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Exercise Training Improves the Defective Centrally Mediated Erectile Responses in Rats with Type I Diabetes

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Abstract

Introduction—Erectile dysfunction is a serious and common complication of diabetes mellitus. Apart from the peripheral actions, central mechanisms are also responsible for the penile erection.

Aim—The goal of the present study was to determine the impact of exercise training (ExT) on the centrally mediated erectile dysfunction in streptozotocin (STZ)-induced type I diabetic (T1D) rats.

Methods—Male Sprague-Dawley rats were injected with STZ to induce diabetes mellitus. Three weeks after STZ or vehicle injections, rats were assigned to either ExT (treadmill running for 3-4 weeks) or sedentary groups to produce four experimental groups: control+sedentary, T1D+sedentary, control+ExT and T1D+ExT.

Main Outcome Measure—After 3-4 weeks ExT, central N-methyl-D-aspartic acid (NMDA) or sodium nitroprusside (SNP)-induced penile erectile responses were measured. Neuronal nitric oxide synthase (nNOS) expression in the paraventricular nucleus (PVN) of the hypothalamus was measured by using histochemistry, real time PCR and Western blot approaches.

Results—In rats with T1D, ExT significantly improved the blunted erectile response and ICP changes to NMDA (50ng) microinjection within the PVN (T1D+ExT: 3.0 ± 0.6 penile erection/rat; T1D+sedentary: 0.5 ± 0.3 penile erection/rat within 20mins, $P < 0.05$). ExT improved erectile dysfunction induced by central administration of exogenous nitric oxide (NO) donor, SNP in T1D rats. Other behavior responses including yawning and stretching, induced by central NMDA and SNP microinjection were also significantly increased in T1D rats after ExT. Furthermore, we found ExT restored the nNOS mRNA and protein expression in the PVN in T1D rats.

Conclusions—These results suggest that ExT may have beneficial effects on the erectile dysfunction in diabetes through improvement of NO bioavailability within the PVN. Thus, ExT may be used as therapeutic modality to up-regulate nNOS within the PVN and improve the central component of the erectile dysfunction in diabetes mellitus.

Keywords

type I diabetes; exercise training; central nervous system; central mechanisms of penile erection

Introduction

Sexual dysfunction is well known consequence of diabetes mellitus in men^{1,2}. Erectile dysfunction, retrograde ejaculation and loss of seminal emission have been described in male diabetic patients. Approximately 35% to 75% of men with diabetes mellitus have

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Conflict of Interest: None

erectile dysfunction³. In animal experiments, diabetic rats show significant deficits in mount, intromission, and ejaculatory behaviors, suggesting that both the sexual arousal (libido) and potency components of male sexual behavior are adversely affected by diabetes⁴. The primary therapy for men with diabetes and erectile dysfunction is oral administration of phosphodiesterase type 5 (PDE5) inhibitors, such as Viagra. Consistent with these observations there is a decreased expression of PDE5 in penile tissue from diabetic animal model⁵. However, approximately 50% of male patients with diabetes are unresponsive to this treatment⁶. Since the actions of PDE5 inhibitors are thought to affect the smooth muscle cells lining the blood vessels supplying the corpus cavernosum of the penis, it is possible that other components of the erectile response including the initiating central mechanisms independent of PDE5 may contribute to the altered erectile dysfunction in diabetic males. The contribution of the central component of the altered erectile dysfunction in diabetes is generally under studied to date.

It is generally accepted that different central and peripheral neural and/or humoral endocrine mechanisms participate in the regulation of sexual response. Penile erection is the result of a complex central and peripheral interaction that induces muscle and vascular changes at the level of the erectile tissues. Regarding the central mechanism, several neurotransmitters and neuropeptides which control erectile function, including excitatory amino acid N-methyl-D-aspartic acid (NMDA), dopamine, nitric oxide (NO), oxytocin, gamma-amino-butyric acid (GABA) and opioid, have been identified⁷. These compounds act in several brain areas, including the paraventricular nucleus (PVN) of the hypothalamus^{8,9}, which convey information to the genitals via projections from the spinal cord.

