Biochemical Pharmacology, Vol. 32, No. 2, pp. 327–335, 1983.
Printed in Great Britain.

0006-2952/83/020327-09 $03.00/0

DIFFERENTIAL EFFECTS OF DIABETES ON MICROSOMAL METABOLISM OF VARIOUS SUBSTRATES

COMPARISON OF STREPTOZOTOCIN AND SPONTANEOUSLY DIABETIC WISTAR RATS*

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(*Received* 18 *February* 1982; *accepted* 18 *June* 1982)

Abstract—We have examined the effect of recent onset diabetes on several aspects of hepatic microsomal metabolism in both streptozotocin (STZ)-induced and spontaneously diabetic BioBreeding (BB) male and female Wistar rats. Differential alterations of the diabetic state on hepatic microsomal enzyme activities were observed. Female diabetic rats exhibited no change in benzo[*a*]pyrene (BP) hydroxylase activity, a decrease in testosterone Δ^4-hydrogenase, and an increase in aniline hydroxylase. On the other hand, male diabetic rats demonstrated a decrease in hepatic BP hydroxylase activity, no change in testosterone Δ^4-hydrogenase, and an increase in aniline hydroxylase. Insulin treatment corrected these effects. No change in kidney BP hydroxylase activity was apparent in either female or male diabetics. There were no marked differences between the chemically induced and genetic models of diabetes with respect to the metabolism studies. Serum testosterone levels were significantly lower than control in male BB diabetics, whereas no change was apparent in female diabetics. Light microscopy and serum insulin determinations indicated that the spontaneously diabetic animals we examined were not severely diabetic. From electrophoresis of hepatic microsomal proteins we determined that spontaneous diabetes of short duration does alter the protein distribution in the cytochrome P-450 region. We conclude that the acute effects of STZ-induced and spontaneous diabetes on hepatic microsomal metabolism are quantitatively and qualitatively similar, despite probable differences in etiology of the diabetic state.

Several laboratories have reported the effects of experimental diabetes on microsomal metabolism of various substrates in rats. In general, it has been observed that alloxan- or streptozotocin (STZ)-induced diabetes decreased androgen-sensitive hepatic drug metabolism in male rats [1–6], whereas in female rats the same sex-dependent activities are increased [2, 3, 5, 6]. On the other hand, sex-independent drug-metabolizing activities are not impaired or may even be enhanced [2, 3, 5, 7]. These effects of diabetes in male and female rats have been shown to be reversed by insulin administration [1, 5, 7]. Most workers have concluded that chemically induced diabetes somehow interferes with androgenic stimulation of hepatic microsomal metabolism, although evidence in this regard has been indirect.

Despite the acceptance of using β-cell toxins to produce a model of diabetes in rats, other extrapancreatic toxicities of these agents cannot be definitively ruled out. There is evidence that STZ, for example, may cause liver damage [8]. Female C3H-S mice were administered a single injection of STZ, and 21 days later ultrastructural changes in hepatocytes were observed. These included degranulation of rough endoplasmic reticulum (ER), swelling of mitochondria, and some dilatation of smooth ER. Furthermore, it was observed that animals exhibited liver damage whether diabetic symptoms were present or not.

In future studies, therefore, it would be advantageous to eliminate possible direct toxic effects of STZ on the liver. In this regard, we have carried out studies in Wistar-derived BB genetically diabetic rats (BioBreeding Laboratories) [9]. The diabetic syndrome in these animals develops spontaneously before 120 days of age with a variable frequency and spectrum of severity. The affected animals are non-obese, hyperglycemic, and glycosuric. Plasma ketone and glucagon levels are elevated in relation to severity of the diabetic state. Progressive pancreatic β-cell destruction with insulin deficiency appears to be a result of marked inflammatory changes in the pancreatic islets [9]. The etiology of the genetic syndrome is unknown.

We have studied hepatic microsomal metabolism towards various substrates in both STZ-induced and BB spontaneously diabetic rats and have followed blood glucose, insulin, and testosterone levels in these animals. We also monitored the effect of diabetes on pancreatic and hepatic histology and on the electrophoretic profile of liver microsomal proteins.

* The majority of this work was submitted by B. L. Warren in partial fulfillment of the requirements for the Ph.D. degree in pharmacology. Supported by the Medical Research Council of Canada, Grant MT-5944.

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MATERIALS AND METHODS

Chemicals. STZ, benzo[*a*]pyrene (BP), testosterone (Δ^4-androsten-17β-ol-3-one), NADP, NADPH, bovine serum albumin, glucose-6-phosphate, sodium dodecyl sulfate, acrylamide, and Coomassie brilliant blue G were obtained from the Sigma Chemical Co. (St. Louis, MO). BP was further purified by dissolving it in benzene and then filtering and recrystallizing it from cold methanol. Aniline HCl, 2-mercaptoethanol, and *N,N'*-methylene bisacrylamide were obtained from the Eastman Kodak Co. (Rochester, NY). Protamine zinc (PZ) insulin (Connaught Laboratories, Willowdale, Ontario) was used to maintain diabetic animals where indicated. *N,N,N',N'*-Tetramethylenediamine and standard proteins for calibration were obtained from Pharmacia Chemical Co. (Piscataway, NJ), and ammonium persulfate from the E-C Apparatus Corp. (St. Petersburg, FL). All other chemicals and reagents were of analytical grade purity.

