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Testosterone Replacement Fails to Reverse the Adverse Effects of Streptozotocin-Induced Diabetes on Sexual Behavior in the Male Rat[1]

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STEGER, R. W. *Testosterone replacement fails to reverse the adverse effects of streptozotocin-induced diabetes on sexual behavior in the male rat.* PHARMACOL BIOCHEM BEHAV **35**(3) 577–582, 1990.—The ability of testosterone to reverse the adverse effects of streptozotocin-induced (STZ) diabetes on male sexual function was tested in adult male rats. Treatment with STZ (50 mg/kg) led to a significant reduction of plasma testosterone (T) levels and in the number of rats exhibiting ejaculatory behavior in a 30-minute test period. A similar reduction in T levels and ejaculatory behavior was seen in rats subjected to caloric restriction to mimic the weight loss seen in the STZ-treated rats. T-replacement (200 μg/day) restored T levels to those seen in control animals, but did not reverse the adverse effects of STZ on copulatory behavior. STZ-induced changes in copulatory behavior were associated with changes in hypothalamic LHRH levels and catecholamine turnover. Caloric restriction also caused endocrine and neuroendocrine changes, but they were not similar to those seen in STZ rats suggesting that these two treatments affect copulatory behavior by different mechanisms.

Diabetes Sex behavior Testosterone Norepinephrine Dopamine LH releasing hormone

CHANGES in sexual and reproductive function are frequently associated with diabetes in men and experimental animals (4, 10, 11, 14, 17, 19, 30, 32). Although erectile impotence due to vascular changes and peripheral neuropathies is the most common form of sexual dysfunction in diabetic men, it is becoming apparent that changes in endocrine function and central nervous system control of sexual arousal may also play an important role (10, 11, 14, 19, 32).

There have been few reports on the effects of diabetes on sexual behavior in the rat. Subtotal pancreatectomy was shown to increase blood glucose levels and significantly reduce sexual behavior (13,15). Sachs and colleagues (26) induced diabetes in 5-week-old rats with streptozotocin, but could show no deficits in copulatory behavior over the next 8 to 9 months. More recently, we have demonstrated that male rats made diabetic with streptozotocin (STZ) when adults, exhibit severe deficits in sexual function (30). These changes in sexual response were accompanied by significant reductions in plasma levels of LH, Prl and testosterone levels. Significant reductions in norepinephrine and variable changes in dopamine metabolism were seen in several brain regions known to be involved with the control of copulatory behavior and gonadotropin secretion.

Since testosterone is required for male sexual behavior (7) and since diabetic rats have low serum testosterone levels (4, 17, 30), the present experiment was designed to determine if testosterone replacement could reverse the adverse effects of diabetes on copulatory behavior in the male rat. Furthermore, since diabetes induces severe weight loss, an additional group of rats was subjected to caloric restriction to mimic weight changes seen in diabetic animals to determine if weight loss caused the same behavioral and neuroendocrine effects as STZ treatment.

METHOD

Adult male Sprague-Dawley rats were purchased from Harlan Industries (Madison, WI). The rats were housed in a temperature-controlled (22°C) room on a 12:12 light:dark cycle (lights on at 01:00 hr). Rats were housed 4 per cage except for the food-restricted group and half of the controls who were individually housed. Food (TekLab Rat Diet; Madison, WI) and tap water were provided ad lib except for the food-restricted group whose food intake was restricted to mimic the weight loss seen in the diabetic rats. Food intake of the restricted rats was approximately 50% of that for the ad lib-fed rats. To provide sexual experience, each

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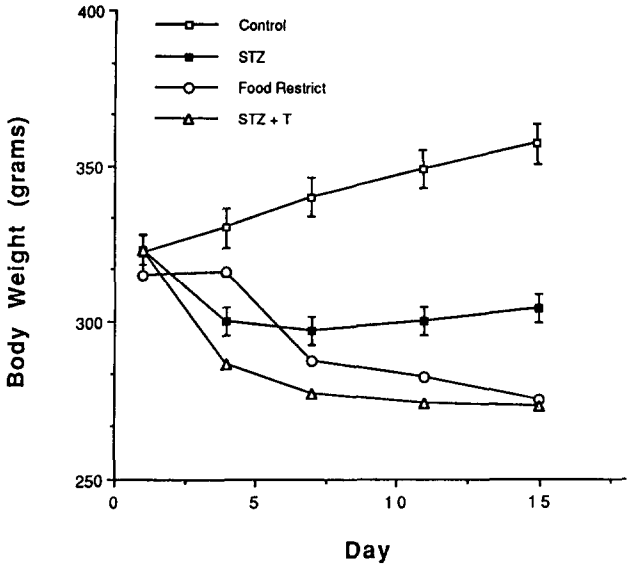