The PVN of the hypothalamus is involved in numerous functions including feeding, metabolic balance, cardiovascular regulation, as well as erectile function and sexual behavior. Bilateral lesions of the PVN dramatically reduce the erectile effects of several compounds¹⁰. Activation of the PVN neurons by central components such as NMDA, or by electrical stimulation leads to penile erection^{11,12}. Our previous study demonstrated that penile erection occurs concomitantly in response to administration of NMDA directly into the PVN¹³. Administration of NMDA within the PVN demonstrated a decreased response in penile erection, yawning and stretching in diabetic rats¹³. This is further observed that the level of neuronal nitric oxide synthase (nNOS) protein is decreased in rats with diabetes compared to control rats. Previously, we also measured penile erection, yawning and stretching before and after the administration of adenoviral transfection of nNOS gene into the PVN of control and diabetic rats¹³. The results showed that restoration of nNOS within the PVN of diabetic rats with viral transfection corrects the behavioral responses (erection and yawning) mediated by microinjection of NMDA. This suggests an abnormality in a central NO mechanism, specifically within the PVN is involved in the altered erectile responses in diabetic rats.

Clinical and experimental studies have shown the benefits of exercise training (ExT) in T1D by insulin sensitivity improvement, reduction in insulin requirement and an attenuation of autonomic and cardiovascular dysfunction^{14,15}. It has been shown that long-term regular ExT benefits to vascular health and erectile function in the patients with erectile dysfunction and diabetes¹⁶⁻¹⁸. However, the mechanisms by which ExT improves the status of diabetic patients, and animals, remain unclear. Running exercise can enhance functional response in rat corpus cavernosum by increasing the NO-cGMP signaling pathway^{19,20}. Exercise training also elicits a beneficial effect on the impaired corpus cavernosum relaxing responses in rats with diabetes²¹. Although ExT has been shown to enhance endothelial function in the patients and animals with T1D^{22,23}, there are little data on the role of ExT in the modulation of central component of erectile function. In other studies we have previously demonstrated that ExT restores the levels of nNOS with the PVN of rats with

chronic heart failure²⁴. Whether ExT improves this central nNOS within the PVN of rats with diabetes remains to be examined.

Aims

The aims of our study were to test 1) whether ExT improves centrally NMDA-induced erectile dysfunction in rats with T1D; 2) whether ExT improves centrally mediated SNP-induced erectile dysfunction in rats with T1D rats; and 3) whether the nNOS system is restored in the PVN of ExT T1D rats.

Methods

Animal and treatment

This study was approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee and conformed to the guidelines for the care and use of laboratory animals of the National Institutes of Health and the American Physiological Society. Male Sprague-Dawley rats (200-220g, Sasco) were randomly injected with STZ (65mg/kg i.p) to induce diabetes or vehicle (citrate buffer) for controls. The percentage of diabetic animals after STZ injection was about 80%. Onset of diabetes was identified by polydipsia, polyuria, and blood glucose levels >250mg/dl. The mortality rate of the STZ rats was about 20%. Rats that exhibit ruffled hair, poor appearance, vocalize, and a lack of appetite were considered to be of poor health and were euthanized.

Three weeks after STZ or vehicle injections, rats were assigned to either ExT or sedentary groups to produce four total experimental groups: control+sedentary, T1D+sedentary, control+ExT and T1D+ExT. For ExT, rats ran on a motor-driven treadmill (Columbus Instruments, Columbus, OH) for a period of 3-4 weeks according to a modified protocol of Musch and Terrell²⁵. Erectile function and nNOS expression experiments were performed 7-8 weeks after STZ injection or vehicle. Initially, low speed (10m/min) and grade (0%) and short duration (10min/day) were used to familiarize the rats with running on the treadmill. The speed, duration, and grade were gradually increased to 20-25m/min, 60min/day, and 5-10%, respectively. Only rats that ran steadily with little or no prompting were used in the study (89%, 32 out of 36 rats).

