Primary Hypoandrogenism in Experimental Diabetes in the Long-Evans Rat

JUDY E. ANDERSON, DON JONES, S. BRIAN PENNER, AND JAMES A. THLIVERIS

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SUMMARY

The hormonal milieu of the testis was examined in streptozocin-induced diabetic (STZ-D) adult male Wistar and Long-Evans rats. Serum testosterone, creatinine, and urea nitrogen (BUN) levels and blood glucose concentrations were determined in diabetic and control Wistar rats (experiment 1). These parameters plus luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were studied in experiments 2 and 3 with Long-Evans rats in untreated diabetic, control, insulin-treated diabetic, nondiabetic STZ-injected, and semistarved groups. Wistar diabetic rats had significantly decreased serum testosterone and increased blood glucose, BUN, and serum creatinine compared with controls. Several findings in Long-Evans rats suggested the existence of a primary Leydig cell defect in steroidogenesis during untreated diabetes that was completely or partially compensated for by increased pituitary gonadotropin secretion. Serum LH and FSH levels increased in Long-Evans diabetic rats. Serum testosterone was significantly reduced only in experiment 2. These hormonal alterations from control levels were not seen in insulin-treated diabetic animals. Semistarved animals, weight matched to the diabetic group in experiment 2, had significantly decreased serum testosterone and increased FSH levels. In addition, Long-Evans diabetic rat BUN and serum creatinine levels increased much less or were unchanged from control values compared with the increase noted in diabetic Wistar rats. In light of the hypogonadism that complicates clinical uremia, these findings suggest the more apt use of the Long-Evans strain rather than the Wistar strain in the study of STZ-D hypogonadal function. Indices to evaluate fertility (sperm count and motility) in diabetic rats in experiment 3 showed no change from those determined in control animals. The same diabetic animals, however, had a complete lack of reproductive success after pairing with cycling control females. These latter findings suggested that, in addition to a primary Leydig cell defect and resulting lowered testosterone, other factors may have contributed to the reproductive dysfunction of Long-Evans male rats with STZ-D. *Diabetes* 36:1104–10, 1987

From the Department of Anatomy (J.E.A.), University of British Columbia, and Departments of Medicine (D.J., S.B.P) and Anatomy (J.A.T.), University of Manitoba, Canada.

Address correspondence and reprint requests to Dr. Judy E. Anderson, Department of Anatomy, Faculty of Medicine, University of British Columbia, 2177 Wesbrook Mall, Vancouver, British Columbia, V6T 1W5 Canada.

Received for publication 13 October 1986 and accepted in revised form 27 March 1987.

Investigation of the pathology underlying decreased testosterone production in experimental diabetes has focused on both endocrine and neurologic parameters. Studies of testicular histopathology (1–4), steroidogenesis (5–8), Leydig cell ultrastructure (9,10), pituitary and hypothalamic structure and hormonal content (11,12), and serum hormonal milieu (13) have attempted to explain reduced fertility in rats (14) and humans (2,15,16). Suggestions of hypogonadism originating at the Leydig cell (8), pituitary (13), and/or hypothalamus (10,11) in different strains and by varying streptozocin (STZ)-induced disease severity and duration have all been reported.

Concurrently, peripheral autonomic neuropathy, noted in clinical studies of diabetic men (17–21), has also been examined in elegant studies on BB Wistar diabetic rats (22,23). Axonopathy (22) and abnormal neuromuscular terminals (23) with reduced conduction velocities have been implicated as significant contributions to the etiology of clinical impotence in diabetic men. Whereas rat penile reflexes have not been found to be a reliable index of fertility (24), presentations of reduced fertility and decreased potency in diabetic men have been reported to participate in reproductive dysfunction (2,25).

This investigation was undertaken to clarify whether a primary Leydig cell defect in Long-Evans STZ-induced diabetic (STZ-D) rats and the resulting endocrine modifications could completely account for the observed hypogonadism noted in an earlier report (8). Parameters determining indices of

fertility and those modulating the endocrine milieu were concurrently examined to distinguish between endocrine and nonendocrine origins for diabetic reproductive dysfunction. Uremia, seen in chronic renal failure with or without clinical diabetes (26–30) and in experimental models of uremia (31,32), perturbs the hypothalamic-pituitary-testicular axis. Therefore, serum chemistry was also closely examined to document the influence of STZ-D on renal function.

