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Centrally Mediated Erectile Dysfunction in Rats with Type 1 Diabetes: Role of Angiotensin II and Superoxide

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Abstract

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

Aim—To determine the contribution of angiotensin (ANG) II in the dysfunction of central N-methyl-D-aspartic acid (NMDA)-nitric oxide (NO)-induced erectile responses in streptozotocin-induced type 1 diabetic (T1D) rats.

Methods—Three weeks after streptozotocin injections, rats were randomly treated with the angiotensin-converting enzyme inhibitor-enalapril, or the ANG II type 1 receptor blocker, losartan, or the superoxide dismutase mimetic, tempol or vehicle via chronic intracerebroventricular infusion by osmotic mini-pump for 2 weeks.

Main Outcome Measure—Central NMDA receptor stimulation or the administration of the NO donor, sodium nitroprusside (SNP)-induced penile erectile responses and concurrent behavioral responses were monitored in conscious rats.

Results—Two weeks of enalapril, losartan or tempol treatment significantly improved the erectile responses to central microinjection of both NMDA and SNP in the paraventricular nucleus (PVN) of conscious T1D rats (NMDA responses – T1D+enalapril: 1.7 ± 0.6, T1D+losartan: 2.0 ± 0.3, T1D+tempol: 2.0 ± 0.6 vs. T1D+vehicle: 0.6 ± 0.3 penile erections/rat in the first 20 min, $P < 0.05$; SNP responses – T1D+enalapril: 0.9 ± 0.3, T1D+losartan: 1.3 ± 0.3, T1D+tempol: 1.4 ± 0.4 vs. T1D+vehicle: 0.4 ± 0.2 penile erections/rat in the first 20 min, $P < 0.05$). Concurrent behavioral responses including yawning and stretching, induced by central NMDA and SNP microinjections were also significantly increased in T1D rats after enalapril, losartan or tempol treatments. Neuronal NO synthase expression within the PVN was also significantly increased and superoxide production was reduced in T1D rats after these treatments.

Conclusions—These data strongly support the contention that enhanced ANG II mechanism/s within the PVN of T1D rats contributes to the dysfunction of central NMDA-induced erectile responses in T1D rats via stimulation of superoxide.

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Keywords

type 1 diabetes; central nervous system; central mechanisms of penile erection; erectile dysfunction

Introduction

Sexual dysfunction is a well known consequence of diabetes mellitus in men[1, 2]. Approximately 35% to 75% of men with diabetes mellitus have erectile dysfunction[3]. It is generally accepted that different central and peripheral neural and/or humoral endocrine mechanisms participate in the regulation of the sexual response. Although there has been considerable advance in elucidating the peripheral component of the response, the central mechanisms remain relatively unexplored. With regard to the central mechanism/s, several neurotransmitters that control erectile function, including excitatory amino acid N-methyl-D-aspartic acid **(**NMDA) and nitric oxide (NO), have been identified[4–6]. These compounds act in several brain areas, including the paraventricular nucleus (PVN) of the hypothalamus[5, 6], which transmits this information to the genital organs via projections to the spinal cord.

The PVN is involved in numerous functions including feeding, metabolic balance, cardiovascular regulation, as well as erectile function and sexual behavior. In the PVN, NMDA-induced penile erection and yawning is mediated by an increased NO synthesis (NOS)[6]. Our previous study also demonstrated that penile erection occurred in response to administration of NMDA directly into the PVN. This response appeared to be linked to the release of NO within the PVN[7]. In streptozotocin (STZ)-induced type 1 diabetic (T1D) rats, the responses of penile erection, yawning and stretching induced by NMDA within the PVN were decreased[7]. These data suggest that the central component of the erectile response is blunted in T1D rats. We further observed that the level of neuronal NOS (nNOS) protein in the PVN was decreased in T1D rats. In T1D rats, restoration of nNOS with viral transfection within the PVN improved the behavioral responses (erection and yawning) mediated by NMDA. These data suggest an abnormal NO mechanism in the central nervous system, specifically within the PVN, may be involved in the altered erectile responses in rats with T1D.

The brain angiotensin (ANG) II system plays an important role in cardiovascular control[8], vasopressin secretion[9] and certain behaviors including thirst and salt appetite[10]. ANG II has been specifically identified as a neuromodulator in the central nervous system, and is known to be involved in neuroendocrine and autonomic function[11, 12]. ANG II type 1 (AT_1) receptors are known to be present on PVN neurons[13, 14]. Functional studies have shown that AT_1 receptors are involved in PVN-mediated autonomic outflow changes and consequent cardiovascular functions[15, 16]. Our previous data has shown an augmented AT_1 receptor function and expression in the PVN of T1D rats[17]. We also found that ANG II down-regulated nNOS expression in cultured neuronal cells[18]. This data corroborates and further supports the concept of a link between ANG II and NO signaling.

Oxidative stress-mediated neurovascular alteration at the level of the penis plays an integral role in the development of erectile dysfunction in the diabetic population[19]. The pathogenesis of diabetes-associated erectile dysfunction is related to the endothelial damage resulting from impaired NO production, which is closely related to oxidative stress[19]. In STZ-induced T1D rats, Jeremy *et al.* reported that superoxide ($O_2^{\bullet-}$) production was markedly elevated in the cavernosum[20]. Over-expressing superoxide dismutase (SOD) via adenoviral-mediated gene transfer results in increased bioavailability of NO by reducing corporal $O_2^{\bullet-}$ levels, and thus restoring erectile function in T1D rats[21]. In addition, NADPH oxidase inhibitor apocynin can ameliorate STZ-induced diabetes-related erectile dysfunction by reducing the reactive oxygen species (ROS) production and inhibiting the activity of RhoA/ROCK signaling pathway[22]. Interestingly, ANG II is known to induce endothelial dysfunction via the ROS, which can counteract the vasodilating, and vaso-protective effects of NO. It is thought that one of the mechanisms for restoration of erection function by ANG II blockade is the alleviation of endothelial dysfunction. Together, these previous studies focused on the peripheral mechanisms suggesting a link between ANG II, $O_2^{\bullet}$, and NO in mediating erectile dysfunction. However, the precise details for the role of central mechanism/s that contribute to erectile dysfunction during T1D remain to be examined.

Aims

The aims of the current study were to determine 1) whether central inhibition of angiotensin-converting enzyme (ACE) or ANG II AT_1 receptor improves NMDA-induced erectile responses in rats with T1D; 2) whether central administration of the SOD mimetic, tempol improves NMDA-induced erectile responses in rats with T1D; and 3) whether nNOS expression in the PVN was restored in rats with T1D after central enalapril, losartan or tempol treatments; 4) whether $O_2^{\bullet-}$ production in the PVN was reduced in rats with T1D after central enalapril, losartan or tempol treatments.

Methods

Animal and Treatment

This study was approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee and conforms to the guidelines for the care and use of laboratory animals according to the National Institutes of Health and the American Physiological Society. Male Sprague-Dawley rats (200–220 g, Sasco) were randomly injected with STZ (65mg/kg, i.p, in a 2 % solution of 0.1 M citrate buffer, Sigma, MO) to induce diabetes or vehicle (citrate buffer) for the control group. The percentage of animals that were diabetic after STZ injection was approximately, 85 %. Onset of diabetes was identified by polydipsia, polyuria, and blood glucose levels >250mg/dl. The mortality rate of the STZ rats was 15%. Rats that exhibited ruffled hair, poor appearance, vocalizations, and lack of appetite were considered to be of poor health and were euthanized. Body weight and blood glucose was monitored weekly. Experiments were performed 6–7 weeks after the injection of STZ or vehicle.