FIG. 1. Body weights of control, STZ, food-restricted and STZ-treated male rats given testosterone (T)-replacement. STZ (50 mg/kg) was injected on Day 0 and T-replacement (200 μg/day) was started 4 days later. Values represent the mean ± SEM of 14–16 animals.

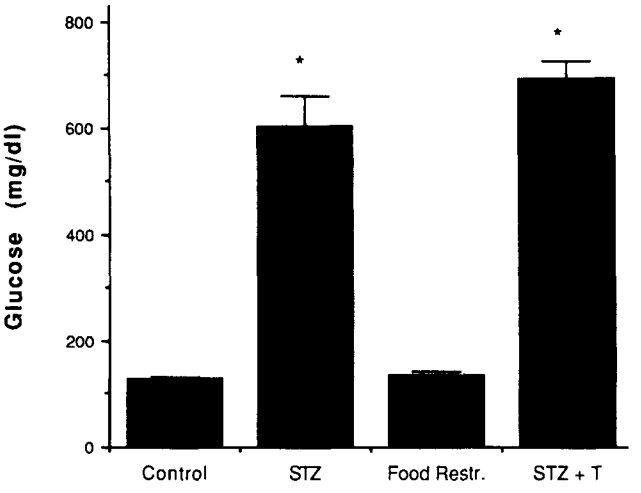

FIG. 2. Plasma glucose levels of rats described in Fig. 1. Values represent the mean ± SEM of 14–16 animals. The asterisk denotes statistical significance ($p<0.05$) vs. the control animals.

male rat was allowed two 30-min exposures to a stimulus female in behavioral estrus several days prior to testing for copulatory performance. The animals were tested 2 times over a 7-day period for copulatory behavior (see below) and divided in 4 groups demonstrating comparable performance. Subjects that failed to ejaculate in any of these two tests were eliminated from the study. The rats were then injected with streptozotocin (STZ; 50 mg/kg IP in 0.01 M citrate buffer, pH 4.5) or the injection vehicle (Control or food restricted). Four days later, a portion of the STZ rats were started on daily injections of testosterone (STZ+T; 200 μg T/rat/day). Rats were tested for copulatory behavior 14 days after STZ injection.

Behavioral testing was conducted between 14:30 and 17:30 hr in a separate room under dim red illumination in bedding-lined, 10 gallon glass aquaria. Ovariectomized Sprague-Dawley female rats implanted with a 5 mm length of 0.125 in. o.d. × 0.095 in. i.d. Silastic (Dow Corning, Midland, MI) capsules filled with estradiol were used as stimulus animals. Behavioral estrus was produced with a subcutaneous injection of 0.5 mg progesterone in 0.1 ml corn oil approximately 5 hr before the beginning of a test. Before each test, each subject was allowed 5 min to acclimate to the test chamber. The test was terminated at the first intromission after ejaculation or when 30 min had elapsed. The behavioral components recorded were the time to first mount (mount latency), the time to first intromission (intromission latency), the time from first intromission to ejaculation (ejaculation latency), the number of mounts preceding ejaculation (mount frequency) and the number of intromissions preceding ejaculation (intromission frequency). A more detailed definition of these components of copulatory behavior have been published by Dewsbury (8).

Three days following the last copulatory test (17 days after STZ) the rats were injected with saline or a tyrosine hydroxylase

TABLE 1

EFFECTS OF STZ, FOOD RESTRICTION AND STZ + TESTOSTERONE ON COPULATORY BEHAVIOR OF MALE RATS

	Control n = 10	STZ n = 9	Restricted n = 7	STZ + T n = 12
Mount				
Latency (sec)	35 ± 11	200 ± 51	322 ± 270	101 ± 42
No. Behaving	10(100%)	7(78%)	4(57%)	10(83%)
Intromission				
Latency	77 ± 22	370 ± 81	216 ± 19	265 ± 95
No. Behaving	10(100%)	7(78%)	3(43%)*	10(83%)
Ejaculation				
Latency	744 ± 116	1191 ± 183	685	977 ± 201
No. Behaving	9(90%)	4(44%)*	1(14%)*	6(50%)*

The values represent the mean ± SEM.
*$p<0.05$ vs. respective control values.