MATERIALS AND METHODS

Experiment 1. In a preliminary experiment, seven adult Wistar rats (Charles River, Wilmington, MA) weighing 250–300 g were anesthetized under ether and injected intravenously via the tail vein with 65 mg/kg STZ (Sigma, St. Louis, MO) in cold citrate buffer (0.1 M, pH 4.5). Within 3 days, all rats became hyperglycemic (blood glucose >22.2 mM) and glycosuric compared with six weight-matched control Wistar animals injected with a citrate buffer vehicle. Animals were housed with food and water ad libitum in controlled temperature (22C°) and light (on at 0600 h, off at 1800 h) conditions. Blood glucose levels of all animals were monitored biweekly for 3 mo. Four of the diabetic animals died during the experimental period. During the final week, animals were placed in metabolic cages for collection of 24-h urine output and urine chemistry. At death (under ether anesthesia), blood was drawn by cardiac puncture for determination of glucose, blood urea nitrogen (BUN), serum creatinine, and serum testosterone levels.

Experiment 2. Seventeen adult male Long-Evans rats (250–300 g) received a tail vein injection of 40 mg/kg STZ, as reported previously (1). Eleven of these animals became hyperglycemic (blood glucose >22.2 mM) and glycosuric within 3 days, whereas six animals remained normoglycemic (blood glucose not significantly different from control level of 7.49 ± 0.8 mM) and aglycosuric. The diabetic group was randomly subdivided into untreated (n = 5) and insulin-treated (n = 6) groups. In the latter group, normoglycemia was achieved by daily (0800 h) subcutaneous injection of 3 U ultralente insulin (Connaught, Toronto). A fourth group of animals (n = 6) was injected with the citrate buffer vehicle to serve as controls. These four groups of rats were maintained in single cages for 3 mo with food and water ad libitum except for the insulin-treated group, whose food was restricted overnight to aid control of glycemia. A fifth group of food-restricted animals (n = 6) was established; their weight loss approximated that seen in the untreated diabetic group by titrating their food allowance. There was one death in the insulin-treated group, and one rat in the STZ-injected nondiabetic group developed a renal tumor.

Urine output was determined as in experiment 1. At death under ether anesthesia, blood was drawn by cardiac puncture; the testes were perfusion fixed, as reported previously (1). Blood samples were analyzed for glucose, BUN, serum creatinine, serum testosterone, and serum follicle-stimulating hormone (FSH) levels. Due to the inclusion of the perfusion-fixation protocol (to permit histological and ultrastructural study) and comprehensive serum chemistry (not reported) in this experiment, serum amounts were insufficient for assay of both gonadotropins.

Experiment 3. To assess fertility and potency of diabetic animals and document serum testosterone in the context of both luteinizing hormone (LH) and FSH levels, experiment 2 was repeated with omission of the perfusion-fixation procedure. With the same protocol as experiment 2, five groups were established: citrate control (n = 10), diabetic (n = 12), STZ-injected nondiabetic (n = 10), insulin-treated diabetic (n = 12), and semistarved (n = 10). During the experimental period, there were three deaths in the insulin-treated group. At the end of 3 mo, animals were weighed, anesthetized under ether, and blood was drawn by cardiac puncture before death.

Fertility assessment. Overall fertility was quantified by housing each of 10 control and 4 untreated diabetic males, randomly selected from the animals in experiment 3, with adult cycling virgin female Long-Evans rats from the same animal colony. Pairs were housed together for the last 2 wk of the experimental period, and their behavior was observed for 0.5 h at least twice each day. Females housed with control males were permitted to deliver their litters. Females housed with diabetic males were anesthetized under ether and killed for subsequent examination of their uterine horns and ovaries. The presence of implantation and resorption sites and the presence or absence or corpora lutea were noted.

