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SHORT COMMUNICATIONS

Liver microsomal cytochrome P-450 and related monooxygenase activities in genetically hyperglycemic (ob/ob and db/db) and lean streptozotocin-treated mice

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Since the first report of Dixon *et al.* [1] on drug metabolism in alloxan-diabetic rats, further pertinent observations have accumulated, using streptozotocin, which leads to a similar hypoinsulinemic diabetes by destroying β cells. Both increase and decrease of oxidative metabolism have been reported in chemically-induced diabetes. For example, an increase of aniline metabolism was observed in alloxan diabetes by Dixon *et al.* [2] and Kato *et al.* [3], as in streptozotocin diabetes by Past and Cook [4] and Donahue and Lindsay [5], whereas Ackerman and Leibman [6] reported an inhibition of this metabolism. Such conflicting reports may be explained by varying degrees of severity of the induced diabetes, as suggested by the study of Donahue and Lindsay [5], but strain, sex and age of the experimental animals may also introduce some variations.

Since strains of genetically hyperglycemic hyperinsulinemic mice are now available, it seemed interesting to study some drug-metabolizing enzymes in those animals, and compare them with the modifications induced by chemical diabetogenics in the corresponding wild strains. In this way, the relative effects of blood glucose and blood insulin levels should be clarified. Furthermore, the two forms of animal diabetes may to some extent be considered as a model for the two similar forms of human diabetes: hyperinsulinemic maturity-onset diabetes on the one hand, hypoinsulinemic juvenile diabetes on the other hand.

Seven- to nine-weeks-old male mice, either obese hyperglycemic (C57BL/6 Orl ob/ob) or of the corresponding lean strain, and 12- to 14-weeks-old male mice, either diabetic (C57BL/Ks Orl db/db) or of the corresponding wild strain, were obtained from the 'Centre d'Elevage du CNRS', Orléans, France. Some lean C57BL/6 mice received a single intraperitoneal (i.p.) injection of streptozotocin (200 mg/kg) freshly dissolved in saline at pH 4.5. The streptozotocin-injected mice were divided into two groups: one remained untreated, while the other one week later received an i.p. injection of 1 unit of protamine–zinc–insulin per 100 g body wt every 12 hr for one week. All mice were killed 2 weeks after the initial injection of streptozotocin, 2 hr after the last injection (at 8:30 a.m.) for the insulin-treated group. Mice of the insulin-untreated group were hyperglycemic but not ketonuric. Animals were decapitated and blood was collected for glucose analysis. Microsomes were prepared as previously described [7].

Plasma glucose was determined by the glucose oxidase method using a Beckman analyser. Microsomal proteins were measured after precipitation by trichloracetic acid as described by Lowry *et al.* [8] using bovine serum albumin as standard. Glucose-6-phosphatase activity was determined as indicated by Swanson [9]. Cytochrome P-450 was measured in homogenates and microsomes as described by Greim [10] and Omura and Sato [11]. Aniline hydroxylase, *p*-nitroanisole-*O*-demethylase and 7-ethoxycoumarin-*O*-deethylase were assayed as previously indicated [12]. Benzo[*a*]pyrene hydroxylase was measured according to the fluorimetric method of Nebert and Gelboin [13] using a Jobin Yvon JYD 3 spectrofluorometer, with 3-hydroxybenzo[*a*]pyrene as reference. *n*-Butylisocyanide (20 μM) spectra were recorded on a Beckman Acta MVI spectrophotometer with microsomes in 30% glycerol–Tris buffer, pH 7.40.

Streptozotocin (STZ) was obtained from Sigma and insulin (endopancrine zinc protamine) from Organon. Benzo[*a*]pyrene (Sigma) was recrystallized from benzene–methanol (1:5). 3-Hydroxybenzo[*a*]pyrene was kindly supplied by Dr. F. Decloitre (Villejuif). 7-Ethoxycoumarin was prepared as described by Ullrich and Weber [14] and hydroxylamines as previously described [12]. SKF 525 A was obtained from Smith Kline & French Laboratories; metyrapone from Ciba; *n*-butylisocyanide from Aldrich.

