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Cytochrome P450-dependent mixed-function oxidase and glutathione *S*-transferase activities in spontaneous obesity-diabetes

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Abstract—The effect of non-insulin-dependent diabetes on the hepatic microsomal cytochrome P450-dependent mixed-function oxidase system and on cytosolic glutathione *S*-transferase activity was determined using the spontaneously obese-diabetic (ob/ob) mouse model. The activities of the xenobiotic-metabolizing cytochrome P450 proteins were monitored by the use of chemical probes. Non-insulin-dependent diabetes did not influence the hepatic metabolism of substrates associated with the P450 I, IIB, IIE, III and IV families of cytochromes. In contrast, cytosolic glutathione *S*-transferase activity was markedly reduced and glutathione levels were significantly lowered. These findings raise the possibility that patients suffering from this disease may be more susceptible to chemicals that rely on glutathione conjugation for their deactivation.

The cytochrome P450-dependent monooxygenases are probably the most important oxidase system, being responsible for the oxidation of endogenous substrates such as fatty acids, steroids, eicosanoids and vitamins, and also for the deactivation and detoxication of xenobiotics that gain entry into the living organism. It achieves this broad specificity by existing as a number of structurally distinct families of proteins, each with characteristic substrate specificity [1]. Certain families, such as the P450 I and to a lesser extent the P450 IIE, have the propensity to metabolize chemicals at positions, the oxidation of which results in the formation of reactive electrophilic species that interact with DNA and other important molecules giving rise to toxicity/carcinogenicity [2]. Clearly any change in the levels and/or composition of cytochrome P450 proteins will have a consequence for the way a living organism deals with a chemical and whether toxicity will ensue.

In the early 60s [3] it was demonstrated that chemically induced type I, insulin-dependent diabetes modified the ability of hepatic preparations to metabolize a number of drugs and this was substantiated and extended by many other workers, employing different model substrates [4]. More recently it was established that the mechanism involved was a profound effect of the disease on hepatic cytochrome P450 proteins that participate in the metabolism of both endogenous and exogenous substrates [5–8]. The outcome of the alterations in cytochrome P450 composition and levels was that the diabetic animal was more susceptible to the toxicity of chemicals such as carbon tetrachloride and other chemical toxins [9] and, moreover, hepatic preparations from diabetic animals were significantly more effective in converting various promutagens, including nitrosamines and aromatic and heterocyclic amines, to mutagenic species in the Ames mutagenicity assay [5, 10, 11]. The present study was undertaken to investigate

whether non-insulin-dependent diabetes causes similar effects in hepatic cytochrome P450 activity and in glutathione conjugation. The studies were conducted in the spontaneously obese-diabetic (ob/ob) mice, which exhibit many features reminiscent of human non-insulin-dependent diabetes.

Materials and Methods

Pentoxyresorufin, ethoxyresorufin and resorufin (Molecular Probes, Eugene, OR, U.S.A.), ethylmorphine (May and Baker, Dagenham, U.K.), 4-nitrocatechol (BDH Chemicals, Poole, U.K.), *p*-nitropherol, lauric acid, 1, chloro-2,4-dinitrobenzene, cytochrome *c* and all cofactors (Sigma Chemical Co., Poole, U.K.) were all purchased.

Six male spontaneously hyperglycaemic obese (ob/ob) mice and six normal lean littermates, all 16 weeks of age, were obtained from the colony maintained at Aston University. Following death by cervical dislocation, the livers were immediately excised, the gall bladders removed, and microsomal and cytosolic fractions were prepared as described previously [12]. The following determinations were carried out on the microsomal fraction: ethoxyresorufin *O*-deethylase [13], pentoxyresorufin *O*-dependentylase [14], ethylmorphine *N*-demethylase [15], *p*-nitrophenol oxidase [16], NADPH-cytochrome *c* reductase [17], cytochrome b_5 and total cytochrome P450 levels [18]. Lauric acid hydroxylase was determined by a TLC method [19] and represents the combined formation of 12(ω)- and 11(ω-1)-hydroxylauric acid metabolites. On the cytosolic fraction: total glutathione levels [20], glutathione *S*-transferase, using 1,chloro-2,4-dinitrobenzene as the acceptor substrate [21], and glutathione reductase [22] were determined. Protein was determined on both fractions [23] using bovine serum albumin as standard. In the plasma, glucose [24] and 3-hydroxybutyrate and acetoacetate [25] concentrations were determined. Statistical evaluation was carried out using the unpaired Student's *t*-test.

Results

The obese-diabetic (ob/ob) mice exhibited hyperglycaemia, plasma glucose levels being double those of the lean controls (Table 1). The plasma ketone levels, measured as the sum of acetoacetate and hydroxybutyrate, did not differ between the two animal groups. Cytochrome b_5 and total cytochrome P450 levels, as well as the NADPH-dependent reduction of cytochrome *c* in obese mice, were not significantly different from those in the lean animals (Table 2). Mixed-function oxidase activity, determined using five model substrates, was similar in the two animal groups (Table 2). However, glutathione *S*-transferase activity was markedly lower in the obese-diabetic mice when compared to the lean animals (Table 3). Cytosolic glutathione levels were also lower in the obese mice but the difference was less pronounced. There was no significant difference in glutathione reductase activity between the two animal groups (Table 3).

