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Original research article

Anti-hypernociceptive and anti-oxidative effects of locally treated dobutamine in diabetic rats

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ABSTRACT

Background: Oxidative stress as a significant factor in the development of diabetes induced neuropathic pain as well as the potential for prevention of this complication. Therefore, we hypothesized that locally administrated dobutamine, a beta-adrenoreceptor agonist, or esmolol, a beta-adrenoreceptor antagonist, can modulate the oxidative stress and ameliorate the diabetes induced neuropathic pain.
Methods: Effects of locally (intraplantar) treated two pharmaceutical preparations used in clinical applications, dobutamine or esmolol, were investigated by measuring thermal latencies, mechanical thresholds and several oxidative stress parameters in streptozotocin (STZ) induced diabetic rats.
Results: Diabetes induced hyperalgesia and allodynia more effectively relieved by dobutamine than esmolol. Anti-hypersensitive action of dobutamine continued through the experiment. Diabetes induced oxidative damage in the paw tissues since STZ rats showed significant increased malondialdehyde (MDA), nitric oxide (NO) and decreased superoxide dismutase (SOD), catalase (CAT), myeloperoxidase (MPO) in the paw. Dobutamine, but not esmolol, restored the tissue oxidative and nitrossive stress parameters to those observed in the non-diabetic rats.
Conclusions: Findings suggest that diabetes-induced oxidative stress may be partially responsible for the development of diabetic neural complications. Amelioration of oxidative stress by locally treated dobutamine can be beneficial in diabetes induced neuropathic pain.

Introduction

Diabetes is characterized by hyperglycemia that produces dysregulation of glucose metabolic pathways, resulting in excess free radical production and oxidative stress [1]. The mechanisms underlying oxidative stress in chronic hyperglycemia and development of neuropathy have been examined in both clinical and experimental studies. Increased production of free radicals, impaired antioxidant defense capabilities, reactive oxygen/nitrogen species play important roles in all stages of diabetes induced neuropathic pain [2–4].

Although heterogeneous etiology of diabetes induced neuropathy, behavioral and physiologic studies involving rodents have revealed indices of sensory dysfunction in animal diabetes models that include hyperalgesia (exaggerated pain sensations as a result of exposure to a mildly noxious stimulus) to thermal stimuli and allodynia (a painful response to a normally innocuous stimulus) to light touch [5–8].

The available treatments of pharmacological options, such as analgesics, anesthetics, antidepressants, opioids, and antiepileptic drugs, for painful diabetic neuropathy do not provide just relieving in all patients. In the clinical setting, despite the use of these agents, the successful therapy of diabetic neuropathy is limited [6,9]. All of these arouse the interest of researchers to new therapeutic choices for the treatment of the diabetes induced neuropathic pain.

Dobutamine is one of the most widely used agents for heart failure in clinic procedures. Dobutamine is predominantly a beta 1 adrenergic agonist, with weak beta 2 activity, and alpha 1 selective activity [10]. It has been well known that peripheral nerve endings contain variety neuroreceptors such as adrenergic, opioid, cholinergic and cannabinoid receptors [11–13]. Previous

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studies have reported that alpha 2 adrenergic agonists produce analgesic effects and beta 2 adrenergic agonists provide anti-inflammatory and analgesic relief for nociceptive conditions [12]. In addition, it has been demonstrated that dobutamine can reduce edema formation and prevent the inflammatory reactions on lungs after hypothermia and ischemia/reperfusion [14].

Esmolol, a beta adrenoceptor antagonist, was characterized in a hydrophilic solution, a short elimination half-life, and more potent beta 1 adrenoceptor selectivity than beta 2 adrenoceptors [15] and are frequently used in patients with cardiovascular diseases to avoid circulatory complications during various types of operations requiring anesthesia [16]. An *in vitro* study has also shown that esmolol has antinociceptive effects through a blockade of tetrodotoxin-resistant sodium channels in a dose- dependent and use-dependent manner [17].

Adrenoceptors are widely distributed in the peripheral and central nervous systems. Therefore, in this study, our working hypothesis is that locally administrated, a beta adrenoceptor antagonist dobutamine or, an antagonist esmolol used in cardiovascular problems may also attenuate the diabetes induced peripheral neuropathic pain through modulation of oxidative stress. The objective of presented study was to assess the possible pain relieving and anti-oxidative effects of locally treated two pharmacological preparations used in clinics, dobutamine and esmolol, by investigating their actions on the hypersensitivities (thermal hyperalgesia, mechanical allodynia) and several biochemical oxidant and antioxidant parameters (malondialdehyde (MDA), nitric oxide (NO), (superoxide dismutase (SOD), catalase (CAT) and myeloperoxidase (MPO)), in streptozotocin (STZ) induced diabetic rats.