Sperm counts and motility assessments were made with standard clinical measurement techniques after the animals were killed (33,34). Nine citrate control and 11 untreated diabetic animals were laparotomized by midline incision, and the testes were drawn by gentle traction into the abdomen. Urine was withdrawn as completely as possible from the bladder through a 21-gauge needle attached to a syringe. Each vas deferens was milked from the testis toward the seminal vesicle and then clamped close to the bladder. Gentle pressure on the seminal vesicles expressed fluid toward the bladder with minimal manipulation of the coagulating glands. The bladder was then incised along its anterior midline, and mixed sperm product and seminal fluid were drawn into a 100-μl mechanical pipette from the region between the ejaculatory papillae (35). A single undiluted drop of this fluid was placed in the center of a Makler counting chamber (Sefi-Medical, Haifa, Israel). Counts of sperm heads were made in duplicate from different diagonal strips of 10-grid squares, with respect to the forbidden line (36), and averaged. Counts were multiplied by 10, such that units were expressed as sperm concentration in millions per milliliter of seminal fluid. Sperm motility was graded + to ++++ from 9 squares of the grid.

Serum and urine chemistry. Routine chemistry on blood and urine samples was used to determine BUN (37) and serum and urine creatinine (38) to monitor animal status. Blood glucose was determined with Dextrostix and a Glucometer (Ames, Elkhart IN).

Radioimmunoassay. Blood samples were allowed to clot and were centrifuged for serum collection. Serum was divided into three allotments and kept frozen at −80C° until assay. Testosterone assays were performed in duplicate in the laboratory of the Endocrinology Section, Dept. of Medicine, Health Sciences Centre (Winnipeg, Canada), with a Radioassay Systems Laboratories (Carson, CA) kit modified to include an extraction procedure (39) and stripped serum standards (40). Interassay variation was 9.8%, although samples from each experiment were assayed in single runs.

Radioimmunoassays for FSH (41) (experiment 2) and FSH

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TABLE 1
Physiological parameters for control and diabetic male Wistar rats in experiment 1

	Control (n = 6)	Diabetic (n = 3)	P*
Blood glucose (mM)	7.5 ± 1.1	>22.2	<.001
Percent weight change	71 ± 6.8	−6.7 ± 12	<.001
Urine volume (ml/h)	0.34 ± 0.12	1.18 ± 0.21	<.02
Blood urea nitrogen (mg/dl)	22.5 ± 2.3	33.2 ± 6.9	.008
Serum creatinine (mg/dl)	0.32 ± 0.016	0.53 ± 0.03	<.001
Creatinine clearance (μl/min)	1993 ± 538	1325 ± 275	
Serum testosterone (ng/ml)	6.1 ± 3.4	1.1 ± 0.75	.04

Values are means ± SE.
*Mann-Whitney U test.

and LH (42) (experiment 3) were carried out in duplicate in single runs for each experiment by Hazelton Laboratories (Vienna, VA). FSH and LH assays had a minimum sensitivity of 114 and 15 ng/ml, respectively. Intra- and interassay coefficients of variation were 9.4 and 10.2% for FSH and 4.9 and 5.7% for LH.

Statistical analysis. Comparisons between group means were made from one-way analyses of variance (ANOVAs) and Tukey's test (43). For LH data, the nonparametric Kruskal-Wallis ANOVA by ranks was applied, due to the appearance of values less than the level of sensitivity. Blood glucose values were also compared by the Kruskal-Wallis test, due to values above the upper limit of the Glucometer. Post hoc analysis of those data was made by Ryan's procedure (44). Frequency data on sperm counts and motility assessments were analyzed with χ^2-contingency tables.

Nonparametric Mann-Whitney U tests were used to determine differences between Wistar control and diabetic group means, due to small group sizes (experiment 1). Two-way ANOVAs were used to determine strain (Wistar vs. Long-Evans), treatment (control vs. diabetic), and interaction effects. In all cases, $P < .05$ was used for rejection of the null hypothesis.

RESULTS

Experiment 1. Blood glucose, body weight change, urine output, BUN, creatinine clearance, and serum testosterone data are given in Table 1. Diabetic Wistar rats had significant increases in blood glucose ($P < .001$) and urine volumes ($P < .02$) and significantly reduced body weight gains ($P < .001$). Diabetic rats also had increased BUN ($P < .008$) and serum creatinine levels ($P < .001$) compared with controls. All six diabetic rats (including 3 that survived only 2 mo) also had significantly increased BUN and serum creatinine levels after 1 and 2 mo (data not shown). Serum testosterone levels were significantly reduced ($P = .04$) in diabetic rats.