Three weeks after STZ or vehicle injections, rats were assigned to eight groups: control +vehicle (VE), T1D+VE, control+enalapril (ENL), T1D+ENL, control+losartan (LOS),

T1D+LOS, control+tempol (TEM), T1D+TEM, n=6–7/group. Experiments for erectile function and evaluation of nNOS expression were performed after 2 weeks of treatment with enalapril, losartan, tempol or vehicle. The total number of rats in the study was 130.

Chronic Intracerebroventricular (ICV) Infusion

Rats were anesthetized with a ketamine/xylazine mixture (90 mg/kg and 5 mg/kg, i.p.) and placed in a stereotaxic apparatus (Davis Kopf instruments, CA). After the bregma was identified, a sterile brain cannula using the Alzet Brain Infusion Kit II connected to an osmotic mini-pump (model 1003D, Alzet, CA) was inserted into the right lateral cerebral ventricle and fixed to the skull with dental cement. The periostia on both sides were sutured together to fasten the brain cannulae. The coordinates were determined from the Paxinos and Watson rat atlas[23], which were 0.8 mm posterior, 1.5 mm lateral to the bregma and 3.8 mm ventral to the 0 level. The brain cannulae, connecting catheter and mini-pump, was pre-filled with ACE inhibitor, enalapril maleate (0.5 mg/ml, Sigma, MO)[24], ANG II AT_1 receptor antagonist, losartan (2 mg/ml, Merck, NJ)[25, 26], SOD mimetic, tempol (50 mg/ml, Sigma, MO)[26], or artificial spinal fluid (aCSF, composition in mM: 132 NaCl, 3.0 KCl, 0.65 MgCl2, 1.5 CaCl2, 24.6 NaHCO3, and 3.3 glucose adjusted to pH 7.4) as vehicle. All drugs were dissolved in aCSF and infused at 1 μl/hr through the mini-pumps for 2 weeks.

Guide Cannula Implantation for PVN Microinjection

After 2 weeks of treatment, each animal was implanted with a stainless steel cannula aimed at the PVN as described previously[7]. The rats were anesthetized with a ketamine/xylazine mixture (90 mg/kg and 5 mg/kg, i.p.) and then placed in a stereotaxic apparatus. Previously implanted ICV cannula was removed prior to the implantation of the PVN cannula. The coordinates for the PVN were determined according to the atlas of Paxinos and Watson[23]. A small burr hole was made in the skull. A stainless steel guide cannula (500 μm outer diameter; Microdialysis AB, Solna, Sweden) was implanted stereotaxically at the following coordinates: 1.5 mm posterior to the bregma, 0.4 mm lateral to midline, and 7.8 mm ventral to the dura. Two stainless-steel anchoring screws were fixed to the skull, and the cannula was secured in place by acrylic dental cement. The animals were then returned to their cages and allowed to recover for 2–3 days.

Erectile Function Protocol

Three days after surgery, penile erection, yawning and stretching were induced by NMDA or sodium nitroprusside (SNP) microinjections in the PVN in 8 groups of freely moving, conscious rats (control+VE, T1D+VE, control+ENL, T1D+ENL, control+LOS, T1D+LOS, control+TEM, T1D+TEM, n=6-7/group). Rats were placed individually into a Plexiglas cage and injected with NMDA (50 ng, Millipore, MA) or SNP (50 ng, Sigma, MO) into the PVN unilaterally in a volume of 100 nL using microsyringe (0.5 μL, Hamilton 7000 series, NV), respectively. After microinjection, the tip of the needle was left in place for at least 30s in order to allow the spread of the injectate. The vehicle solution was aCSF. After NMDA or SNP injections, the rats were monitored to quantify the number of episodes of penile erection, yawning and stretching over 20 min intervals for the next 80 min. Penile erection, yawing and stretching episodes were scored by an observer blind to the treatment.

In separate groups of control and T1D (n=3/group), only aCSF (100 nL) was injected into the PVN as a vehicle control. aCSF injection into the PVN did not induce penile erection, yawning and stretching responses in both control and T1D rats.

Microdissection of the PVN

In separate groups of rats (control+VE, T1D+VE, control+ENL, T1D+ENL, control+LOS, T1D+LOS, control+TEM, T1D+TEM, n=5/group), after euthanasia by pentobarbital (150 mg/kg, i.p.), brains were removed and frozen on dry ice. Six serial coronal sections (100 μm/section) were cut through the hypothalamus at the level of the PVN with a cryostat. The PVN was punched out bilaterally with a blunt 18-gauge needle according to the Palkovits and Brownstein technique[27]. Totally, 12 punches for each brain were placed in 100 L of protein extraction buffer (10 mM Tris, 1 mM EDTA, 1% SDS, 0.1% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride), sonicated, and incubated for 30 min at 37°C to extract the protein.

Western Blot Analysis for nNOS Protein

The protein extraction was used for Western blot analysis of nNOS in samples obtained from the protocols above. The samples were loaded onto a 7.5% SDS-PAGE gel for electrophoresis. The fractionated proteins on the gel were electrophoretically transferred onto the polyvinylidene diflouoride membrane. The membrane was then incubated with primary antibody (rabbit anti-rat nNOS polyclonal antibody, 1:1,000, Santa Cruz Biotechnology, CA) overnight. Then, the membrane was incubated with secondary antibody (goat anti-rabbit IgG, peroxidase conjugated, 1:5,000, Thermo Scientific, IL) for 1 h. An enhanced chemiluminescence substrate (Thermo Scientific, IL) was applied to the membrane, followed by an exposure within an Epi Chemi II Darkroom (UVP BioImaging, CA) for visualization and capture of the images 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 relative to the intensity of the β tubulin band.

Dihydroethidium (DHE) Staining

In separate groups of rats (control+VE, T1D+VE, T1D+ENL, T1D+LOS, T1D+TEM, n=4/group), after euthanasia by pentobarbital (150 mg/kg, i.p.), brains were removed and frozen on dry ice. Serial coronal sections (30μm/section) were cut through the hypothalamus at the level of the PVN with a cryostat. To evaluate the $O_2^{\bullet -}$ production in the PVN, sections were incubated with DHE (2 μmol/L, Molecular Probes, CA) for 30 min in the dark at 37°C. Sections were evaluated under a fluorescent microscope (Leica, Germany) and digitally photographed. Openlab 4.0.3 (Improvision, MA) software was used to identify the total intensity of positive staining. Three alternate sections (1.5 ± 0.1 mm posterior to bregma) representing the PVN were analyzed, and then the mean data from these three images were calculated.

Data Analysis

Data are presented as mean ± SE. For comparison of the measured parameters in the four groups for each treatment (control+VE, T1D+VE, control+treatment and T1D+treatment),

two by two ANOVA analysis was performed. When F values were significant, Newman–Keuls test was applied to identify differences between individual groups. Statistical significance was defined as $P < 0.05$. Table 2 shows the F values for erection, yawning and stretching responses to NMDA and SNP microinjection in the first 20 min.

Main Outcome Measure

After 2 weeks of treatment, in freely moving conscious rats, penile erections induced by NMDA or SNP into the PVN were determined. nNOS expression and $O_2^{\bullet -}$ production in the PVN was also measured by using Western blot and DHE staining approaches.