Surgical Procedures

After 3-4 weeks of ExT, each animal was implanted with a stainless steel cannula aimed at the PVN as described previously¹³. The rats were anesthetized and then placed in a stereotaxic apparatus (Davis Kopf instruments, Tujanga, CA). A longitudinal incision was made on the scalp, and the bregma was exposed. The coordinates for the PVN were determined according to the atlas of Paxinos and Watson²⁶. A stainless steel guide cannula (Microdialysis AB, Solna, Sweden) was implanted at the following coordinates: 1.5mm posterior to the bregma, 0.4mm lateral to midline, and 7.8mm ventral to the dura. The animals were then returned to their cages and allowed to recover for 3 days.

Skeletal Muscle Oxidative Enzyme Activity and Plasma Testosterone Assay

To ensure a similar level of ExT between groups, citrate synthase activity assays on the soleus muscle were performed according to the protocol of Srere²⁷. At the time of death, muscle samples were taken from the soleus muscle, frozen at -70°C, and stored until processed. Citrate synthase activity was measured spectrophotometrically from whole muscle homogenate.

Testosterone level of plasma was measured by ELISA using testosterone immunoassay kit (ALPCO Diagnostic, Salem, NH).

Erectile Function Experimental Protocol

Three days after surgery, NMDA or sodium nitroprusside (SNP)-induced penile erection, yawning, stretching, grooming and chewing within the PVN in freely moving, conscious rats were measured (control+sedentary, T1D+sedentary, control+ExT and T1D+ExT). Rats were placed individually into a Plexiglas cage and injected with NMDA and SNP (50ng) into the PVN in a volume of 100nl, respectively. After NMDA or SNP injection, the rats were observed to count number of episodes of penile erection, yawning, stretching, grooming and chewing over 20 min intervals for the next 80min.

Micropunch of PVN

After the animal was euthanized, the brain was removed and frozen on dry ice. Six serial coronal sections (100µm) were cut through the hypothalamus at the level of the PVN with a cryostat, and, according to the Palkovits and Brownstein technique²⁸, the PVN was bilaterally punched with a blunt 18-gauge needle, such that there were 12 total punches per brain. For each brain, six of the punches were placed in 500µl of Tri-Reagent (MRC, Cincinnati, OH), followed by sonication and extraction of mRNA according to the manufacturer's instructions. The other six punches for each brain were placed in 100µl of protein extraction buffer (10mM Tris, 1mM EDTA, 1% SDS, 0.1% Triton X-100 and 1mM phenylmethylsulfonyl fluoride), sonicated, and incubated for 30min at 37°C to extract the protein.

Real-time RT-PCR measurement of nNOS mRNA

After extraction of mRNA, samples underwent reverse transcription. Real-time RT-PCR measurements were made with the iCycler iQ Multicolor Real-Time Detection System (Bio-Rad). The reaction mixture consisted of SYBR Green Supermix (Bio-Rad), 300nM sense primer, 300nM antisense primer, and the cDNA template of interest. For nNOS, the sense primer was 5'-GCGGAGCAGAGCGCCTTAT and the antisense primer was 5'-TTTGTTGGGAGGACCGAGGG. For rpl19, used as the reference gene, the sense primer was 5'-CCCCAATGAAACCAACGAAA and the antisense primer was 5'-ATGGACAGTCACAGGCTTC. Relative expression of nNOS was calculated with the Pfaffl equation, which relates expression of the target gene (nNOS) to expression of a reference gene (rpl19).

