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Altered cytochrome P450 activities and expression levels in the liver and intestines of the monosodium glutamate-induced mouse model of human obesity

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ABSTRACT

Cytochromes P450 (CYPs) are enzymes present from bacteria to man involved in metabolism of endogenous and exogenous compounds incl. drugs. Our objective was to assess whether obesity leads to changes in activities and expression of CYPs in the mouse liver, small intestine and colon.

Main methods: An obese mouse model with repeated injection of monosodium glutamate (MSG) to newborns was used. Controls were treated with saline. All mice were sacrificed at 8 months. In the liver and intestines, levels of CYP mRNA and proteins were analyzed using RT-PCR and Western blotting. Activities of CYP enzymes were measured with specific substrates of human orthologous forms.

Key findings: At the end of the experiment, body weight, plasma insulin and leptin levels as well as the specific content of hepatic CYP enzymes were increased in obese mice. Among CYP enzymes, hepatic CYP2A5 activity, protein and mRNA expression increased most significantly in obese animals. Higher activities and protein levels of hepatic CYP2E1 and 3A in the obese mice were also found. No or a weak effect on CYPs 2C and 2D was observed. In the small intestine and colon, no changes of CYP enzymes were detected except for increased expression of CYP2E1 and decreased expression of CYP3A mRNAs in the colon of the obese mice.

Significance: Results of our study suggest that the specific content and activities of some liver CYP enzymes (especially CYP2A5) can be increased in obese mice. Higher activity of CYP2A5 (CYP2A6 human ortholog) could lead to altered metabolism of drug substrates of this enzyme (valproic acid, nicotine, methoxyflurane).

1. Introduction

Obesity is currently one of the most important factors affecting human health and quality of life. At least 2.8 million adults die each year as a result of being overweight or obese [28]. Obesity heightens the risk of several illnesses including mainly heart disease and stroke, hypertension, diabetes [5], obstructive sleep apnea [23], osteoarthritis [19], musculoskeletal disorders [27], and cancer [6]. Although the relationships among these pathologies are poorly understood, several studies have demonstrated that obesity is associated with a chronic low-grade inflammation [9].

Recently, progress has been made in understanding the molecular mechanisms of obesity. To study changes in physiological and biochemical parameters in obesity, animal models are used. Several models lacking a single gene or with a dysfunctional single gene have been described. One of these monogenic models, the *agouti* mutation mouse, was reported more than a century ago; however, it was not until 1992 that the first obesity gene in *agouti* was characterized at the molecular level [4]. Although monogenic models provide important information, human obesity is according to current knowledge [17] mediated by multiple genes. Therefore, polygenic models could be more relevant. Frequently used polygenic models include a diet-induced obesity (DIO) or a chemically induced model, which has been also used here. Repeated administration of monosodium glutamate (MSG) to newborn mice has been shown to result in selective destruction of brain arcuate nucleus neurons projecting to the ventromedial hypothalamus and paraventricular nucleus. Mice with MSG-induced lesions are hypophagic and develop obesity with ensuing insulin resistance and hyperinsulinemia [17].

It is important to know if enzymes participating in drug biotransformation are affected by the pathological status of the organism including its obesity. Cytochromes P450 (CYPs) are the most important enzymes

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of drug metabolism [2,7,8]. CYPs are involved not only in the metabolism of drugs and xenobiotics but also in a variety of processes comprising endogenous substances. Although structurally similar, they exhibit differences in the function of individual CYP enzymes reflecting properties of their active sites [2].

The present study was designed to analyze the activities and expressions of hepatic and intestinal CYPs in mice and to reveal possible differences between obese and normal animals. For this purpose, mice with MSG-induced obesity were used as an experimental model. As the enzyme activities and immunoreactivity of mouse CYP forms are in majority analogous to human CYP orthologs or more generally to human CYP subfamilies [20,30] we have used the notation of human CYP forms or subfamilies as well as their characteristic substrates.

The results presented in this report indicate that between the obese and normal mice significant differences exist in CYP specific content (total P450/protein ratio), in relative abundance of the CYP mRNAs and proteins as well as in the corresponding enzyme activities.

2. Material and methods

2.1. Chemicals

Protease inhibitor cocktail tablets (EDTA free Complete Protease Inhibitor Cocktail Tablets) were supplied by Roche (Prague, Roche CZ). Acetonitrile, methanol and HPLC columns were from Merck (Darmstadt, Germany).

For determination of murine CYP activities, substrates of orthologous human CYP forms were used: CYP1A2, phenacetin (murine CYP1A2); CYP2A6, coumarin (murine CYP2A5); CYP2C9, diclofenac and diazepam (murine CYP2C subfamily); CYP2D6, bufuralol (murine CYP2D22); CYP2E1, chlorzoxazone (murine CYP2E1) and CYP3A4, testosterone (murine CYP3A11 and 3A13) as well as respective metabolites 7-hydroxycoumarin, 7-hydroxy-4-(trifluoromethyl)coumarin, desmethyldiazepam and 6-hydroxychlorzoxazone were supplied by Sigma-Aldrich CZ (Prague, Czech Republic); 4-hydroxydiclofenac and 6β-hydroxytestosterone were purchased from Cerilliant Corporation (Round Rock, Texas, USA); 1-hydroxybufuralol was supplied by BD Gentest (Woburn, MA, USA).