Materials and methods

Animals

Female Wistar rats (4–6 months, weight 250–260 g, Medical Sciences Experimental Research Centre of Kahramanmaras Sutcu Imam University (Kahramanmaras, Turkey) were used in these experiments. Throughout the course of the study, the rats were maintained in a climate-controlled and soundisolated room (22–24 °C) under a 12:12-h light:dark cycle (06:00 a.m to 18:00 p.m), with 40–60% relative humidity and *ad libitum* provision of feed (pellets) and water.

Although data were taken without consider to the cycling, our findings shoved no systematic differences in experimental groups as a result of estrous cycles (for 4 weeks in fig). The value of testing female rats at different stages of the estrous cycle is debatable [18]. Because obtaining repeated vaginal samples can be stressors in rodents sensitivity to drugs may change. The other approach for identifying effects of sex hormones in animals is gonadectomy with or without hormone replacement. However, hormone depletion *via* gonadectomy can also alter the physiological status of the animals [19]. Therefore, we used only intact female animals in our study and we cannot discount the possibility that age, sex and estrous cycle may have influenced the results. However, this limitation would not affect our conclusion, as we did not compare the efficacies of drugs between age or sex groups. The comparisons were made with aged-matched controls only.

All the experiments were approved by the Kahramanmaras Sutcu Imam University institutional animal care and use committee (2012/04-6).

Induction of diabetes

Diabetes was induced following an overnight fast by a single intraperitoneal injection of STZ (Sigma–Aldrich GmbH, Taufkirchen, Germany) at 60 mg/kg freshly dissolved in cold 0.9% sterile saline. Rats matched for age at the time of STZ administration were used as non-diabetic control animals (age matched controls). Rats were checked for glycosuria semi quantitatively using Urine-Glucostix reagent strips (Diastix; Bayer, Germany.) 3 days after STZ injection. Rats exhibiting glycosuria were then analyzed for hyperglycemia with a strip-operated glucometer (Accutrend GCT, Roche, Mannheim, Germany). Rats in the diabetes groups were excluded from the study if blood glucose was less than 300 mg/dL. Throughout the experiments, the weights and blood glucose levels were monitored regularly at intervals at the same time of the day. However, drug studies were conducted 4 weeks after onset of hyperglycemia. In all experiments attention was paid to ethical guidelines for the investigation of experimental pain in conscious animals. All animals were given unlimited food and water and were not supplemented with insulin or anti-hyperglycemic agents. The experiments were performed in an observer-blinded fashion with parallel negative placebo (saline vehicle) treatment controls. Control rats were injected with an equivalent volume of the vehicle only.

Drugs and experimental procedures

Esmolol hydrochloride was purchased from Eczacibasi (Istanbul, Turkey) and dobutamine hydrochloride was purchased from Abbott laboratories (Illinois, USA). In this present study, pharmacological preparations of these drugs were examined to assess whether there were any discernible effects in experimental diabetic neuropathy. Sodium metabisulfite is added (in 100 μl, 0.019 mg sodium metabisulfite and 1 mg dobutamine) as a preservative and antioxidants to dobutamine solution by manufacturer. Sodium metabisulfite can eliminate dissolved oxygen in water because dobutamine can be oxidized by oxygen in water. Several studies reported that sodium metabisulfite can have prooxidant properties and can significantly increase neuronal death at high concentrations. However, intraplantarly injected 0.019 mg sodium metabisulfite (in 100 μl, $n = 5$) did not significantly change the thermal latency and mechanical threshold in healthy rats (data not shown). Therefore, it was not tested in diabetic rats.

To limit the period for which animals suffered, while retaining the optimum hyperalgesia and allodynia to observe, effects of local (intraplantar) administration of drugs were examined at 4 weeks post-STZ treatment. In this study, experimental groups were therefore designed as below. Animals were grouped as diabetic (STZ-injected) and non-diabetic (saline (control for STZ)-injected rats). These groups were allocated to two subgroups: drugs-treated rats (0.5 or 1 mg dobutamine or esmolol), saline (control for drugs)-treated rats.

Dobutamine or esmolol (0.5 or 1 mg) were administered as single dose into the plantar side of the right hind paw in a volume of 100 μl using a 30-gauge needle. The needle was inserted at the midline near the heel and advanced anteriorly to the base of the second or third toe, where the drug was injected, forming a swelling (which disappeared approximately 3–5 min after injection) that usually extended back to the initial point of entry. For vehicle-only control groups, equal volumes of saline were injected intraplantarly.

All sensory tests were performed in a quiet room maintained (9:00 a.m. to 1:00 p.m. at 23–25 °C) and were performed on groups of 7 animals.

Assessment of thermal hyperalgesia

The presence of thermal hyperalgesia was determined by measuring paw withdrawal latency to thermal stimulation system (Commat, Ankara, Turkey) [20]. Animals were individually placed in plexiglass chambers (10 cm× 20 cm × 24 cm) on a clear glass