Some physiological characteristics (body and liver weight, blood glucose level) of the mice used in this study are shown in Table 1. Blood glucose is moderately increased (1.8-fold) in ob/ob mice, but highly increased (3.5- to 3.7-fold) in db/db and STZ-injected lean mice. Chemically induced diabetes leads to a loss in both body and liver weight, whereas those parameters increase 1.3- to 1.9-fold in spontaneously hyperglycemic (ob/ob and db/db) mice. Insulin treatment of STZ-injected mice antagonizes the observed abnormalities at least partly.

The hepatic content of cytochrome P-450 (Table 1) is indicated both in the whole homogenate (as nmoles per g liver), and in the microsomal fraction (as nmoles per mg microsomal proteins). In ob/ob mice as compared to their lean controls, liver cytochrome P-450 appears to be slightly increased, unmodified or decreased according to as it is related to 1 mg of microsomal proteins, one whole liver (data not shown) or 1 g of liver, respectively. This apparent discrepancy may be explained by a decreased content of endoplasmic reticulum in ob/ob mouse liver. Determinations of glucose-6-phosphatase activity (results not shown) confirmed this assumption: it was increased 3-fold in microsomes (per mg microsomal protein), but less than 2-fold in crude homogenates (per g liver). Thus, though the microsomal concentration of cytochrome P-450 is slightly increased, its total content per g liver appears to be decreased. However, when the cytochrome P-450 was expressed per whole liver, normal values were recovered, since liver is much larger in ob/ob mice than in their lean controls (Table 1). In db/db mice, the microsomal content of cytochrome P-450 is moderately increased with reference to wild controls.

Table 2 shows the effects of spontaneous or chemically induced hyperglycemic states on some monooxygenase molecular activities. Enzyme activities of ob/ob and db/db mice must be compared to those of the respective wild strains, which appear to be rather different from each other, either genetically or as a consequence of age (C57BL/Ks mice were slightly older when studied). Differences appear between ob/ob or db/db strains and their respective controls, but they are neither very important nor systematic, though usually increased molecular activities are observed in db/db mice. Using qualitative criteria (inhibition of 7-ethoxycoumarin-*O*-deethylase, *n*-butylisocyanide spectra), no significant differences appear between spontaneously hyperglycemic mice and their controls, except for the extent of inhibition of 7-ethoxycoumarin-*O*-deethylase activity by hydroxylamines. The sig-

Table 1. Some physiological characteristics of the mouse strains studied and cytochrome P-450 content in whole homogenates and microsomal fractions*

	C57BL/6 strain				C57BL/KS strain	
	Control mice	ob/ob mice	STZ-injected mice	STZ-injected mice and insulin-treated	Control mice	db/db mice
Body weight (g)	22.5 ± 0.4	28.8 ± 0.8†	16.4 ± 0.8†	18.5 ± 0.7‡	26.0 ± 1.0	38.2 ± 3.3†
Liver weight (g)	1.29 ± 0.02	2.48 ± 0.11†	0.90 ± 0.06†	1.19 ± 0.06	1.19 ± 0.03	2.06 ± 0.30†
Blood glucose ($mmolcs.l^{-1}$)	7.83 ± 0.44	14.44 ± 0.78†	27.61 ± 1.72†	6.11 ± 0.55	6.89 ± 0.17	25.89 ± 2.00†
Total cyt P-450 per g liver ($nmoles.g^{-1}$) (fresh wt)	22.1 ± 2.4	13.7 ± 1.5‡	40.1 ± 1.7†	21.0 ± 1.5	21.4 ± 2.4	26.9 ± 2.4
Microsomal cyt P-450 per mg of protein ($nmoles.mg^{-1}$)	0.41 ± 0.03	0.51 ± 0.05	1.04 ± 0.05†	0.38 ± 0.02	0.58 ± 0.05	0.65 ± 0.02

