Dexmedetomidine Ameliorates Diabetic Neuropathic Pain in Streptozotocin (STZ)-Induced Diabetic Rat Model

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Abstract

Background: Diabetic neuropathy pain (DNP) is one of the most common complications of diabetes mellitus. It is associated with spontaneous pain, hyperalgesia and allodynia and greatly distresses the patients and compromises their quality of life. Dexmedetomidine (DEX) is a selective and potent α2- adrenoceptor agonist which has ananalgesic effect, reducessympathetic nervous tension and decreases release of glutamate. Thus, dexmedetomidine can be used in the treatment of DNP.

Objectives: We investigated the effect of dexmedetomidine on blood glucose level and neuropathic pain by using streptozotocin (STZ)-induced diabetic rat model.

Materials and Methods: Twenty-four adult male Wistar Albino rats were divided into three groups: vehicle group, streptozotocin group (STZ group) and dexmedetomidine plus streptozotocin group (STZ+DEX group).

Results: It was found that rats injected withSTZ only had a decreased pain threshold compared to the vehicle group and this effect was ameliorated by dexmedetomidine administration.

Conclusion: The current data suggest that dexmedetomidine can ameliorate hyperalgesia in diabetic neuropathy.

Keywords: Diabetic neuropathy pain, dexmedetomidine, streptozotocin.

Introduction

Diabetes mellitus (DM) is a chronic health problem characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The hyperglycemia of diabetes is associated with damage and dysfunction failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels **(American Diabetes Association, 2010)**.Diabetes is not a single disease, but it is group of heterogeneous syndromes such as heart attack, stroke, and peripheral vascular disease **(Patel et al., 2011)**.Diabetes prevalence has increased rapidly since there were 382 million people suffering from diabetes in the world in 2013, and the number of such patients is estimated to reach 592 million by 2035 i.e. an increase of 55% **(WHO Bulletin, 2016).**

Diabetic peripheral neuropathy (DPN) is a common complication of diabetes, it is characterized by spontaneous pain, hyperalgesia and allodynia and greatly compromising the life quality of diabetic patients **(Lu et al., 2017; Niu et al., 2017)**. The long-term high blood glucose level causes ectopic discharge and activation of the sympathetic nervous system andleads to peripheral nerves damage, which plays an important role in the

cause and persistence of DNP **(Lu et al., 2017)**.

Incidences of diabetic neuropathy have very high prevalence (50-60 %) in patients associated with both types of diabetes **(Bhattacharya et al., 2018)**. Up to date, tight glycemic control is the only way to prevent or delay the development of neuropathy in patients with type 1 diabetes and to reduce the progression of neuropathy in patients with type 2 diabetes **(Ang et al., 2014)**.

Dexmedetomidine (DEX) is a selective and potent α2- adrenoceptor agonist was approved by the US Food and Drug Administration (FDA) in 1999 for sedation of patients hospitalized in intensive care units **(Gertler et al., 2001)**. It is more selective on α2 than on α1 in ratio of 1:1600 that causes a dose-dependent hypnotic state in rats via a central mechanism.Dexmedetomidine has also analgesic, sympatholytic, and hemodynamic stabilizing properties **(Kim et al., 2018)**. In addition, it relieves sympathetic nervous tension and thus indirectly improves vagus nerve tension and reduces the release of glutamate **(Lu et al., 2017)**. Despite its increased clinical use in critically ill patients, the effects of dexmedetomidine in DM have not yet been investigated completely. In this study, we examine the effect of dexmedetomidine on blood glucose level and the mechanical withdrawal threshold in streptozotocin (STZ)-induced diabetic rat model.

Materials and methods

Animals and Experimental Groups

Male Wistar Albino rats (150-200 g) were obtained from the animal house of Faculty of Medicine, Cairo University, Egypt. All procedures described in the study were conducted in accordance with the Guide for the Care and Use of Laboratory Animals **(Bayne, 1996)** and were approved by the Ethical Committee for Scientific Research at the Faculty of Medicine, South Valley University, Qena, Egypt.

Rats were individually housed at a room temperature of 23-25°C with free access to food and water. A total of 24 rats were randomly divided into three groups (8 rats per group) as follow:

Group 1 (Vehicle group):
Adult rats are injected intraperitoneally with citrate buffer equal to streptozotocin volume/kg injected in the STZ groups.

Group 2 (Streptozotocin group):
Adult rats subjected to single intraperitoneal injection of streptozotocin.