The following antibodies against human CYPs cross-reacting with murine orthologs were used: CYP2C9, CYP2C19 and CYP2D6 primary and secondary antibodies were purchased from Acris (Herford, Germany); CYP1A2, CYP2E1 and CYP3A4 primary and secondary antibodies were acquired from Sigma-Aldrich CZ (Prague, Czech Republic); and the CYP2A6 primary antibody was obtained from BD Gentest (Woburn, MA, USA) and the CYP2A6 secondary antibody from Sigma-Aldrich CZ (Prague, Czech Republic). Western blotting was done using a chemiluminescence kit from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Other chemicals were purchased from Sigma-Aldrich CZ (Prague, Czech Republic). All chemicals were of highest purity available.

2.2. Experimental animals

Male NMRI mice obtained from MediTox (Konárovice, Czech Republic) were housed at a temperature of 23 °C and with a daily cycle of 12 h light and dark (light from 6:00). They were given ad libitum water and standard chow diet (ST-1, Velaz, Koleč, Czech Republic). All experiments followed the ethical guidelines for animal experiments and the Czech Republic Law Against the Misuse of Animals No. 246/1992. The newborn mice were divided into two groups. The first group (11 animals) received MSG treatment, while the second one was treated with an equivalent volume of isotonic saline solution and represents the control group. For hypothalamic lesion-induced obesity, newborn mice were administered MSG (10 mg and 20 mg per mice, s.c.) daily from postnatal day 2 to day 6 and day 7 to day 8, respectively. Controls were treated with saline of osmolality corresponding to the MSG solution [18]). At 7 months of age, parameters such as body weight and food intake were measured for 4 weeks. After this period mice were fasted overnight and were sacrificed the next morning. Blood samples were collected into plastic tubes, and plasma and erythrocytes were separated immediately by centrifugation (3000 RPM, 10 min, 10 °C). The liver, small intestine and colon were removed, washed with phosphate buffer saline (PBS) containing protease inhibitor cocktail tablets and immediately frozen in liquid nitrogen. Small pieces of each tissue were separately placed in RNAlater solution. All biological samples were stored in freezer at −80 °C.

3. Biochemical parameters

Levels of glucose, cholesterol, HDL-cholesterol, LDL-cholesterol and triacylglycerols in pooled plasma were determined on a routine analyzer Modular (Roche, Basel, Switzerland). Leptin and insulin concentrations in plasma were quantified by ELISA assay kits (BioVendor, Brno, Czech Republic) according to the manufacturer's instructions.

3.1. Preparation of subcellular fractions

Microsomal fractions were obtained from the liquid nitrogen-frozen liver, small intestine and colon of mice. Microsomes were prepared according to established protocols [15]. The buffer for homogenization of intestinal samples was supplemented with protease inhibitor cocktail tablets (Roche CZ, Prague). All fractions were stored at −80 °C. Protein concentrations in subcellular fractions were assayed using the bicinchoninic acid method according to Smith et al. [24]. The concentration of CYP enzymes in liver microsomes were determined using difference spectroscopy [22].

3.2. Determination of enzyme activities of individual forms of cytochromes P450 in mouse liver microsomes

Enzyme activities of selected CYP enzymes were measured in hepatic microsomes according to established methods ([10] and references therein). Substrates of orthologous human forms (as listed under Chemicals) were used. The following microsomal CYP activities were tested: CYP1A2, phenacetin O-deethylation; CYP2A5, 7-ethoxy-4-(trifluoromethyl)coumarin O-deethylation; CYP2C, diclofenac 4′-hydroxylation and diazepam N-demethylation; CYP2D22, bufuralol 1-hydroxylation; CYP2E1, chlorzoxazone 6-hydroxylation; and CYP3A activity, testosterone 6β-hydroxylation. The amount of microsomal fraction in reaction mixtures corresponded to 70 pmol P450 for determinations of CYP1A2 and CYP2C; 35 pmol P450 for CYP2A6 and CYP2C9 activity, 67.3 pmol P450 for the assay of CYP2D6 activity; 160 pmol P450 for CYP2E1, and 100 pmol P450 for CYP3A activity. All reaction mixtures were buffered by 100 mM K/PO_4 (pH 7.4) and contained an NADPH generating system consisting of isocitrate dehydrogenase, 0.2 U/ml; $NADP^+$, 0.5 mM; isocitric acid, 4 mM; and $MgSO_4$, 5 mM. Final concentrations of substrates in the corresponding reaction mixtures were as follows: CYP1A2, phenacetin, 25 μM; CYP2A6, coumarin, 10 μM; CYP2C9, diclofenac, 16 μM; CYP2C19, diazepam, 100 μM; CYP2D6, bufuralol, 25 μM; CYP2E1, chlorzoxazone, 50 μM; and CYP3A4, testosterone, 100 μM. All determinations were done in triplicates except for CYP2A6 activity which was determined four times to confirm the increase. Activities of murine CYP forms (with the substrates of the orthologous human CYPs as listed above) were measured using the HPLC Prominence system (Shimadzu; Tokyo, Japan) with a UV/fluorescence detection.

3.3. Western blotting

The amount of CYP proteins was evaluated by Western blotting. Mouse liver, small intestine and large intestine microsomal proteins were separated on 10%-SDS (*w*/*v*) polyacrylamide gel electrophoresis