Animals. In all experiments involving the BB Wistar-derived spontaneously diabetic rats (D), two control groups obtained from the same supplier were followed (BioBreeding Laboratories of Canada Ltd., Ottawa, Ontario). Rats of the directly comparable group for these diabetics were nondiabetic littermates, designated "inside" nondiabetic controls (IC). In addition, we obtained normal "outside" Wistar controls (OC) which were from the same supplier but were not from the diabetic colony. The STZ diabetic rats and the corresponding citrate buffer injected contols were also of the Wistar strain but were obtained from the Canadian Breeding Farms (Montreal, Quebec). All animals were allowed to equilibrate for at least 7 days in controlled light and temperature conditions (6:00 a.m. to 6:00 p.m., 22°). Experimental and control animals were matched for age (120–160 days). Spontaneously diabetic animals were administered subcutaneously 0.8 units/100 g body weight PZ insulin 12 hr after arrival and then on a daily basis. In studies involving insulin-treated diabetics, insulin therapy was maintained until 18 hr before the experiment. Chemically induced diabetic animals received 60 mg/kg STZ via tail vein injection. Control animals received a similar injection with vehicle only (0.1 M citrate buffer, pH 4.5). Experiments were performed either 4 days post-STZ injection in chemically induced animals or 4 days after withdrawal of insulin in genetically diabetic animals. This time period was chosen since we were interested in early detectable changes, and time–course studies showed that at approximately 96 hr this could be achieved. Animals were fasted for 16 hr before being killed unless they were being maintained on insulin. Blood samples were taken for insulin, glucose, testosterone determinations, and the extent of glycosuria was monitored using Tes-Tape (Eli Lilly & Co. Ltd., Toronto, Ontario).

Preparation of microsomes. All procedures were conducted at 4°. Liver and kidney tissues were homogenized in 1.15% KCl (1:4, w/v) in a Potter–Elvehjem tissuer homogenizer and Polytron tissue homogenizer (Brinkmann Instruments, Rexdale, Ontario) respectively. Homogenates were centrifuged at 10,000 *g* for 10 min. Post-mitochondrial fractions were then centrifuged at 100,000 *g* for 60 min. Both liver and kidney pellets were washed and resuspended in 0.1 M phosphate buffer, pH 7.2.

Enzyme assays. BP hydroxylase activities in liver and kidney were measured fluorometrically by the method of Nebert and Gelboin [10] except that albumin was added to the incubation mixture [11]. The requirements for saturating substrate concentration and linearity of enzyme concentration and incubation time in both chemically induced and genetically diabetic rats were established in preliminary studies. The final volume of the incubation mixture was 1.0 ml at pH 7.4. It contained 0.36 μmole NADH, 0.36 μmole NADPH, 3 μmoles $MgCl_2 \cdot 6H_2O$, 8.7 nmoles bovine serum albumin, 150 μl enzyme preparation, 80 nmoles BP in 40 μl acetone, and 25 μmoles Tris buffer, pH 7.5. Protein content in the incubation mixture was 0.2 to 0.4 mg. The incubation was performed for 2.5 min at 37° for liver and for 5 min in kidney. Activity is expressed as pmoles 3-hydroxybenzo[*a*]pyrene produced per min per mg protein. Protein determinations were measured according to the method of Bradford [12], using crystalline bovine serum albumin as the standard.

Hepatic aniline hydroxylase activity was measured colorimetrically by the method of Imai *et al.* [13]. The final volume of the incubation mix was 2.0 ml. It contained 2.5 μmoles aniline, 0.25 ml of 0.1 M phosphate buffer, pH 7.2, 0.25 ml enzyme preparation, 2.5 μmoles NADP, 5 μmoles glucose-6-phosphate, 2.5 units glucose-6-phosphate dehydrogenase, 12.5 μmoles $MgCl_2 \cdot 6H_2O$, and 0.94 ml phosphate buffer. The incubation was carried out for 15 min at 37° and was stopped with 1.0 ml of 20% trichloroacetic acid. The samples were centrifuged to remove the precipitate. To a 1.0 ml aliquot of the supernatant fraction 0.5 ml of 10% Na_2CO_3 was added, followed by 1.0 ml of 2% phenol in 0.2 N NaOH. Color development was measured at 640 nm after incubation for 30 min at 37°. Approximately 2–3 mg of protein was added to the incubation mixture. Activity is expressed as nmoles *p*-aminophenol produced per min per mg protein.

Hepatic testosterone Δ^4-hydrogenase activity was measured colorimetrically by the method of McGuire and Tomkins [14]. The final volume of the incubation mixture was 2.0 ml. It contained 12.5 μmoles $MgCl_2 \cdot 6H_2O$, 1.0 μmole NADP, 20.0 μmoles glucose-6-phosphate, 1 unit glucose-6-phosphate dehydrogenase, 0.5 μmole testosterone, 200 μl enzyme suspension, and 1.72 ml phosphate buffer, pH 7.2. The incubation was carried out for 10 min at 37° and was stopped with dichloromethane. The aqueous phase was aspirated, and the absorption at 240 nm was measured. The decrease in absorption compared to a testosterone blank was related to the amount of testosterone metabolized. Approximately 0.5 to 1.5 mg of protein was added to the incubation mixture. Activity is expressed as nmoles testosterone metabolized per min per mg protein.

Insulin and glucose determinations. Insulin levels were measured by the radioimmunoassay method of Pederson and Brown [15] with modification for application to rat serum. The method employed a guinea pig antibody prepared against human insulin.