Western Blot Analysis for nNOS Protein

The protein extraction was used for Western blot analysis of nNOS in samples obtained above. The sample was loaded onto the 7.5% SDS-PAGE gel for electrophoresis. The fractionated proteins on the gel were electrophoretically transferred onto the polyvinylidene difluoride membrane. The membrane was incubated with primary antibody (rabbit anti-rat nNOS polyclonal antibody, Santa Cruz Biotechnology, 1:1,000) overnight. Then the membrane was incubated with secondary antibody (goat anti-rabbit IgG, peroxidase conjugated, Pierce, 1:5,000), treated with enhanced chemiluminescence reagent, and probed with secondary antibody (peroxidase conjugated goat anti-rabbit IgG, 1:5,000; Pierce). An enhanced chemiluminescence substrate (Pierce) was applied to the membrane, followed by an exposure within an Epi Chemi II Darkroom (UVP BioImaging, Upland, CA) for visualization with the Worklab digital imaging system. Kodak 1D software was used to quantify the signal. The expression of nNOS was calculated as the ratio of intensity of the nNOS band, respectively, relative to the intensity of the β -tubulin band.

NADPH-Diaphorase Activity as a Marker of nNOS Activity

After ExT, the rats from all four groups (control+sedentary, n=7; control+ExT, n=10; T1D+sedentary, n=10; and T1D-ExT, n=10) were anesthetized and perfused through the left

ventricle of the heart with heparinized saline followed by 4% paraformaldehyde. The brain was postfixed in 4% paraformaldehyde solution and then placed in 20% sucrose for 24 h. The brain was blocked in the coronal plane, and sections of 30 μ m in thickness were cut with a cryostat. Every third section was kept from the anterior commissure (0.4mm posterior to bregma) posterior to where the optic tracts were observed to be in their most lateral position on the ventral surface of the brain (2.6mm posterior to bregma). The sections were collected in 0.3% Triton X-100, 0.1mg/ml nitroblue tetrazolium, and 1.0mg/ml β -NADPH. The sections in nitroblue tetrazolium solution were then placed in an oven at 37°C for 1h. After the reaction, the sections were mounted onto slides.

The presence of NADPH-diaphorase in the PVN was examined under a microscope. The density of the staining was evaluated by counting the number of cells that were positively stained for NADPH-diaphorase. The number of NADPH-diaphorase-stained cells was counted for each individual nucleus at the same coronal level. Three adjacent sections were considered to represent one coronal level because the numbers of cells counted were within 5% of each other. The number of cells in the middle section was taken to represent the number of cells within a given nucleus.

Data Analysis

Data were subjected to two-way ANOVA followed by Newman Keuls test (for multiple comparisons) or Student's t-test. $P < 0.05$ was considered to indicate statistical significance.

Main Outcome Measure

After 3-4 weeks ExT, NMDA or SNP-induced penile erection within the PVN of the hypothalamus in freely moving, conscious rats were measured. nNOS expression in the PVN was measured by using histochemistry, real time PCR and Western blot approaches.

RESULTS

General Data

Table 1 summarizes the salient characteristics of control and T1D rats utilized in the present study. T1D rats showed significantly higher glucose level. Body weight was significantly lower in T1D+sedentary rats compared with control+sedentary rats ($P < 0.05$). Plasma testosterone level is significantly lower in T1D+sedentary rats ($P < 0.05$). Exercise training had no significant effects on the blood glucose level, body weight and plasma testosterone level in both control and T1D groups.

The concentration of citrate synthase in the soleus muscle was significantly increased in the ExT groups ($P < 0.05$), consistent with previous reports²⁴. The level of citrate synthase activity in the soleus muscle was comparable in both control and T1D exercise groups, indicative of similar levels of ExT.

Behavioral Responses to NMDA Administration

Between 7-8 weeks after STZ injection, NMDA was microinjected into the PVN of conscious, freely moving, diabetic rats. The results demonstrated blunted erection, yawning, and stretching responses in diabetic rats compared with control rats (Figure 1A-C) (T1D +sedentary 0.5 ± 0.3 penile erection/rat versus control+sedentary 3.4 ± 0.4 penile erection/rat within 20mins, $P < 0.05$). The proportion of responsive rats (those showing at least 1 erectile event) is dramatically different between control+sedentary (100%; 6 out of 6 rats) and T1D +sedentary group (16%; 1 out of 6 rats). There were no significant differences of grooming and chewing responses to NMDA in between control and T1D groups (Figure 1D-E). Vehicle injection within the PVN of control and diabetic rats did not induce penile erection,

yawning, stretching, grooming and chewing responses (data not shown). Exercise training dramatically improved the blunted erection, yawning, and stretching responses in T1D rats compared to the T1D+sedentary rats (T1D+ExT 3.0 ± 0.6 penile erection/rat; T1D+sedentary 0.5 ± 0.3 penile erection/rat within 20mins, $P < 0.05$).