RESULTS

General Data

After a total of 5 weeks, induction and treatment, rats with T1D showed significantly higher glucose level compared to the control group (398 ± 51 mg/dl vs. 112 ± 14 mg/dl, $P < 0.05$). Body weight was significantly lower in T1D rats compared with control rats (249 ± 28 g vs. 398 ± 37 g, $P < 0.05$). Enalapril, losartan or tempol treatments had no significant effects on the blood glucose levels or body weight in either control or T1D groups (Table 1).

Behavioral Responses to NMDA Administration

Three weeks after induction of diabetes plus 2 weeks of enalapril, losartan or tempol treatment, NMDA was microinjected into the PVN of conscious, freely moving rats. The erectile responses to central microinjection of NMDA in the PVN in the conscious animals were significantly improved in T1D rats (T1D+ENL: 1.7 ± 0.6, T1D+LOS: 2.0 ± 0.3, T1D +TEM: 2.0 ± 0.6 vs. T1D+VE: 0.6 ± 0.3 penile erection/rat in the first 20 min, $P < 0.05$; Figure 1). In T1D rats, the erectile responses to central microinjection of NMDA in the PVN were still lower than in control rats with treatments ($P < 0.05$). Concurrent behavioral responses including yawning and stretching, induced by central NMDA microinjection were also significantly increased in the first 20 min in T1D rats after enalapril, losartan or tempol treatments (Figure 2–3, compared to T1D+VE group $P < 0.05$). There was no significant difference between treated and untreated groups in gross behaviour, such as spontaneous locomotor activity.

Behavioral Responses to SNP Administration

We also compared the erectile, yawning and stretching responses to administration of an NO donor, SNP, into the PVN in these 8 groups of rats. After 2 weeks of enalapril, losartan or tempol treatment, the erectile responses to central microinjection of SNP in the PVN in conscious animals were significantly improved in T1D rats (T1D+ENL: 0.9 ± 0.3, T1D +LOS: 1.3 ± 0.3, T1D+TEM: 1.4 ± 0.4 vs. T1D+VE: 0.4 ± 0.2 penile erection/rat at 0-20 min, compared to T1D+VE group $P < 0.05$) (Figure 4). In T1D rats, the erectile responses to central microinjection of SNP in the PVN were still lower than in control rats with treatments ($P < 0.05$). Concurrent behavioral responses such as yawning and stretching, induced by central SNP microinjection were also significantly increased in the first 20 min of observation in T1D rats after enalapril, losartan or tempol treatments (Figure 5–6, compared to T1D+VE group $P < 0.05$).

Expression of nNOS Protein within the PVN

nNOS protein expression, measured by Western blot, is shown in Figure 7. Sample gels showing nNOS and β tubulin protein in the eight experimental groups are presented in Figure 7A. The level of nNOS protein expression in the T1D+VE group was significantly lower than in the control+VE group (relative protein level 0.39 ± 0.10 vs. 0.76 ± 0.13, $P < 0.05$). In the T1D+ENL (0.63 ± 0.08), T1D+LOS (0.72 ± 0.20), T1D+TEM (0.76 ± 0.11) group, nNOS protein expression was significantly higher than in the T1D+VE group (0.39 ± 0.10, $P < 0.05$) and was not significantly different from the control group (0.76 ± 0.13, $P > 0.05$) (Figure 7).

$O_2^{\bullet-}$ Production in the PVN

To provide direct evidence that there was a decrease in intracellular $O_2^{\bullet-}$ levels in the central nervous system of diabetic rats after the three treatments, DHE fluorescence staining was used to estimate $O_2^{\bullet-}$ levels in the PVN of control and diabetic rats, with/without treatment (Figure 8). Quantitative analyses (Figure 8B) showed that there was significant decrease in T1D+ENL (relative DHE density 2.0 ± 0.2), T1D+LOS (1.8 ± 0.4) and T1D +TEM (1.2 ± 0.4) relative to basal fluorescence observed in T1D+VE group (3.2 ± 0.7, $P < 0.05$) and was not different from the control group (1.2 ± 0.4, $P > 0.05$) (Figure 8).

Discussion

The present study shows that 2 weeks of central administration of enalapril, losartan or tempol treatment, significantly improved the erectile responses to microinjection of NMDA and SNP in the PVN in conscious T1D rats. Accompanying behavioral responses such as yawning and stretching induced by central NMDA and SNP microinjections were also significantly increased in T1D rats after central enalapril, losartan or tempol treatments. Concomitant with these behavioral changes, expression of nNOS protein within the PVN was also significantly increased in T1D rats after enalapril, losartan or tempol treatments. The $O_2^{\bullet-}$ production within the PVN was also significantly reduced in T1D rats after central enalapril, losartan or tempol treatments. These data support the contention that altered nNOS mechanism/s within the PVN, possibly mediated by an enhanced ANG II AT_1 receptor mechanism, by stimulation of $O_2^{\bullet-}$, contributes to centrally-induced erectile dysfunction in T1D rats.

As the main active product of the renin-angiotensin system, in the periphery, ANG II has been shown to play a role in the regulation of cavernosal tone. An intracavernosal injection of ANG II can terminate erection in anesthetized dogs[28]. It has been suggested that specificity in systemic hemodynamic control by ANG II is also critical in establishing the optimal erectile environment in rats[29]. Furthermore, ANG II regulates vascular tone by counteracting the effects of NO, the principal mediator of penile erection. Recent results from clinical and experimental studies, show that treatment with ACE inhibitors in hypertensive patients and rats is able to recover/improve erectile function[30].

Our previous study suggested that there is an enhanced ANG II system in T1D[17]. T1D rats have higher plasma ANG II compared to non-T1D rats. In the PVN, T1D rats show an

increased AT_1 receptor mRNA and protein expression[17]. This provides evidence indicating that the central ANG II system is altered in T1D rats. There is a positive relationship between ANG II and NO. Acute administration of ANG II has been shown to activate NOS and increase NO production in various systems[31, 32] including the PVN[33]. However, chronically the interaction between ANG II and NO appears to be organized at the cellular level in such a way as to constitute a mutually inhibitory influence, in which, ANG II causes a decrease in nNOS gene expression and vice versa[34, 35]. Using a neuronal cell line, we have previously shown that ANG II down-regulates nNOS expression in vitro[18]. In the present study, we compared the effects of chronic central administration of the ACE inhibitor, enalapril and the ANG II AT_1 receptor inhibitor, losartan on the NMDA-induced erectile responses between control and T1D groups. These chronic treatments improved the erectile as well as the behavioral responses in TID rats. We have further shown that ACE and ANG II AT_1 receptor inhibitors restored the endogenous levels of nNOS expression in the PVN of T1D rats. The results confirm the potential influence of the ANG II system (via ACE, ANG II AT_1 receptors) on erectile dysfunction and NO mechanism/s within the PVN of T1D rats.