A standard insulin curve was prepared in the presence of rat serum with each assay. Each incubation tube contained 0.7 ml phosphate buffer, 0.1 ml antiserum, 0.1 ml serum sample, and 0.1 ml [^{125}I]insulin. Bound radioactive isotope was separated from free, using the dextran coated charcoal method. Serum glucose levels were measured using a Beckman Glucose Analyzer (Beckman Instruments Inc., Fullerton, CA) with glucose oxidase as the reagent.

Serum testosterone determinations. A direct procedure for radioimmunoassay of serum testosterone was employed using an [^{125}I]testosterone kit (Radioassay Systems Laboratories, Inc., Carson, CA). Total unconjugated testosterone was measured using this method.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). For these studies, animals were not fasted before being killed. Electrophoresis of microsomal preparations was carried out according to Weber and Osborn [16] with modifications. Glass gel tubes approximately 10 cm long with an inner diameter of 6 mm were used for preparation of the gels. The gels consisted of gel buffer: 0.1 M sodium phosphate, pH 7.0, containing 0.1% SDS (w/v), 7.5% (w/v) acrylamide, 0.07% (w/v) methylene bis-acrylamide, 0.06% (w/v) ammonium persulfate, and 0.15% (v/v) *N,N,N′,N′*-tetramethylenediamine. Microsomal preparations (8–12 mg/ml) were diluted 1:10 in 0.1 M phosphate buffer. Forty microliters of the protein suspension was solubilized in 40 μl of 0.5 M sodium phosphate buffer, pH 7.0, containing 3.3% (w/v) SDS, 30% (v/v) glycerol, and 16% (v/v) 2-mercaptoethanol. Bromphenol blue 0.048% was added and the mixture was boiled for 5 min. Approximately 25 μg of solubilized protein which was contained in a volume of 50 μl was applied to each gel. Electrophoresis was carried out at 33–35° with constant current of about 12 mA/gel (Power Supply EPS 500/400, Pharmacia, Montreal) for about 4–5 hr, or until the bromphenol blue tracking dye was within 1 cm of the bottom of the tube. The gels were stained in Coomassie brilliant blue G 0.025% (w/v), with 10% (v/v) glacial acetic acid and 25% (v/v) isopropyl alcohol in distilled water. Gels were destained in 10% acetic acid. The gels were scanned at 550 nm using a Gilford model 250 spectrophotometer.

Light microscopy. Tissue sections were taken from the main lobe of the liver and from the pancreas at the time of sacrifice. Liver specimens were fixed in 10% buffered formalin with calcium acetate 2%. Pancreatic sections were fixed in Bouin's solution. Hematoxylin and eosin staining was performed in the usual manner. Some pancreatic sections from the BB rats were immunostained by the peroxidase antiperoxidase method with antisera to insulin (1:2000) (performed by Dr. Alison Buchan, Department of Physiology, University of British Columbia).

Statistics. Student's *t*-test for unpaired sample means was carried out where indicated. Differences were considered statistically significant if $P < 0.05$.

RESULTS

Effects of diabetes on body weight, serum glucose, and serum insulin levels. Whether rats were chemically induced or spontaneously diabetic, short-term diabetes (4 days) produced a significant decrease in body weight (Table 1). This was apparent in both male and female rats. Control rats had fasted serum glucose levels of approximately 100 mg/100 ml, whereas diabetic rats exhibited significant hyperglycemia (3 to 4-fold above control). It was observed that BB diabetic animals exhibited only slightly lower serum insulin levels than the nondiabetic controls (IC), even though the diabetics were definitely hyperglycemic. On the other hand, the STZ diabetic animals did show a statistically significant reduction in serum insulin levels.

Effects of diabetes on microsomal enzyme activities. We measured hepatic BP hydroxylase activity as an index of sex-dependent drug metabolism. As expected, control female levels were approximately 5 to 6-fold less than male control levels (Fig. 1). Our data indicated that this activity in diabetic females was not different from the appropriate control levels, whether the animal was STZ or genetically diabetic (Fig. 1a). On the other hand, in males, hepatic BP

Table 1. Effects of spontaneous and STZ-induced diabetes on body weight, serum glucose, and serum insulin levels in male and female rats

Animal status	N	Body wt (g)	N	Serum glucose* (mg/100 ml)	N	Serum insulin* (μUnits/ml)
Female OC†	32	265 ± 9‡	7	115.1 ± 5.2	19	28.3 ± 4.7
Female IC	27	247 ± 4	8	92.8 ± 6.6	23	23.3 ± 4.5
Female D	28	215 ± 5§	7	354.9 ± 93.1§	25	19.0 ± 3.1
Female C‖	8	251 ± 2	8	91.3 ± 9.7	7	17.3 ± 5.6
Female STZ	12	233 ± 5§	12	285.8 ± 38.7§	9	6.5 ± 1.3§
Male OC†	32	414 ± 16	7	102.6 ± 10.3	17	25.9 ± 3.7
Male IC	29	415 ± 7	8	119.8 ± 10.7	27	20.2 ± 2.1
Male D	28	352 ± 11§	4	375.8 ± 104.2§	19	19.1 ± 1.5
Male C‖	8	419 ± 7	8	118.9 ± 7.9	12	27.3 ± 3.2
Male STZ	12	377 ± 3§	12	403.8 ± 53.1§	16	14.1 ± 1.6§

* Animals were fasted 16 hr prior to being killed.
† OC = outside Wistar control; IC = inside nondiabetic control; and D = spontaneously diabetic.
‡ Data are means ± S.E.M.
§ Significantly different from appropriate control at $P < 0.05$.
‖ C = citrate-injected control; STZ = STZ, 60 mg/kg i.v.