Experiment 2. Blood glucose, body weight change, urine output, BUN, serum creatinine, creatinine clearance data, serum testosterone, and serum FSH levels are given in Table 2. Diabetic rats had significantly reduced body weight gain ($P < .01$) and increased urine output ($P < .01$) compared with the control group. Blood glucose was also increased over control levels ($P < .01$). Insulin treatment prevented these changes.

BUN values in the untreated diabetic group were not significantly altered from control levels. Interestingly, insulin-treated diabetic rats had statistically lower BUN ($P < .01$) compared with the control group. Plasma creatinine and creatinine clearance in diabetic rats were unchanged from control levels. Serum testosterone was significantly decreased from control levels in both diabetic ($P < .05$) and semistarved ($P < .05$) groups. Serum FSH increased in both diabetic ($P < .01$) and semistarved ($P < .01$) groups.

The two-way ANOVAs of Wistar and Long-Evans control and diabetic groups (Table 3) showed that animals of the Long-Evans strain (both control and diabetic) had significantly greater urine volumes than their respective Wistar control and diabetic rats (strain effect, $P = .03$). Also, the diabetic condition was shown to produce a greater increase in urine volume in the Long-Evans than in the Wistar strain (synergistic interaction, $P = .01$). These results were found

TABLE 2
Physiological parameters for 5 groups of male Long-Evans rats in experiment 2

	Control (n = 6)	Diabetic (n = 5)	Nondiabetic STZ injected (n = 6)	Insulin-treated diabetic (n = 5)	Semistarved (n = 6)
Blood glucose (mM)	7.49 ± 0.8	>22.2*†	9.2 ± 0.8	8.97 ± 0.5	6.57 ± 0.3†
Percent weight change	74 ± 5.3	0 ± 3.1*†	40 ± 8.5*	46.6 ± 2.2*	−6.0 ± 2.0*†
Urine volume (ml/h)	0.26 ± 0.02	1.96 ± 0.27*†	0.41 ± 0.11	0.27 ± 0.02	0.38 ± 0.02
Plasma creatinine (mg/dl)	0.47 ± 0.02	0.58 ± 0.12	0.52 ± 0.05	0.43 ± 0.03	0.38 ± 0.02
Creatinine clearance (μl/min)	1611 ± 83	1241 ± 105†	1731 ± 145	2064 ± 217	1710 ± 45
Blood urea nitrogen (mg/dl)	27.6 ± 0.88†	28.3 ± 2.3†	24.8 ± 3.5	17.2 ± 1.6	22.9 ± 1.2
Serum testosterone (ng/ml)	3.81 ± 0.61	1.02 ± 0.20*†	1.78 ± 0.51	4.38 ± 1.23	1.17 ± 0.49*†
Serum FSH (ng/ml)	147.2 ± 14.7	280 ± 19.1*†	137.7 ± 6.6	148.2 ± 14.8	285.7 ± 76.6*†

STZ, streptozocin. Values are means ± SE.
*Significantly different from control group ($P < .05$).
†Significantly different from insulin-treated diabetic group ($P < .05$).

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TABLE 3
Two-way analyses of variance for strain (Long-Evans vs. Wistar), treatment (control vs. diabetic), and interaction effects

	Strain	Treatment	Interaction
Blood glucose		<.001	
Weight change		<.001	
Urine volume	.03	<.001	.01
Blood urea nitrogen		.01	.01
Serum testosterone		.002	

in addition to the expected effect of diabetes to increase urine volume (treatment effect, $P < .001$). BUN values in diabetic rats increased over controls significantly more in Wistar than in Long-Evans rats (interaction, $P < .01$). This occurred in addition to a significant treatment effect of diabetes to increase BUN levels ($P < .01$).

Experiment 3. Blood glucose, body weight change, plasma creatinine, BUN, serum testosterone, FSH, and LH data are presented in Table 4. Blood glucose in the diabetic group increased significantly over control levels ($P < .001$). Diabetic animals had significantly lower percent body weight gain ($P < .01$) compared with control rats, as did semistarved rats ($P < .01$). All group weight gains were significantly greater ($P < .01$) than those for the semistarved group. Plasma creatinine and BUN levels in untreated diabetic rats were unchanged from those in the control group, as in experiment 2.

Testosterone levels were reduced in the semistarved group ($P < .01$) compared with all other groups. In addition, serum testosterone in the insulin-treated diabetic group was significantly greater than in the diabetic ($P < .05$) or nondiabetic STZ-injected group ($P < .05$). In the diabetic group, however, serum testosterone was reduced from control levels but was not significantly lower.