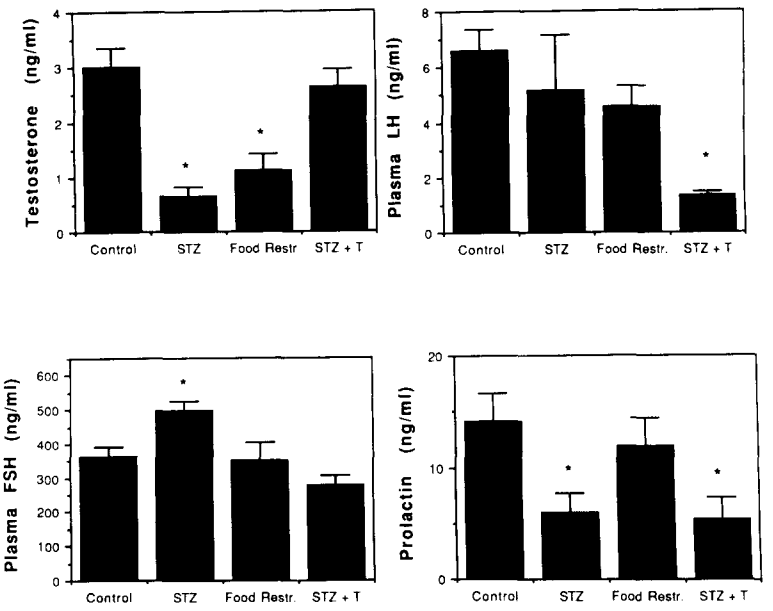

FIG. 3. Plasma T, LH, FSH and Prl levels in control, STZ, food-restricted and STZ + T rats. Rats were killed 17 days after STZ or vehicle injection. One-half of the rats in this experiment were injected with the tyrosine hydroxylase inhibitor, aMPT, but their plasma was not used for hormone determinations. Values represent the mean ± SEM of 7–8 animals. The asterisk denotes statistical significance ($p<0.05$) vs. the control animals.

inhibitor, alpha-methyl-p-tyrosine (aMPT; 250 mg/kg IP) for determination of catecholamine turnover rates in defined brain regions. The rats were sacrificed by decapitation 60 minutes later between 0900 and 1000 hr. At the time of autopsy, the brain was quickly removed and the median eminence was dissected free and frozen. The remaining brain was rapidly frozen on dry ice for subsequent dissection and preparation for amine analysis. Trunk blood was collected and serum was harvested for subsequent assay of LH, FSH, Prl and T.

Prior to assay, the brains were partially thawed, and medial basal hypothalamic (MBH) and anterior hypothalamic (AH) fragments were dissected free as previously described (31). These tissue fragments were weighed and then sonicated in 0.1 M $HClO_4$ containing the internal standards for the catecholamine assay (dihydroxybenzylamine) and the 5-HT assay (methyl 5-HT) and 1 mM sodium bisulfite. The ME was sonicated in the same solution but without the methyl 5-HT. Median eminence supernatants were separated by high performance liquid chromatography (HPLC) and quantitated by electrochemical detection as previously described (31). The MBH and AH fragments were subjected to alumina extraction prior to HPLC separation (31). Serotonin and 5-HIAA levels were determined in the MBH and AH supernatants using HPLC with electrochemical detection according to previously described procedures (31). Catecholamine turnover rates were estimated using the formula $K = k[CA]_o$ where $[CA]_o$ equals the mean catecholamine concentration at zero time (saline controls), and the rate constant, k, represents the $-\log$ of the slope of the line describing the decline of NE or DA concentration during the one hour following the blockade of tyrosine hydroxylase with aMPT (3,31).

Serum, media and tissue levels of LH, FSH and Prl were measured by RIA using reagents provided by the NIADDKD as described previously (29). Testosterone was assayed as previously described (12) with the exception of the use of testosterone antibody S-250 generously supplied by Dr. G. Niswender (Colorado State Univ.). Plasma glucose was assayed by a glucose oxidase procedure using a kit purchased from Sigma (St. Louis, MO).