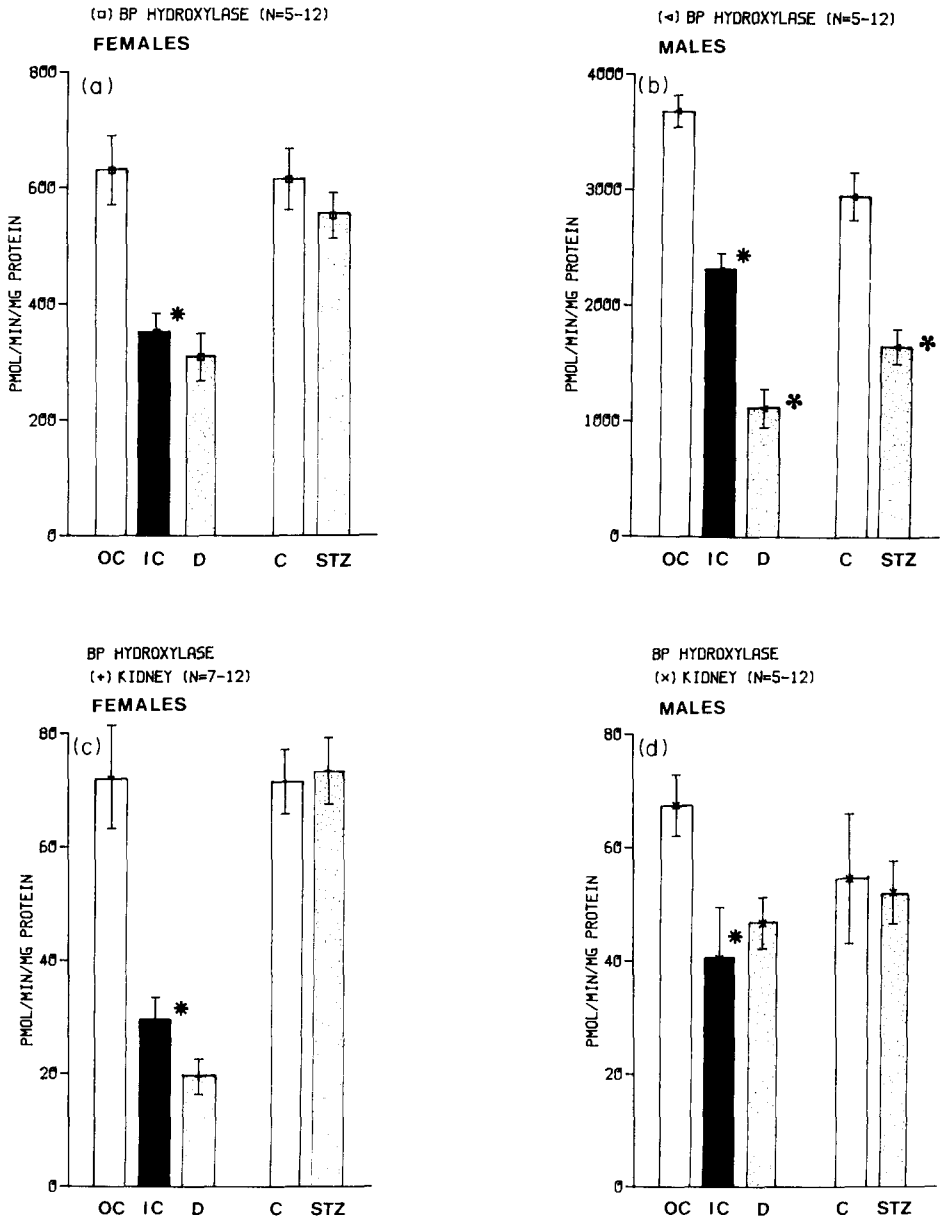

Fig. 1. Microsomal benzo[*a*]pyrene (BP) hydroxylase activity in control and diabetic rats. Panels: (a) female – liver; (b) male – liver; (c) female – kidney; and (d) male – kidney. Abbreviations: OC, outside Wistar control; IC, inside nondiabetic control; D, BB spontaneously diabetic; C, Wistar citrate control; and STZ streptozotocin-induced diabetic. Results are expressed as means ± S.E.M. Key: (*) significantly different from outside Wistar control at $P < 0.05$; and (*) significantly different from inside nondiabetic control at $P < 0.05$ or significantly different from citrate control at $P < 0.05$.

hydroxylase activity was reduced to approximately 50% of control in both STZ and genetically diabetic rats (Fig. 1b). The nondiabetic littermate BB controls (IC) of both sexes exhibited significantly lower hepatic BP hydroxylase activity than the normal Wistar control (OC) animals. To see if this effect was evident in extrahepatic tissue we measured BP hydroxylase activity in kidney microsomes from control and diabetic rats. In both males and females, we observed a similar difference between the nondiabetic BB littermates (IC) and the non-littermate Wistars (OC), although there was no further decrease in activity in the BB diabetics (Fig. 1c and 1d). Kidney BP hydroxylase in STZ diabetic female rats did not differ from control. This is consistent with previous observations in studies of extrahepatic tissues [17]. Similar results were obtained with STZ diabetic males.