Behavioral Responses to SNP Administration

We also tested whether administration of an NO donor, SNP, within the PVN could elicit erection, yawning, stretching, grooming and chewing responses in the four groups of rats (control+sedentary, T1D+sedentary, control+ExT and T1D+ExT) (Figure 2A-D). The proportion of diabetic rats showing at least one erectile event was 16% (1 out of 6 rats) and was significantly lower than control+sedentary rats (100%; 6 out of 6 rats). It should be noted that the erection, yawning and stretching responses to SNP in T1D+sedentary rats were significantly blunted compared with control+sedentary rats (T1D+sedentary 0.5 ± 0.5 penile erection/rat versus control+sedentary 2.8 ± 0.6 penile erection/rat within 20mins, $P < 0.05$). Exercise training significantly improved the blunted erection, yawning, and stretching responses in T1D rats compared to the T1D+sedentary rats (T1D+ExT 1.8 ± 0.4 penile erection/rat; T1D+sedentary 0.5 ± 0.5 penile erection/rat within 20mins, $P < 0.05$).

Expression of nNOS mRNA and protein within the PVN

nNOS mRNA expression, measured by real-time RT-PCR, is shown in Figure 3A. Figure 3A shows the composite real-time RT-PCR data for the four experimental groups. Relative nNOS expression was significantly decreased in the T1D+sedentary group compared with the control+sedentary group. However, in the T1D+ExT group, relative nNOS expression was significantly higher than in the T1D+sedentary group and not different from the control+sedentary or the control+ExT group. These data indicate that ExT normalizes nNOS mRNA expression within the PVN in rats with T1D.

nNOS protein expression, measured by Western blot, is shown in Figure 3B. Sample gels showing nNOS and β -tubulin protein in the four experimental groups are presented. The level of nNOS protein expression in the T1D+sedentary group was significantly lower than in the control+sedentary group. In the T1D+ExT group, nNOS protein expression was significantly higher than in the T1D+sedentary group and was not different from either the control+sedentary group or the control+ExT group.

NADPH-Diaphorase Activity as a Marker of nNOS Activity—As shown in Figure 4A, with the technique of NADPH-diaphorase staining, the PVN is clearly outlined, and cells of medium to large size were observed in the PVN, most of which were concentrated in the ventral and lateral portion of the PVN. The number of NOS-positive cells in the PVN of T1D-sedentary rats was significantly less than that found in the other groups (30% less, $P < 0.05$). In rats with T1D, ExT restored the decreased number of nNOS-positive neurons in the PVN. The number of cells stained for diaphorase in the PVN in the control+sedentary, control+ExT, and T1D+ExT groups was significantly greater than that in T1D+sedentary group (Figure 4B). There were no significant changes in the number of NADPH-diaphorase-positive cells in the SON and LH.

Discussion

The results of the present study indicate that in rats with T1D, 3–4 weeks of ExT significantly improved the blunted erectile response to either NMDA or SNP microinjection within the PVN of the hypothalamus. Consistent with the erectile response, other behavior responses including yawning and stretching, induced by central NMDA and SNP microinjection were also significantly increased in T1D rats after ExT. Furthermore, we

found ExT restored the nNOS expression in the PVN in T1D rats by using real time PCR, Western blot and histochemistry, approaches. Taken together, these data suggest that ExT improves the central component of the erectile response in type I diabetes.