* STZ-injected and STZ-injected insulin-treated animals were lean mice of the C57BL/6 +/+ strain (see text). Results are the mean ± S.E. of mean (six to ten animals). Total cytochrome P-450 was measured in crude homogenates, and microsomal cytochrome P-450 in the microsomal fraction, as described in the text. The yield of cytochrome P-450 recovered in the microsomal fraction is between 26 and 40 per cent of total cytocrome P-450 in homogenates. The significance of differences between means, as compared to controls of the same strain, was determined according to Wilcoxon's *w*-test.

† Significant at the 1% level.

‡ Significant at the 5% level.

Table 2. Cytochrome P-450-dependent monooxygenase activities and *n*-butylisocyanide spectra of the microsomal fraction*

	C57BL/6 strain				C57BL/KS strain	
	Control mice	ob/ob mice	STZ-injected mice	STZ-injected mice insulin treated	Control mice	db/db mice
Aniline hydroxylase	0.60 ± 0.06	0.32 ± 0.10	1.86 ± 0.34‡	1.45 ± 0.12‡	0.76 ± 0.12	0.84 ± 0.16
p-Nitroanisole-*O*-demethylase	4.02 ± 0.68	5.87 ± 0.90	16.51 ± 2.53‡	8.05 ± 1.09‡	15.68 ± 1.66	18.48 ± 2.82
Benzo[*a*]pyrene hydroxylase	0.25 ± 0.02	0.33 ± 0.03	0.38 ± 0.04‡	0.28 ± 0.08	0.10 ± 0.03	0.24 ± 0.08
7-Ethoxycoumarin-*O*-deethylase	1.84 ± 0.23	1.25 ± 0.27	7.42 ± 0.95†	1.84 ± 0.20	0.93 ± 0.30	1.51 ± 0.43
Inhibition(%) of 7-Ethoxycoumarin-*O*-deethylase by:						
Metyrapone	14 ± 2	24 ± 3‡	47 ± 3†	37 ± 4†	19 ± 4	27 ± 5
SKF 525A	9 ± 2	7 ± 2	7 ± 3	6 ± 3	17 ± 5	14 ± 6
2-hydroxylaminopropane	78 ± 2	59 ± 2†	28 ± 3†	46 ± 2†	76 ± 4	60 ± 5
N-hydroxyamphetamine	95 ± 3	74 ± 6†	91 ± 1	92 ± 1	94 ± 1	90 ± 2
n-Butylisocyanide spectra:						
Δ455/Δ430	0.33 ± 0.04	0.29 ± 0.02	0.28 ± 0.02	0.26 ± 0.03	N.D.§	N.D.

* Results are the mean ± S.E. of mean (six to ten animals). Significance of differences between means, as compared to controls of the same strain, was determined according to Wilcoxon's *w*-test. Enzyme activities are expressed as nmoles of product formed . min^{-1} . $nmoles^{-1}$ cytochrome P-450. Inhibitors were used at 10^{-5}M.

† Significant at the 1% level.

‡ Significant at the 5% level.

§ N.D. Not determined.

nificant difference of 7-ethoxycoumarin-*O*-deethylase inhibition by hydroxylamines between ob/ob mice and their lean controls may be explained by a difference in affinity and rate of formation of the inhibitory complex absorbing at 455 nm [12]: towards 2-hydroxylaminopropane, K_s was $2.6 \pm 0.2\ \mu M$ in ob/ob mice and $1.4 \pm 0.2\ \mu M$ in control mice; similarly, the initial rate of formation of the complex (at 0.2 mM substrate) was 0.011 ± 0.001 DO units. min^{-1}. $nmole^{-1}$ cytochrome P-450 in ob/ob mice and 0.021 ± 0.001 DO units. min^{-1}. $nmole^{-1}$ cytochrome P-450 in control mice (means ± S.E. of the mean, three determinations). Thus, it may be postulated that the modification of one of the components of the mixed function oxidase system, leading to modified accessibility of the active centre and consequently to modified affinity towards its substrates, might account for the small variations of drug metabolism observed. A report of Varandani *et al.* [15] indicates that the microsomal phospholipid composition in ob/ob mice is modified when compared to that in lean mice. This fact is in agreement with our hypothesis, since the permissive role of phospholipid on drug metabolism is well known [16].