We also examined the effects of diabetes on the sex-dependent metabolism of an endogenous substrate, testosterone. In this case, reduction of the 4-5 double bond occurs to an extent approximately

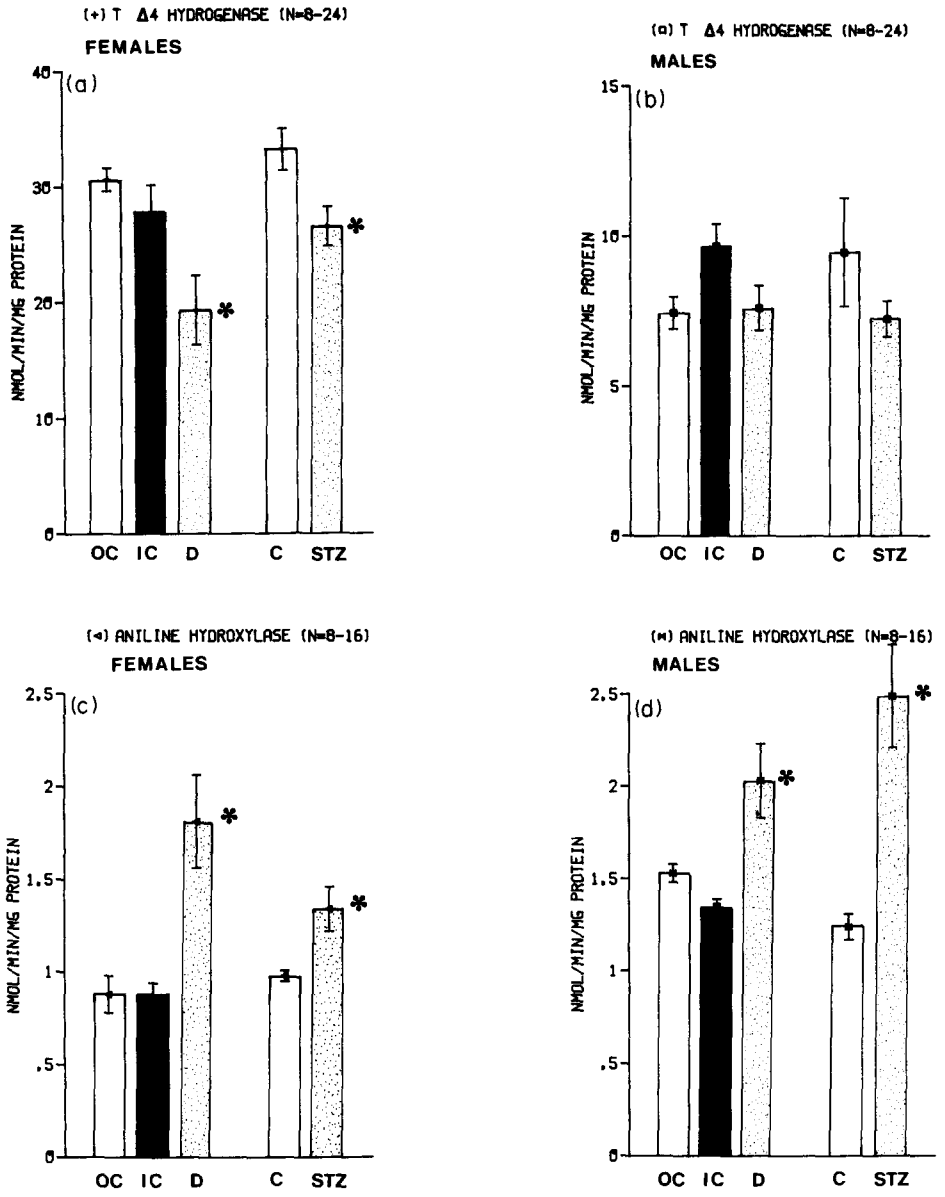

Fig. 2. Hepatic microsomal enzyme activities in control and diabetic rats. Panels: (a) testosterone (T) Δ^4 hydrogenase activity in females; (b) testosterone (T) Δ^4 hydrogenase activity in males; (c) aniline hydroxylase activity in females; and (d) aniline hydroxylase activity in males. Abbreviations: OC, outside Wistar control; IC, inside nondiabetic control; D, BB spontaneously diabetic; C, Wistar citrate control; and STZ, streptozotocin-induced diabetic. Results are expressed as means ± S.E.M. Key: (*) Significantly different from inside nondiabetic control at $P < 0.05$ or significantly different from citrate control at $P < 0.05$.

four times greater in the normal female than in the male (Fig. 2). In female rats, both spontaneous and STZ diabetes led to a significant decrease in testosterone Δ^4-hydrogenase activity (Fig. 2a), whereas in males (Fig. 2b) diabetes had no effect. In contrast to the pattern observed with BP hydroxylase, there was no difference between the two control groups (OC vs IC).

As has been reported previously [2, 3, 5, 7], we observed the sex-independent activity, aniline hydroxylase, to be increased significantly over control in chemically induced male and female diabetic rats (Fig. 2c and 2d). In addition, we found that the same effect was apparent in BB spontaneously diabetic rats. In this instance there was no difference between the non-littermate and littermate controls.

