Published online: 2019-01-04



# Spinal Serotonin and 5HT6 Receptor Levels During Development of Neuropathy and Influence of Blockade of these Receptors on Thermal Hyperalgesia in Diabetic Mice*

Authors
**Cagda Celik Sari, Ozgur Gunduz, Ahmet Ulugol**

Affiliation
Department of Medical Pharmacology, Faculty of Medicine, Trakya University, Edirne, Turkey

Key words
diabetic neuropathy, neuropathic pain, serotonin, 5-$HT_6$ receptors, thermal hyperalgesia

**received** 15.11.2018
**accepted** 04.12.2018

Bibliography
**DOI** https://doi.org/10.1055/a-0817-5464
Published online: 4.1.2019
Drug Res 2019; 69: 428–433
© Georg Thieme Verlag KG Stuttgart · New York
ISSN 2194-9379

Correspondence
Prof. Ahmet Ulugol, MD
Trakya University
Department of Medical Pharmacology
Faculty of Medicine
22030 Edirne
Turkey
Tel.: + 90/284/2353 925, Fax: + 90/284/2353 925
aulugol@trakya.edu.tr; aulugol@yahoo.com

**ABSTRACT**

Little is known about the role of 5-$HT_6$ receptors in the pathophysiology of neuropathic pain. The aim of this study is firstly, to investigate the influence of spinal and systemic 5-$HT_6$ receptors on thermal hyperalgesia, one of the most significant symptoms of neuropathy occurring in diabetes; and secondly to determine spinal lumbar serotonin and 5-$HT_6$ receptor levels during development of diabetic neuropathy in mice. Diabetes was produced in Balb/c mice with a single injection of streptozocin (150 mg/kg, i.p.). Using the hot plate test, the 5-$HT_6$ antagonist SB-258585 was given systemically (3, 10, 30 mg/kg) and intrathecally (0.01, 0.1, 1 nmol/mouse) to determine its effect on thermal hyperalgesia. Furthermore, on days 7 and 15 of diabetes, development of thermal hyperalgesia was evaluated in relation to changes in spinal serotonin and 5-$HT_6$ receptor levels by using LC/MS/MS and Western blot analyses, respectively. Two-way analysis of variance and unpaired t-tests were used to evaluate data from hot-plate tests and 5-HT levels/ 5-$HT_6$ receptor expression, respectively. Thermal hyperalgesia was observed in neuropathic mice, starting from day 5 after streptozocin administration. On day 15, systemic, but not intrathecal, SB-258585 attenuated thermal hyperalgesia in neuropathic mice. Spinal serotonin levels did not change during development of hyperalgesia after induction of diabetes, whereas spinal 5-$HT_6$ receptor levels were significantly reduced on days 7 and 15. Our findings show that systemic, but not spinal, blockade of 5-$HT_6$ receptors may exert antihyperalgesic effects in neuropathic mice and suggest that systemic 5-$HT_6$ receptors contribute to the pathophysiology of diabetic neuropathy.

## Introduction

Peripheral neuropathic pain is defined as "pain caused by a lesion or disease of the peripheral somatosensory nervous system" [1, 2]. Traumatic injuries, certain medications such as cytotoxic agents, and systemic diseases such as diabetes mellitus are among the most common causes of peripheral neuropathic pain. Allodynia (pain response to normally non-painful stimulus) and hyperalgesia (augmented pain response to normally painful stimulus) are prominent clinical symptoms associated with neuropathic patients [1, 3, 4]. Current pharmacological treatments for neuropathic pain are typically insufficient at reducing pain symptoms. Even most of the powerful painkillers like morphine and other opioid drugs are inadequate; they are generally effective at higher doses when their adverse effects are seen. In general, antidepressants (e. g., amitriptyline and nortriptyline) and some anticonvulsant drugs (e. g., gabapentin, pregabalin) are recommended for first-line treatment of neuropathic pain; however, it is clear that there is a lack of specific medications that effectively treat this indication [5–7].

Serotonin (5-hydroxytriptamine; 5-HT) is a biogenic amine extensively distributed both in peripheral and central nervous systems. Serotonin contributes to pain modulation by interacting with

* Presented at the "17th World Congress on Pain", Boston, USA, 12–16 September 2018.