In contrast to the slight modifications observed in spontaneously hyperglycemic mice, STZ treatment produces a large increase of cytochrome P-450 (2.5-fold; Table 1) and some monooxygenase activities, especially *p*-nitroanisole-*O*-demethylase and 7-ethoxycoumarin-*O*-deethylase (Table 2). Specific activities (per mg microsomal protein) increase 10-fold (not shown), and molecular activities (per nmole of cytochrome P-450) 4-fold, which suggests both quantitative and qualitative differences at the cytochrome P-450 level. The existence of qualitative differences is confirmed by the inhibition pattern of 7-ethoxycoumarin-*O*-deethylase: STZ treatment increases inhibition by metyrapone and decreases inhibition by 2-hydroxylaminopropane. Insulin corrects most STZ-induced modifications either completely or partially.

The marked increase of cytochrome P-450 and some related monooxygenase activities in STZ-injected animals might result from a simple inducing effect of the drug. The inhibition pattern of 7-ethoxycoumarin-*O*-deethylase, *n*-butylisocyanide spectra and the minute increase of benzo[*a*]pyrene hydroxylase activity certainly exclude the involvement of cytochrome P-448-like species, as was also shown in rat by the lack of effect of STZ on biphenyl-2-hydroxylation, a 3-methylcholanthrene-induced enzyme activity [17]. However, the correction of STZ effects by insulin, also observed in rat for some activities [17], is quite inconsistent with a direct inducing action of STZ on the monooxygenase system, as are also the following observations: analogues of STZ without diabetogenic properties fail to modify drug-metabolizing activities [17]; moreover, in our experiments, one single injection of STZ would retain inducing properties two weeks later, which is quite unusual among common inducers. It must also be stressed that STZ has no effect on drug-metabolizing enzymes when added *in vitro* to a microsomal suspension [6].

A direct effect of elevated blood glucose may also be ruled out: as compared with STZ-treated animals, genetically hyperglycemic mice exhibit very moderate increases of cytochrome P-450 and related monooxygenase activities, although their blood sugar (especially for db/db) is in the same range.

The main difference between STZ-treated and spontaneously hyperglycemic animals lies in their blood insulin level, decreased in the former, increased in the latter. Glucagonemia is high in both cases [18–20]. Results and interpretations of Ackerman and Leibman [6] about the possible inhibitory effects of glucagon and cAMP upon drug metabolism in rat certainly do not hold in diabetic mice whose enzyme activities are increased. Thus, it might be postulated that, at least in male mice, insulin regulates monooxygenase activities, and behaves as a 'repressor' of certain forms of cytochrome P-450, but not of cytochrome P-448. A more precise identification of the forms possibly regulated by insulin, as compared with phenobarbital-induced cytochromes P-450, is presently in progress.

In summary, as compared with the corresponding wild strains, spontaneously hyperglycemic mice exhibit some slight qualitative and quantitative differences in their mixed-function oxidase system, whereas a marked increase of some monooxygenase molecular activities is observed in streptozotocin-treated lean mice. Insulin treatment counteracts the effects of streptozotocin. In streptozotocin-treated animals, neither the increased blood level, nor a simple inducing effect of streptozotocin is responsible for the observed modifications of the monooxygenase system, which appear to be related to the decreased level of blood insulin.

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