Serum FSH values showed significant alteration by treatment ($P < .001$) in a one-way ANOVA. Serum FSH increased over control levels in the diabetic ($P < .05$) and the semistarved group ($P < .01$). Serum LH increased significantly in the diabetic group compared with controls ($P < .01$). The semistarved group had significantly lower LH values compared with those of control ($P < .05$) and diabetic ($P < .01$) groups. Serum LH values are given as means ± SE where possible or accompanied by a < sign where values were recorded below the level of assay sensitivity within a group.

Sperm count and motility data are presented in Table 5. The χ^2-tests for significant dependence on treatment applied to sperm motility ($\chi^2 = 0.56$, df = 3, $P = .51$) and sperm count ($\chi^2 = 1.67$, df = 4, $P = .68$) data indicated the lack of a change produced by diabetes.

During behavioral observation of the four breeding pairs, which included diabetic males, mating behavior or mounting was never encountered. Control breeding pairs, in contrast, exhibited all phases of active mating behavior. There were no implantation or resorption sites in the uterine horns of females housed with four diabetic males, despite observation of many corpora lutea in their ovaries. In comparison, the litter size produced in 10 cycling females housed with 10 control males (12.0 ± 0.7) indicated that a significant reduction in successful matings by diabetic animals had occurred, viewed in the context of their normal sperm motility and count data.

DISCUSSION

The results of the experiments with Long-Evans rats show that diabetes induces a primary Leydig cell defect, with reduced testosterone levels and increased pituitary secretion of both LH and FSH. In experiment 2, diabetic animals had reduced weight gain and increased blood glucose and urine output, similar to the Wistar diabetic rats, and significantly reduced serum testosterone. The reduction of testosterone in diabetic rats was statistically separate from that due to semistarvation, as determined by the orthogonal-contrast method (8). Insulin treatment of diabetes prevented testosterone decrease, suggesting the contribution of a primary hypoandrogenism originating at the Leydig cell. In as much as increased gonadotropins may compensate for Leydig cell hypofunction, increased FSH levels in diabetes (and semistarvation) suggested two possibilities: either FSH has a direct influence on Leydig cell steroidogenesis, or more likely, there is an indirect effect of FSH on steroidogenesis through Sertoli cell–Leydig cell paracrine functions (45,46). Such paracrine regulatory mechanisms have recently been reported in two quantitative studies after FSH treatment of immature hypophysectomized rats (47,48) and in ethane-1,2-methanesulfonate-perturbed Leydig cell populations (49).

Analysis of data from treatment groups in experiment 3 showed significant reduction of serum testosterone in semistarvation, although not in diabetes. This was observed in

TABLE 4
Physiological parameters for 5 groups of male Long-Evans rats in experiment 3

	Control (n = 9)	Diabetic (n = 11)	Nondiabetic STZ injected (n = 9)	Insulin-treated diabetic (n = 7)	Semistarved (n = 9)
Blood glucose (mM)	7.53 ± 0.35	>22.2*	8.90 ± 0.50	14.8 ± 1.8	7.7 ± 4.8
Percent weight change	74 ± 5.7	29 ± 7.0*†	62 ± 2.3	54 ± 4.9	−3.0 ± 1.7*†
Plasma creatinine (mg/dl)	0.56 ± 0.04	0.71 ± 0.16†	0.44 ± 0.03	0.43 ± 0.02	0.55 ± 0.08
Blood urea nitrogen (mg/dl)	25.3 ± 0.80	29.2 ± 4.0†	22.5 ± 0.93	17.9 ± 0.77	28.1 ± 2.4†
Serum testosterone (ng/ml)	4.08 ± 0.72	3.57 ± 0.40†	3.61 ± 0.50†	10.3 ± 2.5*	1.04 ± 0.34*†
Serum FSH (ng/ml)	248.7 ± 16.0	323.5 ± 22.5*	236.2 ± 8.2	264.4 ± 17.1	329.1 ± 14.0*
Serum LH (ng/ml)	<27.1	40.1 ± 5.7*	29.3 ± 2.8	29.1 ± 3.4	<16.4*

STZ, streptozocin. Values are means ± SE.
*Significantly different from control group ($P < .05$).
†Significantly different from insulin-treated diabetic group ($P < .05$).