It has been known for the past several years that ANG II is a potent stimulator of ROS[36]. In the periphery, at the level of the penis, it is thought that one of the mechanisms for restoration of erectile dysfunction in diabetes by ANG II blockers, is the alleviation of endothelial dysfunction. Further, ANG II is known to induce endothelial dysfunction via the increased production of ROS. The increased generation of ROS is thought to be responsible for NO degradation because it mediates the uncoupling of NO synthesis and inactivates NO via peroxynitrite formation. In the brain, it has been shown that ANG II signaling is mediated by ROS, particularly $O_2^{\bullet-}$, produced from NADPH oxidase[37]. However, virtually nothing is known regarding ANG II-induced activation of NAD(P)H oxidase and subsequent $O_2^{\bullet-}$ production on the neural control of erectile dysfunction. In the present study, we found ACE and ANG II AT_1 receptor inhibitors reduced $O_2^{\bullet-}$ production in the PVN, suggesting ANG II via AT_1 receptors mediate $O_2^{\bullet-}$ signaling which may be involved in the erectile dysfunction observed in diabetes.

Previously, we observed an increase in NAD(P)H oxidase and $O_2^{\bullet-}$ levels with concomitant decrease in CuZnSOD levels in the PVN of rats that were diabetic for 6 weeks[17]. Further, the AT_1 receptor antagonist losartan reduced NAD(P)H oxidase subunit expression, and $O_2^{\bullet-}$ in the PVN of T1D rats. Considering $O_2^{\bullet-}$ reacts with NO in a diffusion-limited fashion, we speculated that increased $O_2^{\bullet-}$ levels might contribute to the impaired erectile function in T1D by diminishing the bioavailability of NO. We also found that CuZnSOD protein in the PVN was less in T1D than in non-T1D rats, suggesting that this antioxidant enzyme is deficient in the PVN from T1D rats[17]. As a SOD mimetic agent, tempol has been shown to decrease excitatory effect of ANG II in the PVN. In the present study, we have shown that tempol has similar effects on the erectile response. This is because tempol, a SOD by mimetic agent, enhances $O_2^{\bullet-}$ scavenging and reduces the excitatory effects of ANG II in the PVN. These data provide crucial evidence for the involvement of $O_2^{\bullet-}$ in the erectile dysfunction during T1D. Increased ANG II levels in diabetes increases $O_2^{\bullet-}$ levels in the PVN, that may contribute to the reduced bioavailable NO leading to impaired centrally mediated erectile responses in T1D. These data provide a novel mechanistic insight for erectile dysfunction commonly seen in diabetes.

Although the present data suggest that in T1D rats ANG II activity is increased leading in turn to an increased $O_2^{\bullet-}$ production that decreases NO content in the PVN, other possibilities cannot be ruled out. For instance, the improvements in penile erection and yawning induced by NMDA injected into the PVN after the chronic administration of enalapril, losartan or tempol may be due to a direct effect of these drugs on NMDA receptors, i.e., changes in the number or affinity of NMDA receptors in the PVN. Previously we have observed that ANG II treatment stimulated NMDA NR_1 protein expression and losartan significantly ameliorated the NR_1 expression induced by ANG II in a neuronal culture model[38]. This would support the hypothesis that chronic administration of enalapril, losartan or tempol may have their effect due to a direct effect of these ANG II blockers on NMDA receptors.

Furthermore, excitatory amino acids are not the only substances that induce penile erection when injected into the PVN, there are several other neurotransmitters that could also change after enalapril, losartan or tempol treatments in diabetes, such as oxytocin, dopamine, GABA, NPY. All these neurotransmitters influence the pro-erectile activity and could change the penile erection[39–41]. It would be of interest to examine if enalapril, losartan or tempol influence other neurotransmitters involved in the pro-erectile activity in this model of diabetes in the future.

Long-term complications of diabetes have been ascribed to both the effects of prolonged hyperglycemia and increased oxidative stress[42, 43]. The Diabetic Control and Complications Trial (DCCT) has established the importance of hyperglycemia and other consequences of insulin deficiency in the pathogenesis of diabetic autonomic dysfunction. Hyperglycemia has been implicated in the etiology of diabetic neuropathy. It has become apparent that in insulin-deficient conditions, such as T1D, both insulin and insulinomimetic C-peptide must be replaced in order to gain hyperglycemic control and to combat complications[44, 45]. It has been found that in response to chronic hyperglycemia, the activity of ANG II is significantly up-regulated in the peripheral tissue[46]. High glucose stimulates angiotensinogen synthesis and ROS generation in renal tubular cells[47]. In light of this evidence, and the data presented in this manuscript, it is important to amalgamate the changes in the central nervous system with those in the periphery by further investigations.

Conclusion

Previously, we have demonstrated that erectile dysfunction in diabetes is partially due to a selective defect in the central nNOS mechanism, specifically, within the PVN[7]. The present set of experiments validate and support the hypothesis that enhanced ANG II AT_1 mechanisms via stimulation of $O_2^{\bullet-}$ contributes to centrally induced erectile dysfunction in T1D rats. Thus, any and all mechanisms that up-regulate nNOS within the PVN, such as ANG II inhibitors and superoxide dismutase may be used as therapeutic modalities to consequently improve the central component of the erectile dysfunction in diabetes mellitus in humans.

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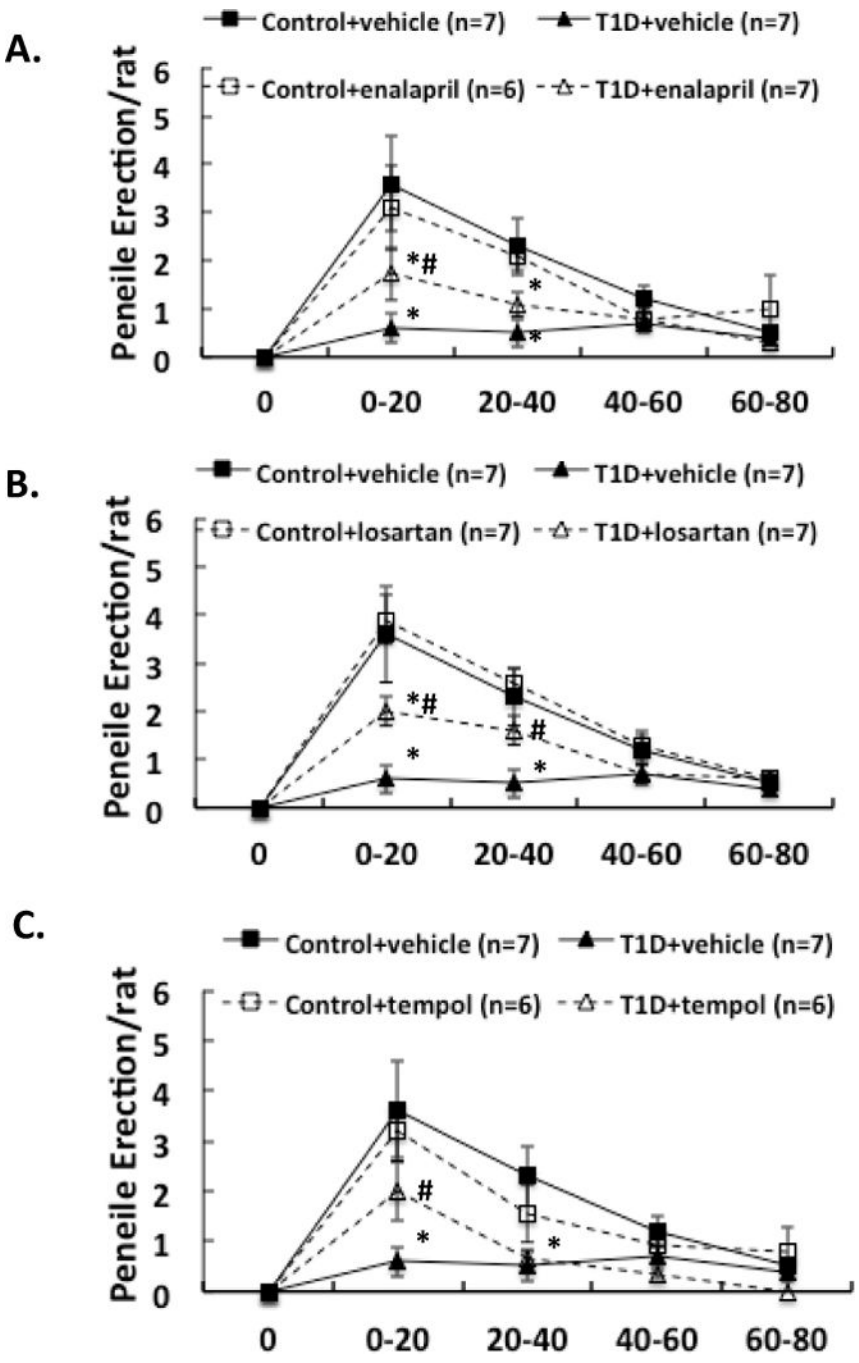