Effect of insulin replacement in BB diabetic rats. Various microsomal enzyme activities were measured in nondiabetic littermate controls (IC), BB diabetics (D) and insulin-treated BB diabetics

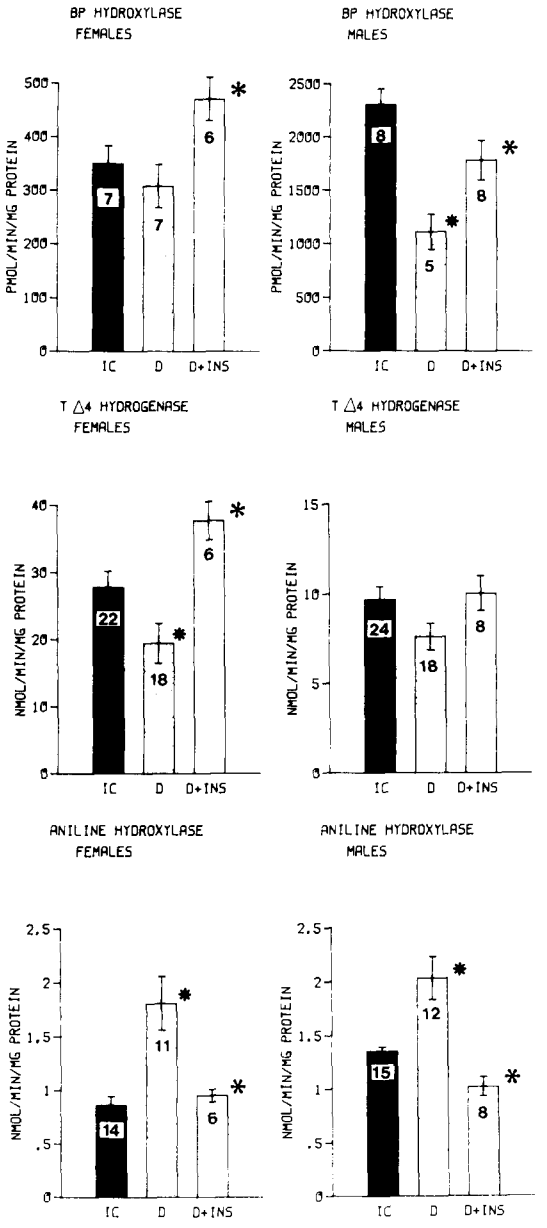

Fig. 3. Effect of insulin (INS) replacement on hepatic microsomal enzyme activities in BB spontaneously diabetic rats. Abbreviations: IC, inside nondiabetic control; D, BB spontaneously diabetic; and D + INS = BB diabetic maintained on daily insulin therapy. Key: (*) significantly different from inside nondiabetic control at $P < 0.05$; and (*) significantly different from untreated BB diabetic at $P < 0.05$.

(D + INS) (Fig. 3). Insulin maintenance reversed the effects of spontaneous diabetes on these activities. Hepatic BP hydroxylase activity in female insulin-treated BB diabetics was increased compared to that in untreated diabetics. These levels, however, are within the overall range observed for the nondiabetic controls in our laboratory.

Serum testosterone levels. BB diabetic (D) male rats were observed to have significantly reduced serum testosterone levels compared to nondiabetic littermate controls (IC) (Table 2). Furthermore, the nondiabetic littermates had lower testosterone levels than the nonlittermate controls (OC). The steroid levels in females were significantly lower than in the males but no difference was observed between controls and diabetics.

Table 2. Effect of spontaneous diabetes on serum testosterone levels in male and female rats

Animal status	N	Serum testosterone (ng/ml)
Female OC*	4	0.18 ± 0.04†
Female IC	4	0.12 ± 0.04
Female D	2	0.15 ± 0.02
Male OC	4	6.40 ± 1.25
Male IC	7	2.99 ± 0.42‡
Male D	4	1.16 ± 0.44§

* OC = outside Wistar control; IC = inside nondiabetic control; and D = BB spontaneously diabetic.
† Data are means ± S.E.M.
‡ Significantly different from OC at $P < 0.05$.
§ Significantly different from IC at $P < 0.05$.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The effect of diabetes on the appearance of microsomal proteins separated by electrophoresis is shown in Fig. 4. We did not observe major changes in the overall pattern of protein bands (28,000–130,000 molecular weight) in diabetic animals. However, some bands did change in relation to one another. For instance, the BB diabetic females in comparison to the nondiabetics showed an increase in the 52,000 molecular weight band without a decrease in the 46,000 and 61,000 molecular weight regions. On the other hand, in male BB diabetics, the prominent series of four peaks (46,000–61,000) evident in nondiabetic controls (IC) was altered. The 52,000 molecular weight band increased, the 56,000 decreased, and the 46,000 and 61,000 regions nearly disappeared. Insulin treatment reversed these changes in both male and female BB diabetics. Citrate-injected controls (C) and BB nondiabetic controls (IC) demonstrated some differences in the protein bands compared to one another. For example, in males, the 46,000 and 61,000 molecular weight regions were not prominent in citrate controls. Generally, within each strain of animal there were no major differences observed between male and female densitometric scans.

Histology. Light microscopy studies of pancreas and liver were performed. Minimal histological change was apparent in tissues of STZ or spontaneously diabetic animals. In the STZ diabetic rats there was a slight decrease in the number of pancreatic islets present. The spontaneously diabetic pancreas did appear to have an increased number of lymphocytes in the islets but this trend was not entirely consistent. The number of immunoreactive insulin-containing cells in the islets of the BB diabetics was reduced compared to the nondiabetic controls. Livers of the diabetic animals were essentially normal.