Group 3 (STZ + DEX group)
Dexmedetomidine hydrochloride was administrated via intraperitoneal route at the 5th week after injection of streptozotocin. Dexmedetomidine hydrochloride 100 µg/kg, (Precedex 100 µg/2 ml, Abbott®, Abbott Laboratory, North Chicago, Illinois, USA).

The rat model of diabetes was performed as previously described **(Chen and Pan, 2003)**.Food was removed from rats for 16 h while water was replaced by glucose prior to injectionof streptozotocin. STZ and STZ+ DEXgroups were injected with a single intraperitoneal injection of freshly prepared streptozotocin (STZ; Sigma, MO, U.S.A.) in a dose of 45 mg/kg dissolved in 2.5 mM sodium citrate buffer (pH 4.5)

To prevent hypoglycemia induced by streptozotocin, rats received 10% dextrose solution after 6 h of streptozotocin administration for the next 24 h **(Sabitha et al., 2011)**.

Rats were kept alive 4 weeks after streptozotocin injection to allow development of chronic diabetes before they were exposed to dexmedetomidine. Blood glucose level (non-fasting) was determined using tail blood samples 72 h after STZ administration and 30-60 min after DEX administration using glucometer (AccuChek Sensor Comfort (Roche Diagnostics, Berlin, Germany)) to assess hyperglycemia. Rats with blood glucose concentration of 250 mg/dl or above were considered diabetic. Blood glucose level (non-fasting) and body weight were checked once a week throughout the study duration.

The paw pressure (PP) test

Nociceptive threshold was evaluated using a paw pressure (PP) test (Randall-Selitto test, UgoBasile). Rats were restrainedgently with a towel surrounding the trunk to keep them calm. Incremental pressure was applied during the experiments using 7200 Analgesy-Meter onto the dorsal surface of the hind paw that is placed on a small plinth under a cone-shaped pusher with a rounded tip. A force is exerted. It increases at a regular rate (a certain number of grams/second). This force is measured by a pointer which move on a linear scale. When the animal strives, the pedal is removed and determine at which the animal felt pain on the scale is determined. The slide is returned, after each test, to the start pointby moving it to the left. The cut-off pressure was 200g to avoid damaging the tissue. The intensity of the pressure which result in struggle reaction was defined as the pain threshold (measured in grams). The PP test was conducted 30min after dexmedetomidine administration. Measurements were performed six times at about 90-sec intervals and the mean value was taken as the threshold. The nociceptive thresholds correspond with the maximal pressure (expressed in grams) tolerated by the animal. In order to avoid tissue damage, only a trial was performed at each time **(Bianchi and Panerai, 1996)**.

Statistical analysis

Data are expressed as means ± SEM and were analyzed using two-way analysis of variance (ANOVA) followed by post-hoc comparisons. P values <0.05 were considered significant (Field, 2013).

Results

Dexmedetomidine significantly decreased blood glucose level in streptozotocin (STZ)-induced diabetic rats

After injection of streptozotocin, all rats in STZand STZ+ DEXgroups showed polydipsia, polyphagia and polyuria comparedwith the vehicle group. Compared with the vehicle group, these rats had significant increase in blood glucose levels. Dexmedetomidine was administrated at the 5th week after injection of streptozotocin, blood glucose level was significantly decreased in rats injected with DEX compared with STZ group (P<0.001, Table 1 and Figure 1).

Table 1.Means, standard error of mean and two-way analysis of variance for change in blood glucose levels in each group over 5 weeks. STZ: streptozotocin, DEX: Dexmedetomidine.

Week	Groups n=8 in each group		
	Vehicle	STZ	STZ + DEX
1	111.30 ± 2.47	581.3±9.15	460.8±23.79
2	108.8±2.06	570±20.7	448.8±4.41
3	114.8±1.8	546.3±10.68	461.5±7.14
4	123.8±5.88	572.5±8.18	477.5±29.32
5	119±3.97	527.5±8.18	449±12.65