Figure 1.

Effect of NMDA microinjections in the PVN on penile erection in the enalapril (A), losartan (B) and tempol (C) treatment groups of rats. Each value is the mean ± SE. *P < 0.05 with respect to control+vehicle group. #P<0.05 with respect to T1D+vehicle group.

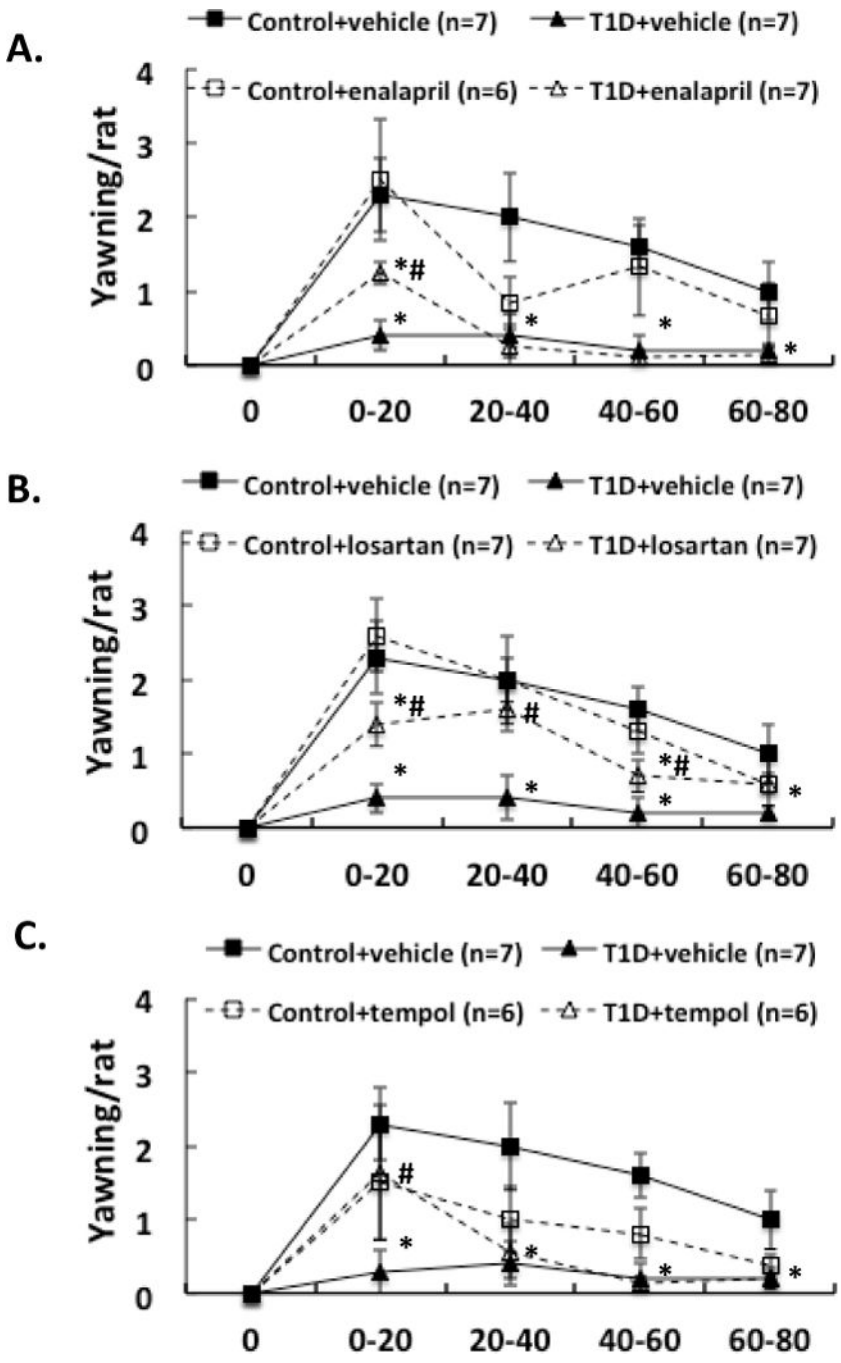

Figure 2.

Effect of NMDA microinjections in the PVN on yawning in the enalapril (A), losartan (B) and tempol (C) treatment groups of rats. Each value is the mean±SE. *P < 0.05 with respect to control+vehicle group. #P < 0.05 with respect to T1D+vehicle group.