In the present study, administration of NMDA within the PVN demonstrated a decreased response in penile erection, yawning, and stretching in T1D rats confirming our previous observations¹³. That these responses were mediated by altered levels of nNOS are supported by the observation that the levels of nNOS mRNA and protein are decreased in rats with T1D compared with control rats. Administration of NMDA within the PVN did not show a significantly decreased response in grooming and chewing in T1D rats. This may imply a different activation of neural circuit controlling different behavioral responses²⁹⁻³¹

The presence of the penile erectile responses to SNP administration in the PVN in diabetic rats, albeit smaller, implies that the peripheral mechanisms are involved in the overall response. However, because the response to SNP was blunted in diabetic rats, these results could not differentiate between an additional peripheral abnormality and a central abnormality in the diabetic rats. In the present study, ExT improved the nNOS within the PVN with concomitant improvement in SNP response within the PVN in diabetic rats. It is of interest to note that adenoviral mediated up-regulation of nNOS within the PVN was able to restore the NMDA responses in diabetic rat. Presumably there was no peripheral component improvement in that study¹³. Taken together, we suggest that of the central restoration of nNOS within the PVN in the ExT diabetic rats was crucial for the improvement of the central component of the erectile response. Furthermore, ExT improved the blunted responses to SNP suggesting that the peripheral mechanisms may also be improved as suggested by others²⁰.

In the present study, we have further shown that ExT improved the blunted erectile, yawning and stretching responses to either NMDA or SNP microinjection within the PVN. We also found ExT restored the nNOS expression in the PVN in T1D rats. The results of these studies may have a more general implication of a central NO mechanism within the PVN involved in erectile dysfunction. This is consistent with the observation that mice that lack nNOS display less erectile responses to the electrical stimulation around the cavernous nerve³². This implies that nNOS may be a major mediator of sexual behavior that may be affected in various disease states that are associated with erectile dysfunction reported in various disease conditions.

The mechanism by which central NMDA induces penile erectile function appears via the release of NO, which causes in turn the activation of oxytocinergic neurons in the PVN. Pharmacological, electrophysiological and immunocytochemical studies have identified oxytocinergic neurons, which regulated penile erection, projecting from the PVN to the brain stem and finally to the spinal cord^{33, 34}. In addition to this well documented peripheral action, evidence is accumulating which indicates that NO may also function as a neurotransmitter in the central nervous system to modulate sexual behavior and penile erection³⁵. The PVN is a primary site within the forebrain that has been implicated in NO-mediated penile erection^{33, 34}. Delivering NO or NO donors to the PVN of conscious rats elicits episodes of penile erection, and an increase in intracavernous pressure (ICP), consistent with the observations in this study. Interestingly, we have observed a marked reduction in the central NO mechanism in the PVN, which, as least in part, leads to a lack of NMDA-mediated penile erection in T1D rats¹³.

The mechanisms responsible for the ExT-induced increase in nNOS within the PVN remain unknown. However, it is well known that the PVN receives information from various cardiopulmonary receptors including myocardial vagal afferents^{36, 37}. Some studies suggest

that ExT increases various hemodynamic parameters within the heart that dictate cardiac vagal activity^{38,39}. Accordingly, the activation of cardiac vagal activity during ExT may mediate an increase of nNOS within the PVN. This is consistent with the enhanced inhibitory influence of cardiac vagal afferents on directly measured sympathetic activity and regional vascular resistance after ExT⁴⁰.

Modulation of NO synthesis and the interaction of NO with angiotensin II (AngII) in the central nervous system may be another important mechanism for the regulation of central NMDA-induced erectile function during ExT in T1D. AngII has been implicated as a contributor in the erectile dysfunction observed in diabetes. Plasma levels of AngII are increased in diabetes⁴¹, while angiotensin-converting enzyme (ACE) inhibitors and AngII type 1 (AT₁) receptor antagonists improve erectile function in diabetic patients⁴². Increased AngII may enhance oxidative stress and decrease NO bioavailability in diabetes. Interestingly, it has been shown that ExT reverses the increased oxidative stress in NO-deficient rats and ameliorating the erectile dysfunction. So it is possible that ExT may be reducing elevated levels of AngII in diabetes⁴³ and thus improving or modulating the central levels of nNOS within the PVN of diabetic rats. This possibility remains to be examined more thoroughly in the future.