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TABLE 5
Sperm count and motility data (experiment 3)

		Sperm concentration (10^6/ml)					Sperm motility			
	n	<20	21–60	61–100	101–140	>140	+	++	+++	++++
Control	9	1	1	3	0	4	2	3	3	1
Diabetic	11	4	1	1	2	3	2	4	4	1

Values are numbers of animals.

the hormonal milieu of increased FSH levels in both groups but with increased LH in diabetic animals and reduced LH in semistarved rats. A Leydig cell defect had been produced in untreated diabetes and, in this experiment, was probably compensated for by increased release of both LH and FSH in a pituitary response to that defect. This increase in gonadotropins is in sharp contrast to previous reports of decreased gonadotropins in STZ-D in Wistar (9–11,50) and Sprague-Dawley (12,13) rats. The disparity between primary (reported herein) and secondary-tertiary (9–13,50) gonadal failure may be due to strain differences in response to the diabetic condition or in the timing of that response. In the latter case, testing of Long-Evans rats with long-term diabetes may reveal similarly decreased gonadotropin levels subsequent to pituitary failure after an interval of compensatory stimulation. Such surprisingly large differences in endocrine regulation have been reported previously in strain-response studies of Leydig cell LH-receptor sensitivity, maximal testosterone production (51), and susceptibility to the induction of diabetes by STZ in mice (52,53).

The lack of implantation sites in ovulating females housed with diabetic males having normal serum testosterone levels occurred despite unaltered sperm motility or number in the diabetic group. The effect of diabetes may therefore extend beyond a primary Leydig cell defect in testosterone production (compensated for by increased LH and FSH in experiment 3). Although there are many etiologies for reduced potency, neuropathy causing sexual dysfunction would seem a distinct possibility, especially in view of recent reports of autonomic neuropathy in BB Wistar diabetic rats (22,23). It would have been useful in this regard to determine the sperm content of urine (if any) from male diabetic rats during the breeding study to test for retrograde ejaculation.

A primary Leydig cell origin of hypoandrogenism in diabetes was suggested by previous examination of ultrastructural changes. Increased smooth endoplasmic reticulum, lipid droplets, and mitochondria were reported in the Leydig cells of diabetic rats (8). Two situations may be postulated. First, successful pituitary compensation for partial Leydig cell failure resulted in statistically normal testosterone levels in experiment 3. Second, ineffective compensation for a Leydig cell defect produced significantly reduced testosterone in experiment 2. In either case, the marked reduction in mating behavior and pregnancy rate despite normal sperm count and motility may be best explained by some sexual dysfunction affecting potency or mating behavior. This is in contrast to a previous report of normal libido and penile reflexes in Sprague-Dawley rats with long-term diabetes (9 mo) (24). Although seminal vesicle, penile, and body weights decreased in that study, total serum androgen levels were normal. However, fertility was not assessed by sperm count, motility, or pregnancy rates.

The observation of normal or above-normal testosterone levels in insulin-treated diabetic rats (from experiments 2 and 3, respectively) reports successful prevention of lowered testosterone by insulin treatment alone in experimental diabetes in the rat, as found previously after 4 wk of diabetes (54,55). This may be due to adequate control of blood glucose by the combination of overnight food restriction, followed by insulin injection before eating, and the use of the Long-Evans strain. The necessary inclusion of human chorionic gonadotropin to stimulate pituitary LH, and thereby stimulate testicular function, in a study by Paz et al. (56) was probably not required in our investigation, because the pituitary in Long-Evans diabetic rats was able to effectively compensate for low testosterone by increased gonadotropin secretion. This further suggests the primary nature of the Leydig cell defect in Long-Evans rats.

Viewed as a whole, the results of the three experiments showed Long-Evans rats to be as subject to the diabetogenic effects of STZ as the more commonly studied Wistar rats. However, the onset of diabetes occurred with different susceptibilities to STZ β-cell cytotoxicity. A dose of 40 mg/kg in Long-Evans rats resulted in ~60% incidence of diabetes, with no deaths in the untreated group. The same dose was ineffective in Wistar rats, which needed 65 mg/kg for the induction of diabetes, producing 100% affected animals and 50% subsequent deaths. The latter dose of STZ given to Long-Evans rats in a preliminary study produced 100% diabetic rats and 90% mortality. In addition, diabetic Long-Evans rats were not as uremic as diabetic Wistar rats. Comparative results between strains indicated greater susceptibility of the diabetic Wistar rats to compromised renal function, studied herein by BUN and creatinine clearance, than the Long-Evans rats with STZ-D.