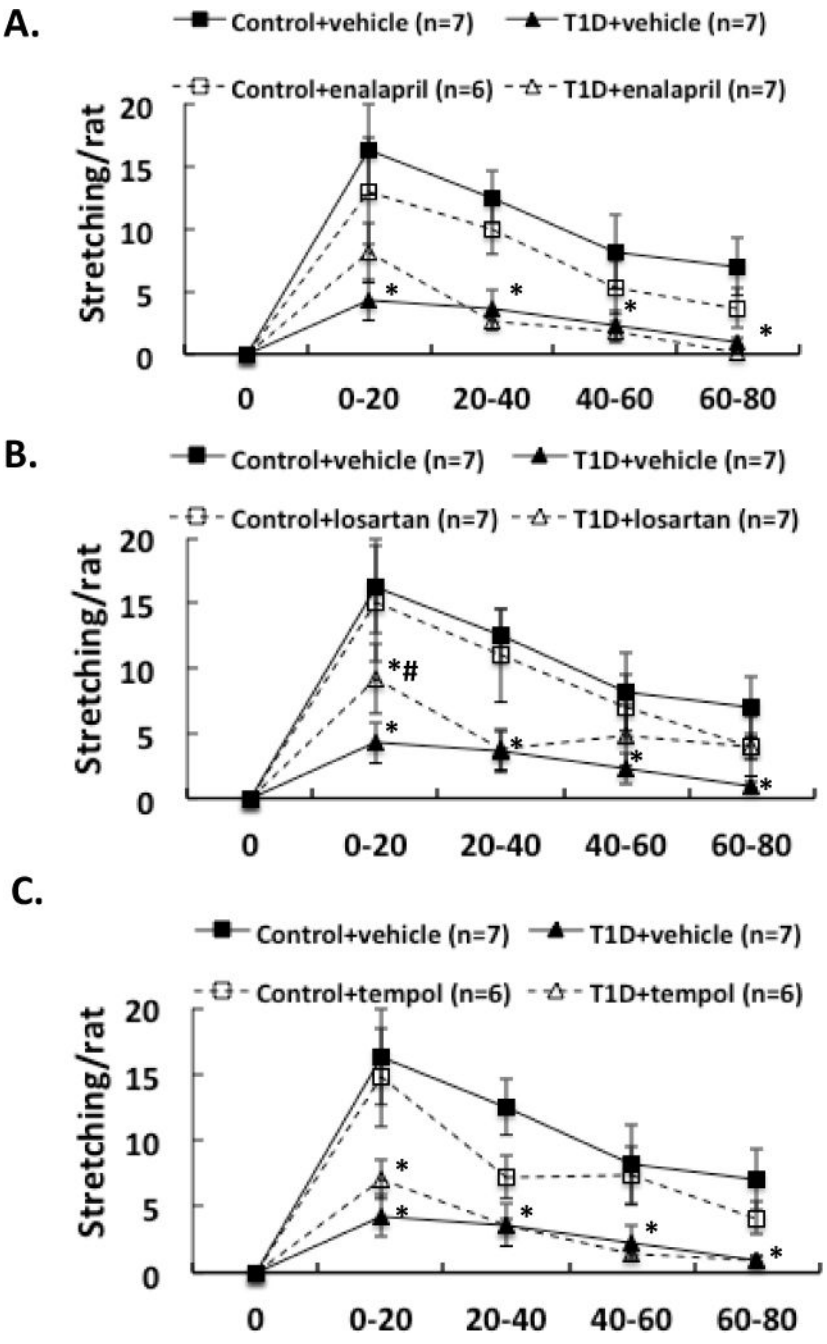

Figure 3.

Effect of NMDA microinjections in the PVN on stretching in the enalapril (A), losartan (B) and tempol (C) treatment groups of rats. Each value is the mean ± SE. *P < 0.05 with respect to control+vehicle group. #P < 0.05 with respect to T1D+vehicle group.

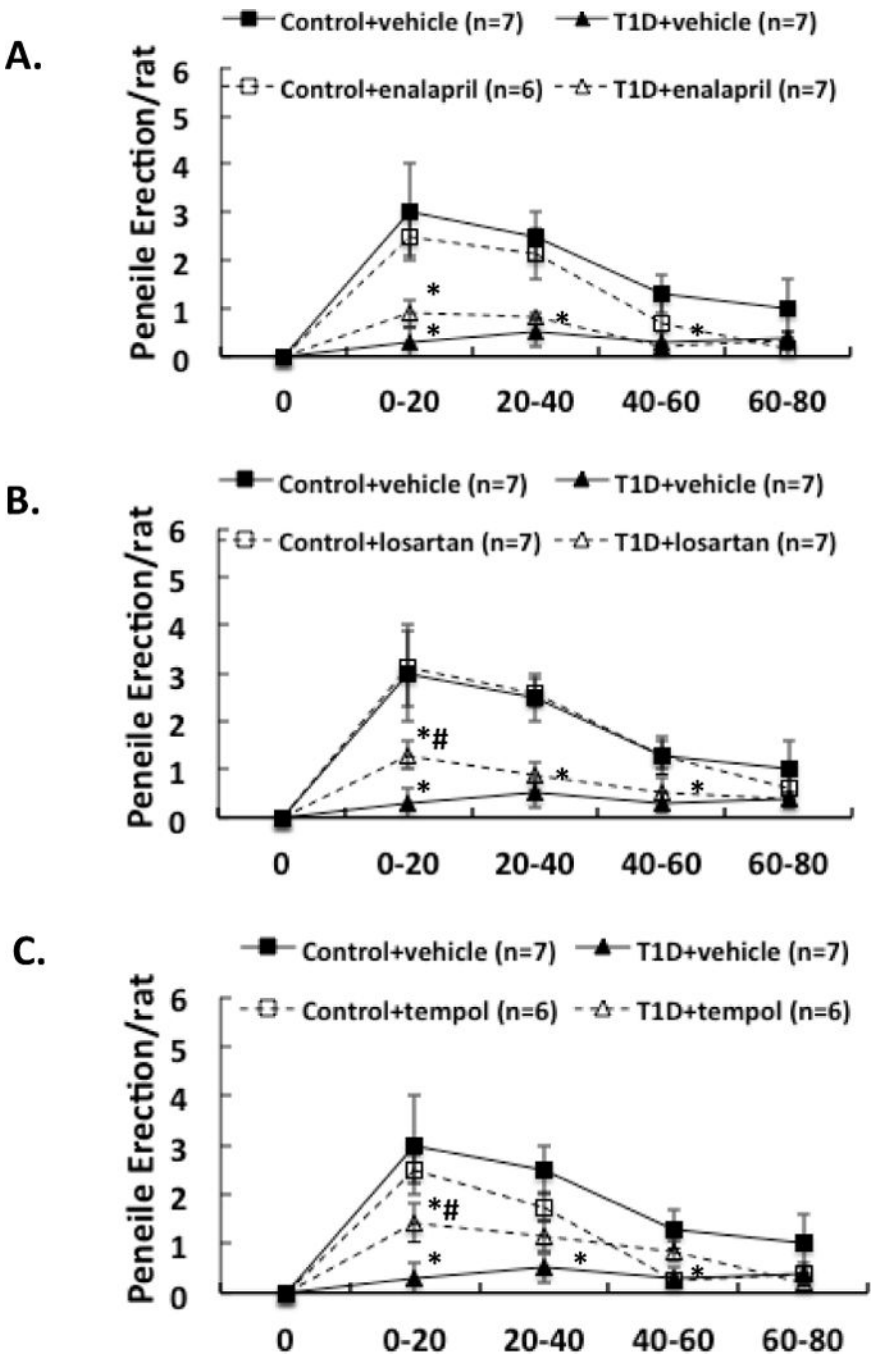

Figure 4.

Effect of SNP microinjections in the PVN on penile erection in the enalapril (A), losartan (B) and tempol (C) treatment groups of rats. Each value is the meanSE. *P < 0.05 with respect to control+vehicle group. #P < 0.05 with respect to T1D+vehicle group.