The pathophysiology of diabetes-related erectile dysfunction is well known to be multifactorial⁴⁴, including neuropathy, vascular disease, metabolic abnormalities, endocrine disorders, and psychogenic factors. NO plays key role in regulating erectile function by balancing vasodilation, which leads to the rigid state, and vasoconstriction, which results in detumescence of the penis. Although our results show ExT improving centrally mediated erectile function in T1D, the impact of ExT on systemic endothelial dysfunction and corpus cavernosum responses is also confirmed by other studies¹⁹⁻²¹. Claudino et al showed that running exercise for 8 weeks in rats increases the relaxation responses of corpus cavernosum through the NO-cGMP signaling pathway activation^{19,20}. Recently, they have shown that the physical preconditioning markedly restores the reduced relaxation response of corpus cavernosum for the muscarinic agonist acetylcholine and electrical field stimulation in rats with diabetes²¹. Regular ExT up-regulates both eNOS and nNOS expressions in the aged and young rat penis to improve penile erection⁴⁵. Since eNOS expression has been previously documented to be reduced in the penis of T1D rats⁴⁶, we are assuming that ExT may prevent erectile dysfunction associated with diabetes by improving both nNOS and eNOS function at the level of the penis. In the case of the central nervous system, eNOS is seen in blood vessel-like structures in the PVN, while nNOS is localized in the PVN neurons⁴⁷. Although, in the PVN neurons, nNOS is predominant NOS, the details of central eNOS within the PVN involvement in erectile response and dysfunction in diabetes remain to be examined.

Furthermore, the endocrine disorders (such as reduced testosterone levels, hypothyroidism, or hyperthyroidism) associated with diabetes may compound the problem, making the etiology of erectile dysfunction in men with diabetes a complex issue. Sex steroid hormones play an important role in maintaining endothelial health and sex steroid deficiency is associated with endothelial dysfunction, vascular disease and erectile dysfunction⁴⁸. STZ-induced T1D has been previously described to alter erectile function due to an underlying condition of hypogonadotropic hypogonadism^{5,49}. These observations suggest that, at least in the male, consolidation of a state of diabetes is linked to suppression of the functionality of hypothalamic system. Reported histomorphological analysis of penile tissue shows that neuronal damage is associated with penile hypoxia and tissue alteration from diabetic and hypogonadic condition^{50,51}. Our result shows the plasma testosterone is significantly lower in T1D rats. This is consistent with other reports from patients and animal experiments^{52,53}. Testosterone replacement therapy may lead to symptomatic improvement

in diabetes with erectile dysfunction^{5, 54}. The contractile RhoA/Rho-kinase (ROCK) signaling pathway is upregulated in penile tissue in animal models of experimental diabetes and has been proposed to contribute to diabetes-related erectile dysfunction. Treating hypogonadism in course of diabetes may maintain erectile function also by normalizing RhoA/ROCK pathway upregulation⁵⁵. However, our data shows that despite the lack of change in plasma testosterone to ExT in rats with T1D, there was restoration of centrally mediated erectile responses suggesting that altered levels of plasma testosterone level in T1D, do not contribute to the centrally mediated erectile response in this paradigm. The effects of ExT on the centrally involved sex steroid levels in controlling erectile function remains to be investigated.