The importance of these results is twofold. First, inferences from one strain to another within a species must be made with great caution. This is applicable to the appearance of secondary effects (e.g., uremia) and to pituitary responsiveness. The increased LH and FSH levels in the serum of Long-Evans diabetic rats, regardless of whether serum testosterone was significantly reduced in a particular experiment, implies primary Leydig cell failure, as do the prevention of these changes with insulin treatment. These results are further supported by our previous report of normal seminiferous tubule diameter in insulin-treated diabetic rats and its reduction in untreated diabetic animals (1). In a morphometric study (8), smooth endoplasmic reticulum, lipid, and mitochondrial fractions of Leydig cell cytoplasm increased in diabetic animals and were reduced to control (or less) pro-

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portions in insulin-treated diabetic rats. The increased gonadotropin stimulation observed in these diabetic rats appears to confirm the enlargement of the steroidogenic organelle fractions in their Leydig cells.

Second, the appearance of significant uremia (high BUN and serum creatinine) in diabetic Wistar rats, although in a small group ($n = 3$), and its absence in diabetic Long-Evans rats is not a trivial difference. Although renal function has not been previously investigated in the STZ model of diabetes, uremia has been well documented in clinical studies to be accompanied by decreased testosterone, altered gonadotropin levels, decreased seminiferous tubule diameter, maturation arrest, and reduced sperm count and potency (26–30). In one study, parameters of fertility were corrected toward normal values after renal transplantation (29). Experimental uremia has also been recently reported to be accompanied by decreased testosterone, LH, and FSH and infertility after subtotal nephrectomy (31,32). The similarity between the above portrait of the uremic influence on testicular pathology and function and those noted in the literature on experimental diabetes would strongly suggest that renal function and BUN levels be monitored in future investigations and that Long-Evans rats may be more applicable to those studies than Wistar animals.

The apparent dissociation of direction in LH and FSH alterations in semistarvation may indicate the operation of a temporal feature in the regulation of pituitary responsiveness or a third strain difference. FSH levels may eventually have decreased with more severe food restriction, as in previous reports of semistarved Wistar rats (50), or with restriction over a longer term. Alternatively, distinct LH and FSH pools of secretory granules within certain gonadotropes (57) and different age-related patterns of synthesis and storage between LH and FSH pituitary content in response to castration have been reported (58,59). Such a difference in specific gonadotropin responsiveness to semistarvation may be present.

Interpretation of the differences in serum testosterone levels between experiments 2 and 3 may be due to differences in severity of diabetes, marked by reduced body weight gain. In experiment 2, the semistarved and diabetic groups were well matched in weight. In experiment 3, body weight gain among diabetics was significantly greater than in semistarved animals. In both experiments, however, serum testosterone and body weight change were significantly correlated.

Although neuropathic influence on overall fertility in diabetes has been discussed in clinical literature and related to the appearance of cardiovascular (17) and gastrointestinal (18) signs of autonomic neuropathy, its overt involvement in this investigation was suggested only by the lack of matings by the diabetic animals. The histopathology of seminiferous tubules in animals in experiment 2 (1) indicated an alteration in structure and a change in the frequency distribution of the stages of spermatogenesis. The ultrastructural changes and reduced hydroxysteroid dehydrogenase activity in Leydig cells of diabetic animals (8) suggested a primary Leydig cell defect in testosterone production. This may be sufficient to contribute to a decrease in fertility, but the lack of a significant decrease in serum testosterone, sperm count, or motility while the pregnancy rate was reduced suggests that other aspects of sexual dysfunction, possibly reduced potency, contributed to decreased fertility. Thus, anatomical and functional parameters of autonomic neuropathy should be investigated in diabetic Long-Evans rats with decreased fertility and a primary Leydig cell defect but not uremia.

ACKNOWLEDGMENTS

We express our gratitude for the excellent technical assistance of Donna Love of the Department of Anatomy, University of Manitoba.

J.E.A. was supported during the term of this project by a Manitoba Health Research Council Studentship.

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