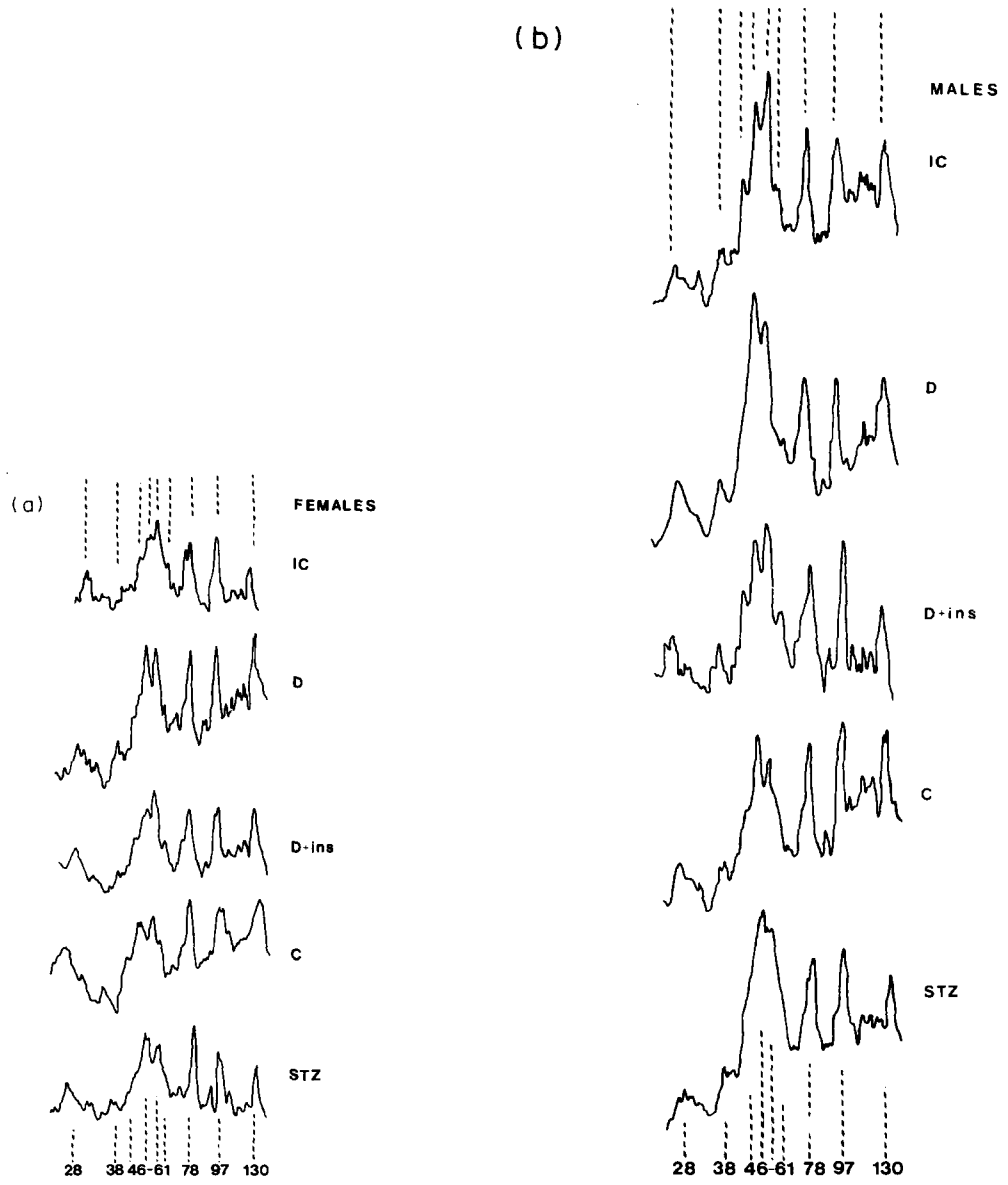

Fig. 4. Densitometric scans of Coomassie-blue stained microsomal proteins separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis from control and diabetic rats. Molecular weight ranges are as shown times 10^{-3}, with the range 46–61 designating 46,000, 52,000, 56,000 and 61,000 molecular weight regions. Panels: (a) female rats; and (b) male rats. Abbreviations: IC, inside nondiabetic control; D, BB spontaneously diabetic; D + INS, BB diabetic treated with insulin; C, Wistar citrate control; and STZ, streptozotocin-induced diabetic.

DISCUSSION

We have observed that hepatic BP hydroxylation, a sex-dependent enzyme activity, was reduced in BB spontaneously diabetic and STZ diabetic male rats. The mechanisms responsible for this alteration are unclear. Kato and Gillette have observed that alloxan diabetes in castrated male rats did not cause a reduction of sex-dependent metabolism although it did in methyltestosterone-treated castrates [2]. This is an indication that diabetes interferes with androgenic stimulation of certain drug-metabolizing activities.

It is possible that androgen sensitive mechanisms are impaired due to a lack of circulating testosterone. Tesone *et al.* [18] and Murray *et al.* [19] found that prostate and other sexual tissue weights were reduced in STZ diabetic male rats as a result of a 50% decrease in serum testosterone levels. Baxter's group has also demonstrated a decrease in serum testosterone levels in STZ male diabetic rats [20]. Our data indicate that BB spontaneously diabetic males have lower than control serum testosterone levels.