The effects of treatment on hormone levels and neurotransmitter content and/or turnover were evaluated using analysis of variance or Student's *t*-test. Mean values between groups were considered significantly different when the p value was <0.05.

RESULTS

At the time of STZ injection there were no differences in body weight among the four groups of rats, but the control animals gained weight throughout the experiment compared to weight losses exhibited by the other 3 groups (Fig. 1). At autopsy, blood glucose levels in the control and food-restricted animals were significantly lower than in the STZ-treated rats (Fig. 2). Injection of aMPT did not affect glucose levels and, therefore, the values for saline and aMPT animals were combined. Testosterone treatment did not significantly affect blood glucose levels. One STZ-treated animal had a body weight >290 grams and glucose levels <130 mg/dl and was eliminated from the experiment.

The numbers of animals achieving ejaculation during the 30-min test of copulatory behavior were significantly greater in the control group than in the STZ, food-restricted or STZ + T groups (Table 1). Mount, intromission and ejaculation latencies and the number of animals exhibiting these behavioral responses are presented in Table 1. Only the times for animals exhibiting behavior was used in the latency calculations. When a latency equal to the time of the test, 1800 sec, was assigned to nonperforming animals, ejaculation and intromission latencies were significantly less in the control than in any of the other animals.

Plasma T levels were significantly depressed in both the STZ and the food-restricted rats (Fig. 3). The daily T injections restored levels to those seen in the control rats. Prolactin levels were

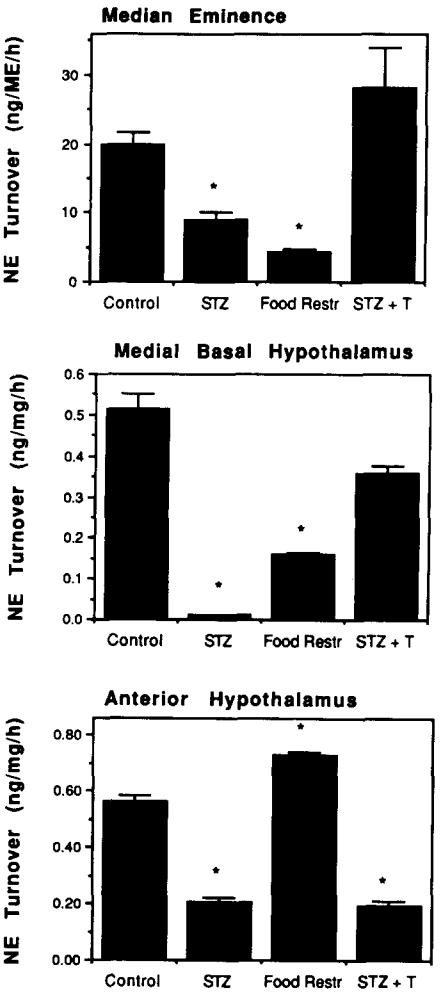

FIG. 4. NE turnover in defined brain regions of control, STZ, food-restricted and STZ + T rats. NE turnover, an index of neuronal activity, was calculated from the decline of NE content after inhibition of tyrosine hydroxylase with aMPT. Values represent the mean ± SEM of 7–8 animals. The asterisk denotes statistical significance ($p<0.05$) vs. the control animals.

