. It enhances several phase I reactions (like oxidations and reductions) as well as phase II reactions (like conjugations)[10]. Moreover, as shown in the present study, phenobarbital also induces to a considerable extent the activity of glucose-6-phosphatase, which is an important enzyme in intracellular glucose metabolism.

Cimetidine is a narrow-spectrum enzyme inhibitor, which causes an interference in cytochrome P-450 mediated phase I reactions only. Cimetidine does not affect conjugation reactions[15] or glucose-6-phosphatase (present study). Since cimetidine does not alter fasting blood glucose levels either, it is reasonable to assume that the glucose-lowering effect of phenobarbital is primarily caused by the induction of glucose processing enzymes.

A development of specific enzyme inducers affecting only glucose metabolism is an interesting new aspect of testing in the treatment of NIDDM patients.

REFERENCES

1 Sotaniemi EA, Arranto AJ, Sutinen S, Stengård JH, Sutinen S. Treatment of noninsulin-dependent diabetes mellitus with enzyme inducers. *Clin Pharmacol Ther* 1983; 33 : 826

2 Willms B, Ben-Ami P, Söling HD. Hepatic

enzyme activities of glycolysis and gluconeogenesis in diabetes of man and laboratory animals. *Horm Metab Res* 1970; 28 : 135
3 Seidman I, Horland AA, Teebor GW. Glycolytic and gluconeogenic enzyme activities in the hereditary obese-hyperglycemic syndrome and in acquired obesity. *Diabetologia* 1970; 6 : 313
4 Weber G, Singhal RL, Stamm NB, Srivastava SK. Hormonal induction and suppression of liver enzyme biosynthesis. *Fed Proc* 1965; 24 : 745
5 Hellerström C, Hellman B, Larsson S. Some aspects of the structure and histochemistry of the adrenals in obese hyperglycemic mice. *Acta Pathol Microbiol Scand* 1962; 54 : 365
6 Weber G, Singhal RL, Stamm NB, Fisher EA, Mentendiek MA. Regulation of enzymes involved in gluconeogenesis. *Adv Enzyme Regul* 1963; 2 : 1
7 Longhurst PA, LaCagnin LB, Staats DA, Colby HD. Changes in hepatic drug metabolism in alloxan-diabetic male rabbits. *Biochem Pharmacol* 1986; 35 : 1768
8 Zysset T, Sommer L. Diabetes alters drug metabolism- in vivo studies in a streptozotozin-diabetic rat model. *Experientia* 1986; 42 : 560
9 Lahtela JT, Gachalyi B, Eksymä S, Hämäläinen A, Sotaniemi EA. The effect of liver microsomal enzyme inducing and inhibiting drugs on insulin mediated glucose metabolism in man. *Br J Clin Pharmacol* 1986; 21 : 19
10 Conney AH. Pharmacological implications of microsomal enzyme induction. *Pharmacol Rev* 1967; 19 : 317
11 Puurunen J, Sotaniemi EA, Pelkonen O. Effect of cimetidine on microsomal drug metabolism in man. *Eur J Clin Pharmacol* 1980; 18 : 185
12 Harper AE. Glucose-6-Phosphatase. In: Bergmeyer H-U, ed. *Methoden der enzymatischen Analyse*, 1. Aufl. Weinheim: Verlag Chemie GmbH, 1963 : 788
13 Williams CH Jr, Kamin H. Microsomal triphosphopyridine nucleotide-cytochrome *c* reductase of liver. *J Biol Chem* 1962; 237 : 587
14 Kitchin KT. Laboratory methods for ten hepatic toxification/detoxification parameters. *Methods Findings Exp Clin Pharmacol* 1983; 5 : 439
15 Somogyi A, Gugler R. Drug interactions with cimetidine. *Clin Pharmacokinet* 1982; 7 : 23

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苯巴比妥和西咪替丁对先天性肥胖小鼠血糖浓度和肝代谢酶系活性的影响

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提要　苯巴比妥显著降低 II 型糖尿病动物模型-先天性肥胖小鼠的血糖水平，使肝重/体重比率、肝匀浆中微粒体蛋白含量、NAPDH 细胞色素 C 还原酶以及葡萄糖-6-磷酸化酶活性明显提高，而西咪替丁却没有这些作用。提示苯巴比妥是通过激活糖代谢酶系而产生降血糖效应的。

关键词　酶诱导；酶抑制剂；苯巴比妥；西咪替丁；葡萄糖耐量试验；肥胖小鼠