STZ-induced diabetic rats provide an interesting and relevant model to study the effects of diabetes on male sexual dysfunction, since they exhibit several deficits in copulatory behavior similar to those in diabetic men^{56, 57}. Four weeks-STZ-induced diabetic rats have shown an endocrine and metabolic disorder often associated with erectile dysfunction and peripheral neuropathy⁵⁸. Diabetic rats show significant deficits in mount, intromission, and ejaculatory behaviors, suggesting that both the sexual arousal (libido) and potency components of male sexual behavior are adversely affected by diabetes^{56, 59}. Examination of these responses early in the diabetic condition, as done in this experiment (7-8 weeks after STZ injection), also further emphasizes the importance of these observations since minimal/none of the chronic functional effects of metabolic dysfunction or complications are evident at this early stage. Time-dependent change in erectile function and responsiveness to PDE5 inhibitor were investigated in STZ-induced diabetic rats⁶⁰. Erectile responses were significantly decreased in 10 weeks diabetic rats, and administration of PDE5 inhibitor resulted in partial recovery of normal responses. At more than 12 weeks, rats demonstrated severe deterioration of erectile function, which did not fully respond to PDE5 inhibitor. It is possible that the impairment of centrally mediated erectile function in the present study might be the early pathophysiology of diabetes-related erectile dysfunction. The results are compatible with those of previous cohort studies showing that risk of erectile dysfunction increased with duration of diabetes⁶¹⁻⁶³. Perhaps restoration of this central component with ExT may represent a critical early window for therapeutic intervention for this disease process.

Conclusion

In conclusion, we have demonstrated that erectile dysfunction in diabetes is partially due to a selective defect in the central NO mechanisms within the PVN. This defect is a result of a loss in the synthetic enzyme, nNOS for the production of NO within neurons of the PVN. ExT restores reduced levels of nNOS within the PVN in this study as well as other studies in rats with heart failure reported previously²⁴. Thus, ExT may be used as therapeutic modality to up-regulate nNOS within the PVN and consequently improve the central component of the erectile dysfunction in diabetes mellitus in humans.

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Figure 1. Effect of NMDA microinjections within the PVN on penile erection (A), yawning (B), stretching (C), grooming (D) and chewing (E) in the four groups of rats (control+sedentary, T1D+sedentary, control+ExT and T1D+ExT). Each value is the mean±SE. *P<0.05 with respect to control+sedentary group. # P<0.05 with respect to T1D+sedentary group.

Figure 2. Effect of SNP microinjections within the PVN on penile erection (A), yawning (B), stretching (C), grooming (D) and chewing (E) in the four groups of rats (control+sedentary, T1D+sedentary, control+ExT and T1D+ExT). Each value is the mean $\pm$ SE. * P <0.05 with respect to control+sedentary group. # P <0.05 with respect to T1D+sedentary group.

Figure 3.

A. Composite data of real time PCR of nNOS of punched PVN tissue in the four groups of rats. B. Representative Western blotting of nNOS protein and composite data of nNOS protein expression in the four groups of rats. * $P < 0.05$ with respect to control+sedentary group. # $P < 0.05$ with respect to T1D+sedentary group.

Figure 4.

A. NADPH-diaphorase labeled neurons in the PVN of four groups of rats (control +sedentary, T1D+sedentary, control+ExT and T1D+ExT). B. Summarized data of NADPH-positive cells in the PVN. * $P < 0.05$ with respect to control+sedentary group. # $P < 0.05$ with respect to T1D+sedentary group.

Table 1

Characteristics of control and diabetic rats in the experiments

	Control+sedentary	T1D+sedentary	Control+ExT	T1D+ExT
Body weight (g)	36±817 (n=10)	223±19* (n=10)	354±23 (n=7)	246±11* (n=7)
Glucose level (mg/100ml)	95±9 (n=10)	396±28* (n=10)	86±7 (n=7)	366±33* (n=7)
Testosterone level (ng/ml)	1.80±0.11 (n=6)	0.81±0.07* (n=6)	1.85±0.13 (n=6)	0.78±0.06* (n=6)
Citrate synthase (umol/g/min)	12.6±1.0 (n=10)	11.5±0.7 (n=10)	17.3±1.1 [#] (n=7)	16.8±1.4 [#] (n=7)

Values are presented as mean ± SE

* indicates P < 0.05 vs. control rats

[#] indicates P < 0.05 vs. sedentary rats