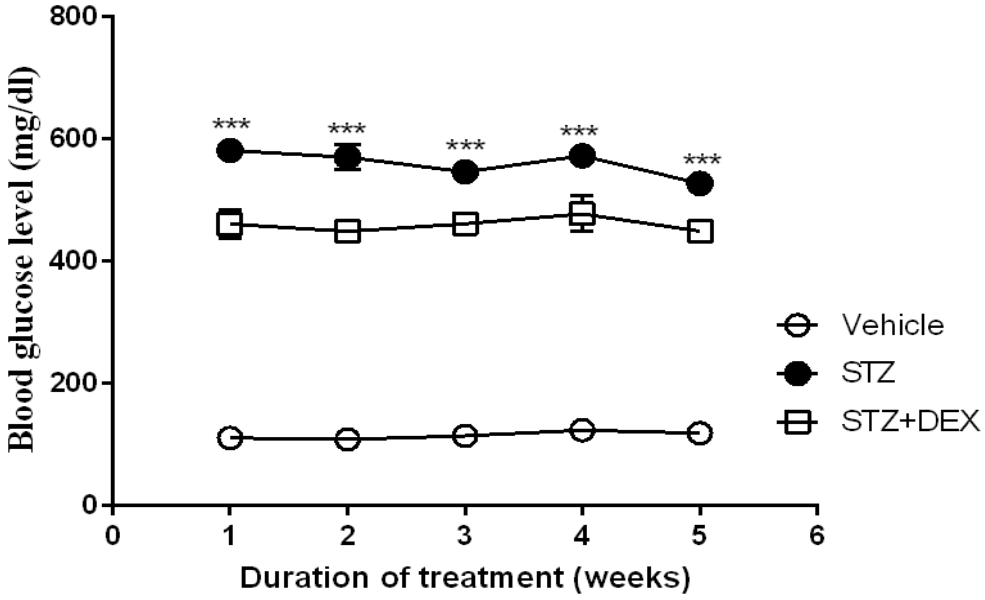

Fig.1. Difference in blood glucose levels in rats. Groups are vehicle, streptozotocin (STZ), streptozotocin+dexmedetomidine (STZ+DEX). n=8 for each group. ***P < 0.001 for comparisons shown.

Dexmedetomidine increases pain threshold in streptozotocin (STZ)-induced diabetic rats

Mechanical withdrawal threshold (MWT) (Randal Sellito test) was performed over six-time intervals in minutes (0, 15, 30, 45, 60, and 90) in all groups.Compared with the vehicle group, pain threshold was significantly decreased in STZ groupat all-time points (P<0.001, Fig 2 and Table 2). Compared with the STZ group, pain threshold was significantlyincreased in STZ+DEX group at all-time points (P<0.001, Table 2 and Figure 2).

Table 2.Means, standard error of mean and two-way Analysis of Variance for the mechanical withdrawal threshold in each group over six-time intervals. STZ: streptozotocin, DEX: Dexmedetomidine.

Time (min)	Groups n=8 in each group		
	Vehicle	**STZ**	**STZ + DEX**
0	233.1±2.66	237.5±2.99	238.1±2.83
15	215±3.54	126.3±2.95	142.5±3.41
30	222.5±2.84	117.5±2.11	134.4±3.17
45	219.4±2.75	114.4±1.75	122.9±2.08
60	211.3±3.50	105.6±2.75	121.9±2.83
90	201.9±3.27	113.8±4.30	114.0±2.49