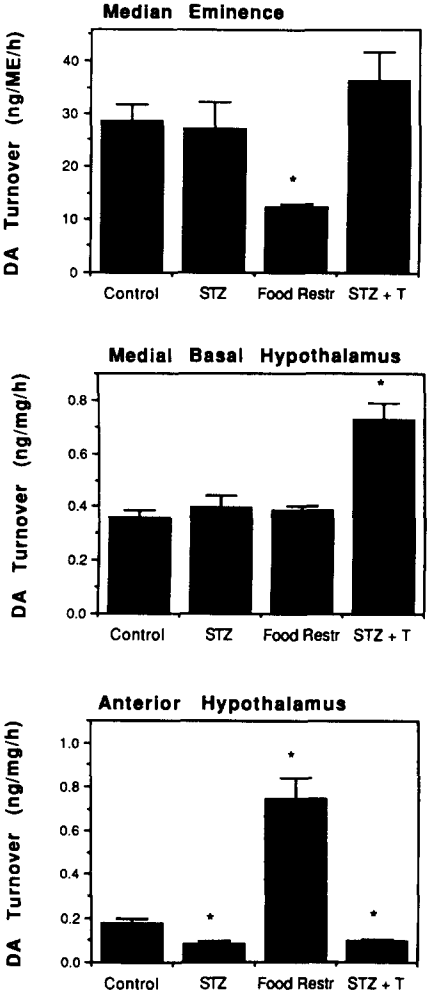

FIG. 5. DA turnover in defined brain regions of control, STZ, food-restricted and STZ + T rats. DA turnover, an index of neuronal activity, was calculated from the decline of DA content after inhibition of tyrosine hydroxylase with aMPT. Values represent the mean ± SEM of 7–8 animals. The asterisk denotes statistical significance ($p<0.05$) vs. the control animals.

depressed by STZ treatment, while FSH levels were increased and LH levels were unchanged (Fig. 3). T-treatment reduced LH, but not FSH levels. Food restriction had no significant effects on LH, FSH or Prl levels despite its marked effect to lower T levels.

Norepinephrine turnover in the ME, MBH and AH were all suppressed by treatment with STZ (Fig. 4). Turnover of DA was decreased in the AH, but unchanged in the ME and MBH of the STZ as compared to the control rats (Fig. 5). Food restriction led to a decrease in NE turnover in the ME and MBH, but NE turnover was slightly, but significantly increased in the AH. DA turnover was decreased in the ME, unchanged in the MBH and increased in the AH. Testosterone reversed the effects of STZ on ME, MBH NE turnover, but had no similar effect on AH NE turnover. The STZ + T rats tended to have higher DA turnover rates in the ME and MBH, but the effect was only significant for the MBH.

Levels of LHRH were unchanged in the ME and AH, but reduced in the MBH of STZ as compared to control rats (Fig. 6). T-treatment did not effect the levels of LHRH in the STZ rats, but food restriction increased ME LHRH levels. Levels of 5-HT were unchanged in any of the tested areas (data not shown).

DISCUSSION

The results of the present study confirm our previous observations that STZ-induced diabetes is associated with marked reductions in male copulatory behavior that are accompanied by changes in plasma hormone levels and hypothalamic neurotransmitter turnover (30). In addition, the present study demonstrates that changes in copulatory behavior may be induced by caloric restriction. However, as discussed below, the changes in sex behavior associated with diabetes and caloric restriction may be due to entirely different mechanisms.

Both the STZ and the food-restricted rats had reduced plasma T levels, but this factor alone does not appear responsible for changes in copulatory behavior since T-replacement did not reverse the adverse effects of diabetes on sexual behavior. T-replacement has previously been shown to restore copulatory behavior in castrate rats (7). Furthermore, it has been shown that T levels similar to those seen in the STZ rats and as low as 0.2 ng/mg can support ejaculatory behavior (7). STZ-induced deficits in copulatory behavior might also be due to reduced T-responsiveness resulting from alterations in steroid receptors or due to

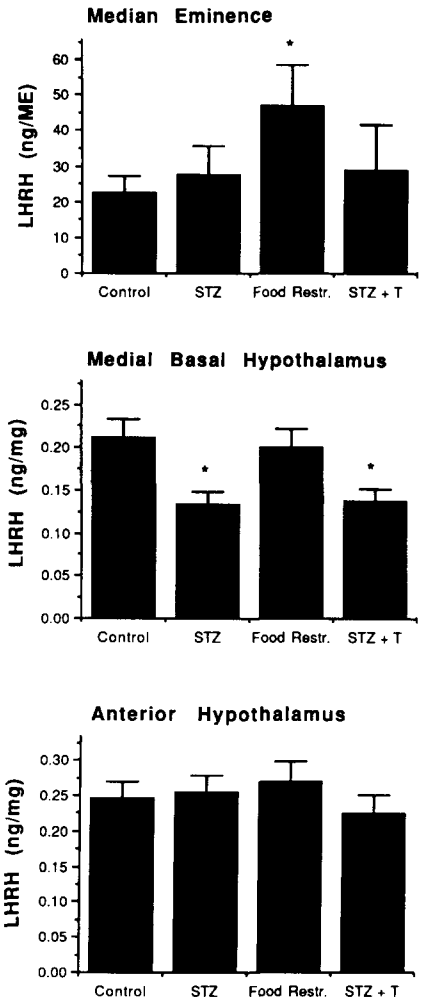

FIG. 6. LHRH content of defined hypothalamic regions of control, STZ, food-restricted and STZ + T rats. Values represent the mean ± SEM of 7–8 animals. The asterisk denotes statistical significance ($p<0.05$) vs. the control animals.

reduced formation of behaviorally active aromatized metabolites of testosterone. In this regard, estrogen binding and sexual receptivity is reduced in diabetic female rats (16,27). Despite the inability of T to reverse behavioral deficits, T-replacement caused a significant reduction in plasma LH levels and reversed the STZ-induced elevation of plasma FSH levels.