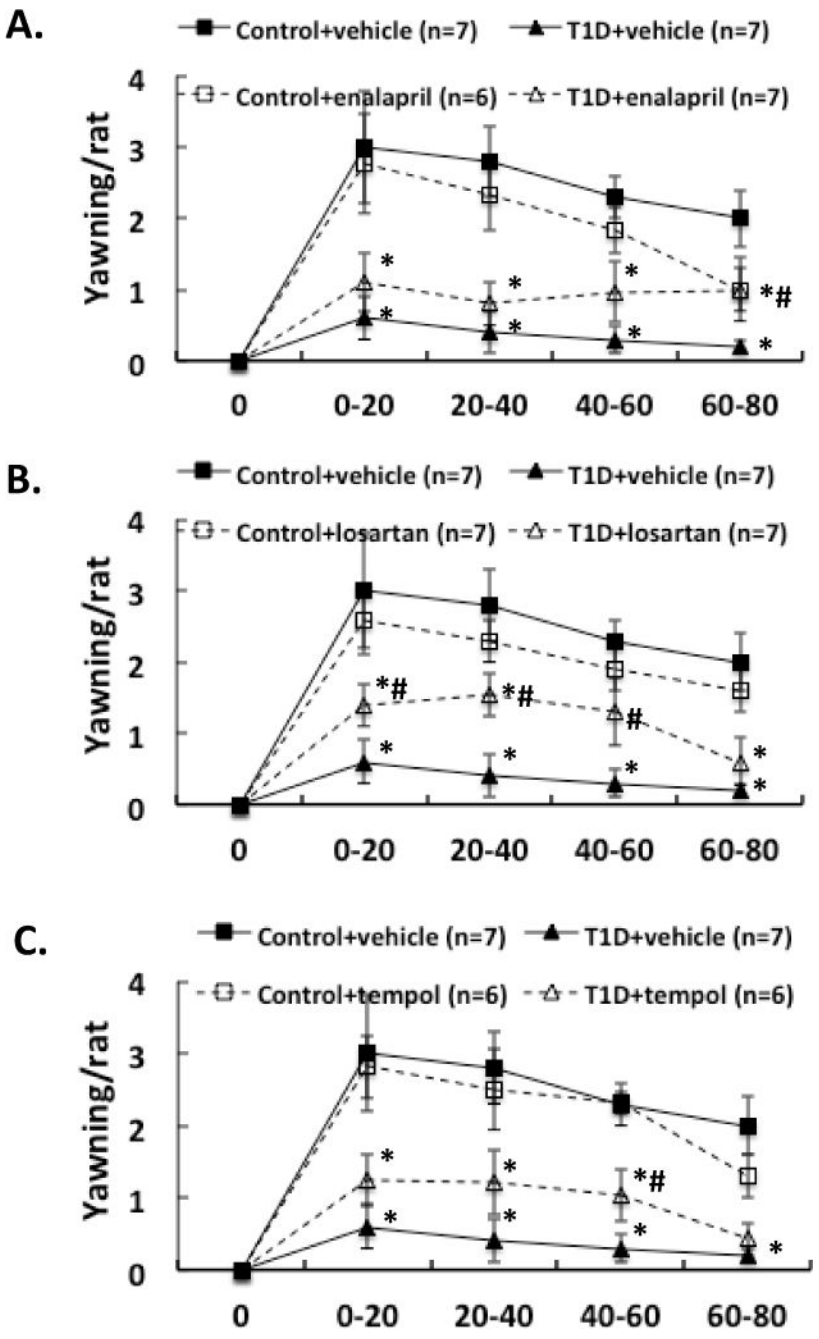

Figure 5.

Effect of NMDA microinjections in the PVN on yawning in the enalapril (A), losartan (B) and tempol (C) treatment groups of rats. Each value is the mean±SE. *P < 0.05 with respect to control+vehicle group. #P < 0.05 with respect to T1D+vehicle group.

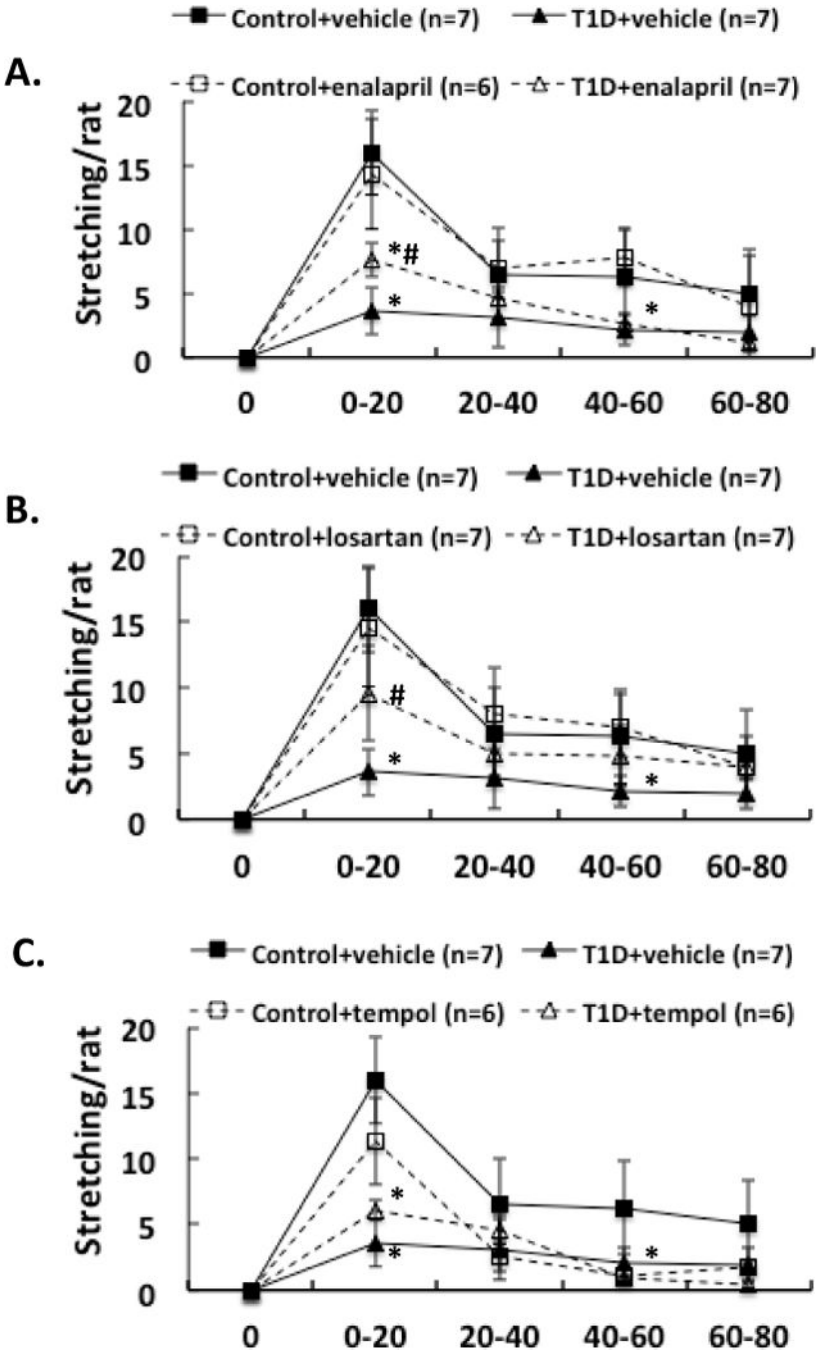

Figure 6.

A. Effect of NMDA microinjections in the PVN on stretching in the enalapril (A), losartan (B) and tempol (C) treatment groups of rats. Each value is the mean±SE. *P < 0.05 with respect to control+vehicle group. # P < 0.05 with respect to T1D+vehicle group.