Baxter *et al.* [20] reported an increase in serum testosterone levels in female STZ diabetic rats com-

pared to control. This could be related to previous reports where sex-dependent metabolism has been observed to increase in STZ diabetic female rats [2, 3, 5, 6]. However, in our studies using either model of diabetes, female rats did not demonstrate an increase in hepatic BP hydroxylase activity compared to control, even when the sample size was increased to 26–32 or when we examined 10-day rather than 4-day diabetic animals. In addition, we did not observe any increase in serum testosterone levels in these females. At present, we are unable to account for this difference between our studies and those of others.

The other sex-dependent activity we measured was testosterone Δ^4-hydrogenase, an index of endogenous substrate metabolism. Generally, androgens exert opposite effects on steroid Δ^4-hydrogenases compared to mixed function oxidases [21]. In control rats, Δ^4-hydrogenase activity is approximately 4-fold greater in females than in males (Fig. 2). We observed a significant decrease in activity in female diabetics and no change in male diabetics. The trends were the same in STZ-induced and spontaneously diabetic animals. The data are not what would be predicted in relation to the lowered serum testosterone levels observed in diabetic male rats. In this situation one would expect an increase in hydrogenase levels in diabetic males in association with the decreased serum testosterone levels but this was not observed (Fig. 2b). These data suggest that there are more factors than the serum testosterone levels involved in the control of sex-dependent drug metabolism.

Although it is still speculative, there is indirect evidence to suggest that androgenic stimulation of drug metabolism may be mediated at least to some extent by hepatic steroid binding protein(s) [22–24]. Since STZ-induced diabetes has been associated with a decrease in androgen receptor content and binding capacity in rat prostate cytosol [18] a similar situation could exist in the liver. Notably, Roy and Leonard [25] observed that the androgen receptor-associated synthesis of hepatic α_{2u}-globulin was greatly reduced in diabetic male rats. In this regard, we are currently investigating the possibility that alterations in hepatic binding proteins could be related to the changes in enzyme activity associated with diabetes.

Our observations are consistent with previous reports which indicate that the sex-independent activity, aniline hydroxylase, is increased in both male and female diabetic rats [2, 3, 5, 7]. This trend was evident in both STZ and spontaneously diabetic rats. Since, in this case, diabetes exerted a common effect in males and females, it might be predicted that a relatively nonspecific mechanism is responsible for this alteration. For instance, there is evidence to suggest that ketonic compounds stimulate various microsomal enzyme activities, including aniline hydroxylase [26].

Other workers have found that total hepatic cytochrome P-450 is not decreased in diabetic rats [17, 27]. In the present study, we obtained densitometric scans of diabetic microsomal proteins which had been separated electrophoretically. These tracings suggested that the composition of proteins in the P-450 region (~50,000 molecular weight) was altered in STZ and BB diabetic rats compared to their corresponding controls. Insulin treatment reversed the observed alterations. In addition, Past and Cook [28] demonstrated quantitative differences between alloxan diabetic and control rats in similar electrophoretic studies. It is possible that relatively subtle changes in individual P-450 proteins could effect marked alterations in substrate specific microsomal metabolism. It may be that the regulatory processes involved in the control of hepatic metabolism are disrupted in STZ and BB diabetic animals, thereby altering specific cytochromes P-450 and producing differential changes in enzyme activities.

We observed that significant changes in various hepatic microsomal enzyme activities occurred in both STZ and spontaneously diabetic rats at a very early time point in the course of the disease (4 days). It is of interest that these alterations occurred even though animal weights, serum insulin levels, and pancreatic histology revealed relatively minor effects due to diabetes. Therefore, it would appear from these parameters that the spontaneously diabetic animals employed in our study tended toward the stable diabetic syndrome, as opposed to the unstable ketotic syndrome of diabetes [9].

Tannenbaum *et al.* [29] have monitored BB rats over the entire spectrum of the diabetic syndrome. The stable diabetics in their study demonstrated hypoinsulinemia 1–5 days post-detection. In contrast, we observed no difference between BB diabetics and littermate controls with regard to serum insulin levels (Table 1). This difference between our observations and theirs, however, is not unexpected in view of certain differences in experimental protocol. For instance, the BB diabetic animals we employed were those that had survived the stress of a long airflight. Such preselection of "healthier" animals in our case was evident in the observed body weight ranges. The diabetic males in our study were approximately 100 g heavier than the animals monitored by Tannenbaum *et al.* [29]. In addition, the diabetic animals which we received had been maintained on insulin prior to shipment and then received insulin for at least 1 more week in our laboratory. The diabetic rats in Tannenbaum's study never received insulin. It is apparent that there is a marked spectrum of severity of genetic diabetes, and it is important to monitor several parameters of the disease process when studying this model of diabetes.

In conclusion, we have studied BB spontaneously diabetic rats withdrawn from insulin therapy for 4 days, and compared these to 4-day STZ-induced diabetic rats. Significant differential effects on hepatic microsomal metabolism were evident in both males and females at this early time period. These changes could be reversed by insulin treatment. Preliminary evidence, including altered serum testosterone levels and changes in relative disposition of microsomal proteins, is consistent with the suggestion that diabetes affects the hormonal control of sex-dependent microsomal enzyme activity. We are currently studying the mechanism(s) by which this could occur.

Acknowledgements—We are grateful to the Animal Resources Division, Health Protection Branch, Health and

Welfare Department, Canada, for supplying the BB Wistar rats used in these studies. We acknowledge the contribution of Dr. Alison Buchan in performing the immunocytochemical studies on the BB pancreas sections (publication in preparation).

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