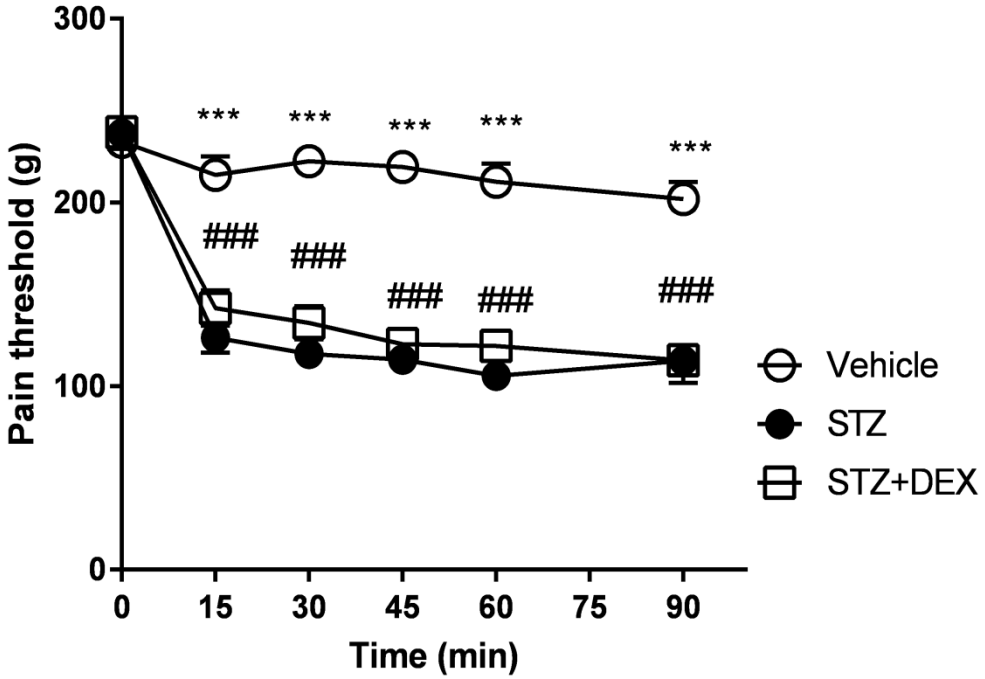

Fig.2. Difference in mechanical withdrawal threshold in rats. Groups are vehicle, streptozotocin (STZ), streptozotocin+dexmedetomidine (STZ+DEX). n=8 for each group. ***P < 0.001 STZ versus Vehicle. ###P < 0.001 STZ+DEX versus STZ.

Discussion

Diabetic neuropathy (DN) is a serious, chronic complication of diabetes with numerous clinical manifestations. Neuropathic pain is very disturbing and interferes with the physical activity, quality of life **(Pop-Busui et al., 2017)**. In addition to foot ulceration and amputation which are serious consequences of diabetic neuropathy **(Bril et al., 2018)**.Strong glycemic control is the effective way to prevent or delay the development of neuropathy in patients with type 1 diabetes and to reduce the progression of neuropathy in patients with type 2 diabetes **(Ang et al., 2014)**. Although, there are multiple treatments and published

guidelines concerning treatment of neuropathic pain, it is important to note that only few patients show complete relief of the painful symptoms with any treatment **(Bril et al., 2018)**.

Dexmedetomidine is highly selective α2A adrenergic receptors (α2AR).The α-2 adrenergic receptor has three subtypes; α2A, α2B and α2C which bind α-2 agonists and antagonists **(Ma et al., 2004)**. Alpha-2 receptor subtypes are responsible for the variety of the pharmacodynamic effects of dexmedetomidine. For example, acting on α-2A receptor appears to promote sedation, hypnosis, analgesia, sympatholysis, neuroprotection and inhibition of insulin secretion **(Paris and Tonner, 2005)**. Acting on α-2B receptor suppresses shivering centrally, promotes analgesia at spinal cord sites, and induces vasoconstriction in peripheral arteries. The α-2C receptor is associated with modulation of cognition sensory processing, mood and stimulant-induced locomotor activity, and regulation of epinephrine outflow from the adrenal medulla **(Paris andTonner, 2005**). It is believed that the neuro-protective effects of dexmedetomidine are produced by activating α2AR adrenal receptors **(Ma et al., 2004)**.

Lu and his colleagues have reported that dexmedetomidine administration had no effect on blood glucose level in type 1 diabetic rats **(Lu et al., 2017)**. However, a recent study by Mostafa and his colleagues have demonstrated that perioperative administration of dexmedetomidine had significantly decreased blood glucose level in morbid obese patients (**Mostafa et al., 2018)**.

Previous studies have revealed that dexmedetomidine can relieve hyperalgesiain rats with diabetic neuropathy pain **(Lu et al., 2017; Zhong et al., 2018)**.

Some researchers have indicated that increasing dexmedetomidine doses in healthy subjects resulted in linearly decreasing pain sensation and hemodynamic responses **(Ebert et al., 2000)**.

Other researchers also reported that dexmedetomidine intervention was able to significantly reduce the pain scores and postoperative diclofenac sodium consumption and improve duration of analgesic effect in patients undergoing knee arthroscopy **(Li and Qu, 2017).**

In a previous study done by Gupta and his fellows, results showed that postoperative pain was significantly diminished with dexmedetomidine during endoscopic sinus surgery **(Gupta al., 2016)**.

Based on the findings from past studies and the current data, we have found that dexmedetomidine administration can increase the mechanical withdrawal threshold in DNP induced in rats. We have confirmed that dexmedetomidine administration significantly decreased blood glucose level in type 1 diabetic rats. Our results indicate that dexmedetomidine can reduce diabetic symptoms which can provide a novel insight into the ameliorative effects mediated by dexmedetomidine through decreasing blood glucose level in rats with diabetic neuropathy.

In summary, data from the present study showed that dexmedetomidine could ameliorate DNP in rats by decreasing blood glucose level and increasing mechanical withdrawal threshold. However, the underlying mechanisms need to be investigated in the future.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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