The possibility exists that reductions in MBH LHRH content as seen in the present and previous (30) studies may reflect changes in LHRH release that might lead to reductions in copulatory behavior. There is considerable evidence that LHRH can directly stimulate sexual behavior independent of its effects to stimulate gonadotropin secretion (1, 22, 25). The inability of food restriction to change MBH LHRH levels despite adversely affecting copulatory behavior does not support the hypothesis of LHRH involvement in behavioral changes in diabetic, but it is possible that food restriction and diabetes may affect sex behavior by entirely different mechanisms. Although food-restricted rats are often used as controls for diabetic animals, this may be inappropriate since the metabolic states of the animals are undoubtedly quite different despite the fact that they both lose weight.

Changes in anterior hypothalamic catecholamine metabolism may also have been responsible for the changes in copulatory behavior observed in the present study. A number of studies have shown a facilitory role of dopamine in controlling sexual behavior (2, 6, 21), while the role of NE is much less clear (5,21). Thus, the attenuation of AH DA turnover as seen in the STZ-treated rats could be responsible for the observed reduction in copulatory behavior. In support of a role for reduced DA turnover as a cause of altered sexual behavior, we have recently demonstrated that insulin replacement reverses the effects of STZ on AH DA turnover and sexual behavior (Steger and Kienast, unpublished observations). Again, the food-restricted animals are clearly different since DA turnover in the AH was markedly elevated and although this increase would be expected to enhance sexual activity, this was clearly not the case. Again, it should be emphasized that sex behavior is controlled by a complex interaction of many neurotransmitter and neuromodulatory factors and that much additional work needs to be done to resolve what CNS changes are responsible for the behavioral changes associated with diabetes.

As demonstrated in previous studies (30), STZ treatment led to significant changes in anterior pituitary function that may also have been secondary to changes in central nervous system function. Food restriction also altered the pituitary hormone secretion, but the profile of the change was different from the diabetic animals. As previously observed, plasma Prl levels were significantly depressed in the STZ rats, but in contrast to previous observations, plasma LH was unchanged and FSH levels were significantly elevated (30). However, it should be noted that LH levels in the STZ-treated rats and the food-restricted rats were the same as control rats despite the very low levels of plasma T which would normally be expected to cause elevated LH levels. It is probable that the length of time between the STZ injection and autopsy may have accounted for differences in the magnitude of the LH change in the 2 studies since the animals in the present study were autopsied 17 days after STZ, while in the previous study, animals were not autopsied until 29 days after STZ treatment.

The lack of a significant rise in LH levels despite low T levels in the STZ and food-restricted rats may have been due to a reduction in ME NE turnover since a number of studies have shown that NE is a primary stimulus of LHRH and, therefore, LH release (18, 20, 24). Interestingly, T-replacement reversed the effects of STZ on ME and MBH NE turnover. These results were somewhat unexpected since T has been previously been shown to reduce MBH NE turnover (28). However, NE turnover rates were still lower in the STZ + T animals than in untreated controls. The ability of T to increase NE turnover in STZ-treated rats was also seen in recent experiments where it was shown that insulin replacement increased plasma T levels in STZ-treated rats and reversed the effects of STZ on ME and MBH NE turnover (Steger and Kienast, unpublished observations). The ability of T to increase NE turnover while decreasing LH release can be explained by the direct effect of T on the anterior pituitary to inhibit LH release (9,23).

In conclusion, STZ-induced diabetes leads to significant reductions in copulatory behavior that may be secondary to changes in hypothalamic catecholamine metabolism. STZ-induced changes in copulatory behavior are not reversed by normalization of serum T levels with exogenous T-replacement. Restriction of food intake that mimics the body weight loss seen in diabetic rats also severely attenuates copulatory behavior but the mechanism may not be similar to that in the diabetic animal.

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