Discussion

Non-insulin-dependent diabetes is a milder, but a much more prevalent form of the disease that tends to afflict the old and the obese, and is effectively treated by oral medication and manipulation of the diet. In the present study, mixed-function oxidase activity was determined in the obese-diabetic mice and their normal littermates using

Table 1. Plasma ketone and glucose concentrations in lean and obese-diabetic (ob/ob) mice

Parameter	Lean	Obese-diabetic
Glucose (mM)	5.6 ± 0.2	11.2 ± 1.4*
Acetoacetate + 3-hydrohybutyrate (mM)	0.40 ± 0.01	0.38 ± 0.10

Results are presented as means ± SEM for six animals.
* $P < 0.05$.

Table 2. Hepatic microsomal parameters and mixed-function oxidases in lean and obese-diabetic (ob/ob) mice

Parameter	Lean	Obese-diabetic
Ethoxyresorufin *O*-deethylase (pmol/min/mg protein)	9.9 ± 2.1	8.6 ± 2.8
Pentoxyresorufin *O*-dependentylase (pmol/min/mg protein)	0.4 ± 0.2	0.5 ± 0.2
p-Nitrophenol oxidase (nmol/min/mg protein)	0.80 ± 0.07	0.91 ± 0.09
Ethylmorphine *N*-demethylase (nmol/min/mg protein)	14.0 ± 1.1	15.1 ± 2.4
Lauric acid hydroxylase (nmol/min/mg protein)	1.8 ± 0.7	2.3 ± 0.6
NADPH-cytochrome *c* reductase (nmol/min/mg protein)	5.5 ± 0.7	7.1 ± 2.0
Cytochrome P450 (nmol/mg protein)	0.21 ± 0.05	0.19 ± 0.01
Cytochrome b_5 (nmol/mg protein)	0.45 ± 0.10	0.51 ± 0.10
Protein (mg/g liver)	26.0 ± 0.9	28.1 ± 1.2

Results are presented as means ± SEM for six animals.

Table 3. Hepatic cytosolic glutathione conjugation in lean and obese-diabetic (ob/ob) mice

Parameter	Lean	Obese-diabetic
Glutathione *S*-transferase (μmol/min/mg protein)	1.48 ± 0.06	0.34 ± 0.06†
Glutathione reductase (nmol/min/mg protein)	64.4 ± 2.4	78.2 ± 7.2
Total glutathione (mM)	7.8 ± 0.5	4.8 ± 0.7*

Results are presented as means ± SEM for six animals.
* $P < 0.01$; † $P < 0.001$.

five substrates that serve as chemical probes for specific cytochrome P450 families: ethoxyresorufin *O*-deethylase for P450 I [26], pentoxyresorufin *O*-depentylase for P450 IIB [14], *p*-nitrophenol oxidase for P450 IIE [27], lauric acid hydroxylase for P450 IV [28] and ethylmorphine *N*-demethylase for P450 III [29]. None of these activities was influenced by non-insulin-dependent diabetes in the model examined which is in marked contrast to the effect observed in insulin-dependent diabetes where all the above activities were induced [5, 7, 8]. Similarly, using less specific substrates, Rouer and Leroux [30] did not observe significant differences between the ob/ob mice and their lean controls. The insulin-dependent diabetes-induced changes in hepatic P450 activity were ascribed to two physiological changes that accompany this condition, namely hyperketonaemia and the lower circulating levels of growth hormone, the consequence of impaired secretion [7, 8, 31, 32]. The present model of non-insulin-dependent diabetes exhibits normal plasma ketone concentrations and plasma growth hormone levels are normal or slightly reduced [33, 34]. The normal plasma ketone and growth hormone levels account for the lack of effect of non-insulin-dependent diabetes on the mixed-function oxidase system.

Reactive intermediates produced through metabolism may be detoxicated by phase II reactions, the most prominent being the conjugation with the tripeptide glutathione, the reaction being catalysed by the glutathione *S*-transferases. In chemically induced insulin-dependent diabetes rat hepatic glutathione *S*-transferase activity diminished [9] although other workers, whose studies were conducted in mice, reported increases in streptozotocin-treated mice, which, however, could be attributed to the diabetogen rather than the diabetic state [35]. In the present study, glutathione *S*-transferase activity was reduced markedly in obese-diabetic (ob/ob) mice, being 25% of that seen in the lean animals. The mechanism through which glutathione *S*-transferase activity is inhibited in both diabetes syndromes is not clear, but the fact that hyperglycaemia is a common characteristic of both conditions, indicates that it may, at least partly, mediate these changes; however, other mechanisms may be involved and may not be necessarily common to the two conditions. Glutathione levels were also lower in the obese-diabetic mice when compared to the lean controls. Chemically induced insulin-dependent diabetes has also been associated with changes in glutathione levels, both increases and decreases [36, 37]. Finally, non-insulin-dependent diabetes did not influence glutathione reductase activity which ensures that glutathione is maintained in the reduced form. The decreased capacity to conjugate with glutathione observed in this model of non-insulin-dependent diabetes raises the possibility that patients suffering from this disease may be particularly susceptible to the toxicity of chemicals. Moreover, the glutathione levels being low, they can be depleted with lower doses of a given chemical toxin, further exacerbating the toxic response.

In conclusion, the present study demonstrates that non-insulin-dependent diabetes, as manifested in obese-diabetic (ob/ob) mice, decreases glutathione conjugation capacity but, in contrast to insulin-dependent diabetes, it does not modulate the hepatic microsomal cytochrome P450-dependent mixed-function oxidase system.

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