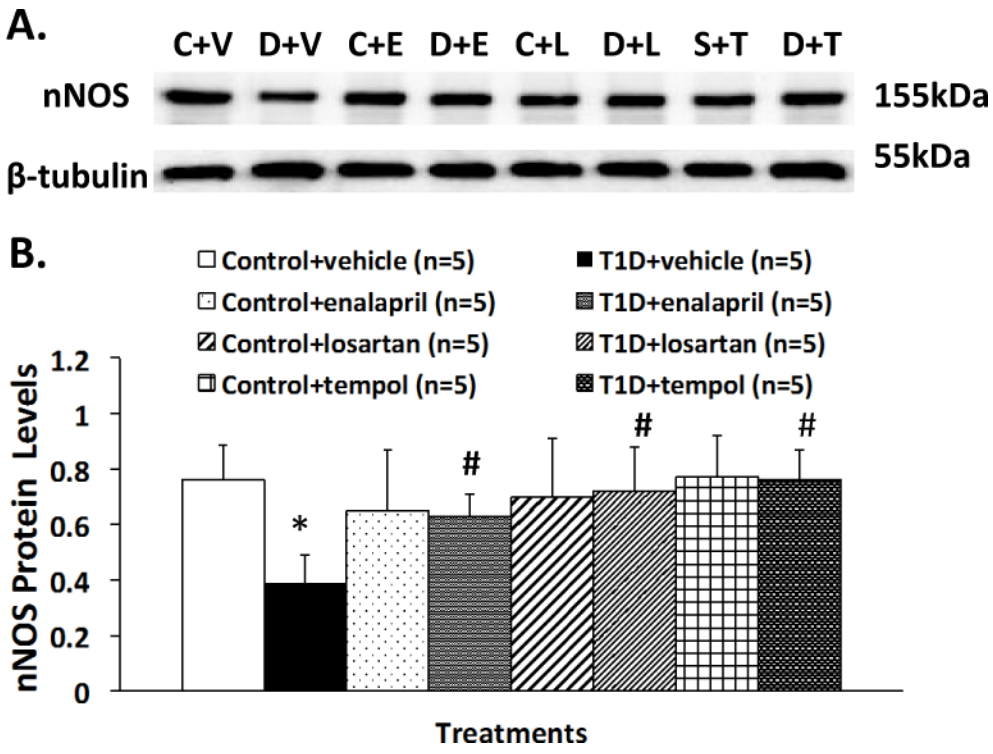

Figure 7.

Representative Western blotting of nNOS protein (A) and composite data of nNOS protein expression (B) of PVN in the eight groups of rats. *P < 0.05 with respect to control+vehicle group. #P < 0.05 with respect to T1D+vehicle group. C: control; V: vehicle; E: enalapril; L: losartan; T: tempol.

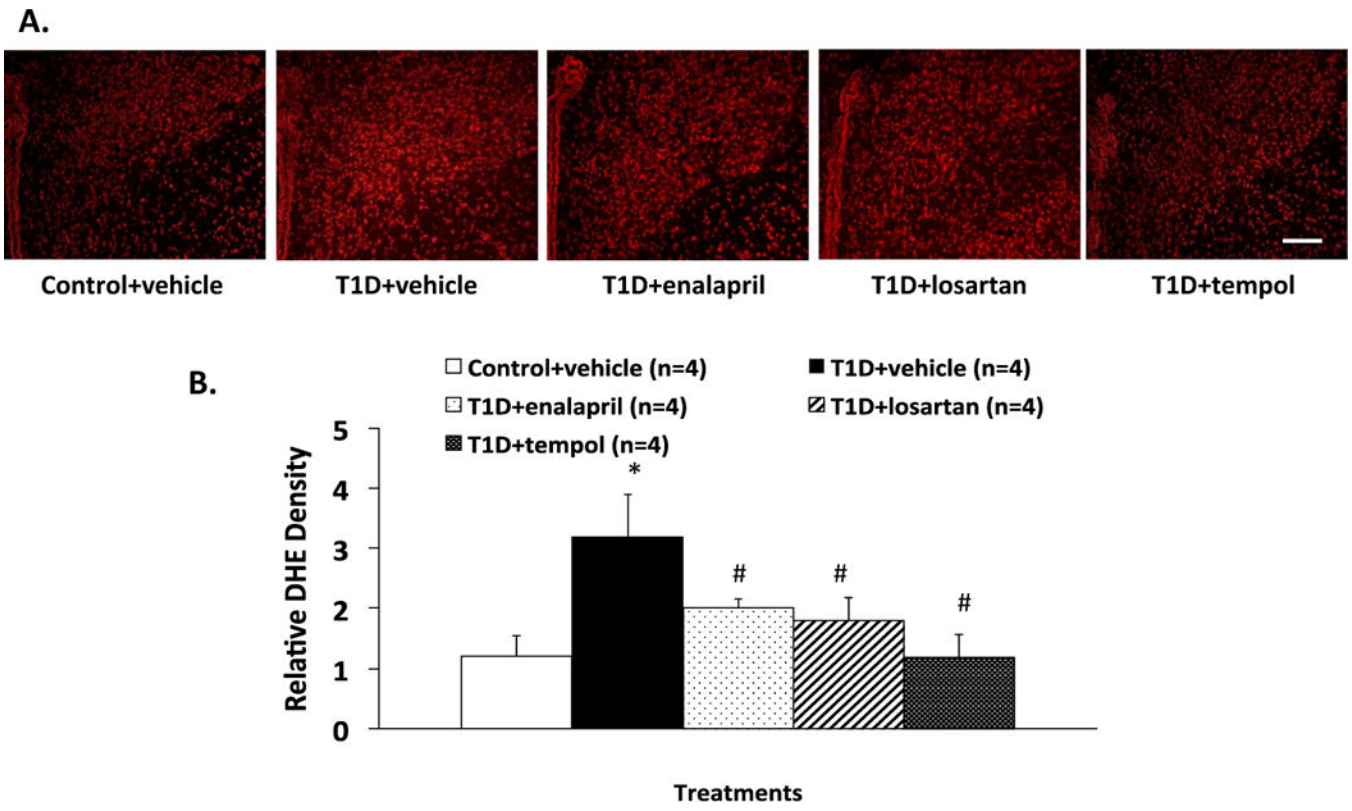

Figure 8.

Representative DHE staining of PVN (A) and composite data of (B) in the five groups of rats. *P < 0.05 with respect to control+vehicle group. #P < 0.05 with respect to T1D+vehicle group. Scale bar=100 μm.

Table 1

Characteristics of control and diabetic rats

	Body weight (g)	Blood glucose (mg/dl)
Control+vehicle (n=12)	398 ± 37	112 ± 14
T1D+vehicle (n=12)	249 ± 28*	398 ± 51*
Control+enalapril (n=12)	379 ± 26	106 ± 14
T1D+enalapril (n=12)	255 ± 36*	374 ± 33*
Control+losartan (n=12)	377 ± 29	101 ± 9
T1D+losartan (n=12)	257 ± 29*	357 ± 44*
Control+tempol (n=12)	377 ± 24	108 ± 7
T1D+tempol (n=12)	248 ± 29*	355 ± 19*

Values are presented as mean±SE;

* indicates $P < 0.05$ vs. control rats

Table 2

F values for erection, yawning and stretching responses to NMDA and SNP microinjection in the first 20 min

	Erection	Yawning	Stretching
NMDA-induced responses			
Enalapril treatment	*F=4.7 (p=0.010)*	*F=6.2 (p=0.003)*	*F=5.8 (p=0.004)*
Losartan treatment	*F=8.2 (p=0.001)*	*F=6.7 (p=0.002)*	*F=5.5 (p=0.005)*
Tempol treatment	*F=6.4 (p=0.003)*	*F=4.2 (p=0.016)*	*F=6.0 (p=0.003)*
SNP-induced responses			
Enalapril treatment	*F=5.4 (p=0.006)*	*F=6.0 (p=0.003)*	*F=5.2 (p=0.067)*
Losartan treatment	*F=3.9 (p=0.020)*	*F=5.9 (p=0.004)*	*F=4.1 (p=0.018)*
Tempol treatment	*F=5.0 (p=0.008)*	*F=6.0 (p=0.003)*	*F=6.6 (p=0.002)*