Downloaded by: Trakya University. Copyrighted material.

different 5-HT receptors [8–10]. Up to now, 7 families of 5-HT receptors (5-$HT_{1-7}$) have been identified. At the periphery, 5-HT has been shown to exert pronociceptive actions mediated via 5-$HT_{2A}$, 5-$HT_{2B}$, 5-$HT_{2C}$, 5-$HT_3$, 5-$HT_4$, 5-$HT_6$ and 5-$HT_7$ receptors [10–13]. On the contrary, its spinal effects appear to be more complicated than peripheral effects. 5-HT exerts antinociception at lower doses, whereas it produces pronociceptive effects at higher doses; physiological or pathophysiological status of the animal (e. g., neuropathic pain, inflammation) may also have an impact on the net spinal outcome. It is generally accepted that activation of spinal 5-$HT_{1A}$, 5-$HT_{1B}$, 5-$HT_{1D}$, 5-$HT_{1F}$ and 5-$HT_{5A}$ receptors reduce nociception, while spinal 5-$HT_2$, 5-$HT_3$, 5-$HT_4$, 5-$HT_6$ and 5-$HT_7$ receptors augment nociception when activated [10, 11, 14–17].

5-$HT_6$ receptor subfamily is one of the least known 5-HT receptor subtypes. They are positively coupled to adenylate cyclase, which in turn produces cyclic AMP, and may exert an excitatory action on neuronal activity [18]. 5-$HT_6$ receptors are exclusively localized in pain-related areas, such as periaqueductal gray, spinal cord and dorsal root ganglion [19], indicating that these receptors may play a pivotal role in pain modulation at different levels of central nervous system. 5-$HT_6$ receptors have been shown to be pronociceptive in formalin-induced pain [20], and 5-$HT_6$ antagonists have been shown to reduce nociceptive behavior in the same test [21]. Both peripheral and spinal 5-$HT_6$ receptors have been shown to play role in the development and maintenance of secondary mechanical allodynia and hyperalgesia [12, 14]. Recent studies on neuropathic pain states suggest that 5-$HT_6$ receptor antagonists attenuate neuropathic pain behaviors in spinal nerve ligated rats [22–24]; however, there are also contradictory results indicating that 5-$HT_6$ receptor antagonists do not exert antinociception in neuropathic rats [25].

Taking into consideration all these data on the effect of 5-$HT_6$ receptors and their antagonists on neuropathic pain conditions, we aimed to investigate the effect of systemically and spinally administered 5-$HT_6$ receptor antagonists on thermal hyperalgesia, and to determine spinal lumbar serotonin and 5-$HT_6$ receptor levels during development of diabetic neuropathy in mice.

# Materials and Methods

## Animals and ethics

Male Balb/c mice (Center of the Laboratory Animals, Trakya University) weighing 20–30 g (n = 8 for each group) were used through these experiments. Animals were housed under a 12–12 h light/dark cycle at a constant temperature of 21 ± 2 °C and had free access to food and water and food. All experiments were carried out between 10:00 and 17:00. The experiments were conducted in strict accordance with "Guide for the Care and Use of Laboratory Animals" published by National Academy of Sciences and the experimental protocols were approved by the ethics committee of Trakya University.

## Assessment of antinociception and motor coordination

The nociceptive response was assessed using a standardized cold/hot plate apparatus (Ugo Basile, Comerio, Italy). To conduct the hot plate test, mice were put on a heated surface kept at a temperature of 55 ± 0.1 °C, and response latencies for jumping or paw licking were documented. To prevent tissue damage, a cut-off time of 25 s was set. Test latencies in the hot plate test were converted to the percentage of the maximal possible effect (%MPE) according to the formula: %MPE = [(postdrug latency − baseline latency)/cut-off time − baseline latency] x 100 [26].

The changes in locomotor function after intrathecal injections and in diabetic mice were evaluated using a rotarod apparatus (Commat, Ankara, Turkey). The animals were placed on the apparatus and allowed to explore it during 5 min. Then, mice were placed on the rod rotating at 16 rpm and the performance time until the mice fell from the rod were recorded. A cut-off time of 180 s were set before the assessments [27].

## Study design and drugs

Streptozocin (STZ, 150 mg/kg, i.p.), freshly prepared in 0.1 N citrate buffer at pH 4.5, was administered to induce diabetes. The development of diabetes was confirmed 1 week after STZ injection by measurement of tail-vein plasma glucose levels, using IME-DC (Germany) test strips. Mice were considered diabetic when blood glucose levels were at least 200 mg/dl [28]. Thermal thresholds for the hot plate tests were determined on days 5, 9 and 15 after injection of STZ.

To determine the effect of 5-$HT_6$ blockade on thermal hyperalgesia in diabetic mice, different doses of the 5-$HT_6$ antagonist, SB-258585, was given systemically (3, 10, 30 mg/kg, i.p.) and intrathecally (0.01, 0.1, 1 nmol/mouse/i.t.) on day 15 after induction of diabetes. Thermal thresholds for the hot plate tests were investigated before and 30, 60, 90 and 120 min after the injections. Intrathecal injections were made using a widely accepted technique [29]. Additionally, on 7$^{th}$ and 15$^{th}$ days of diabetes, a different group of drug-naïve mice were euthanized and lumbar spinal cord was extracted to examine serotonin and 5-$HT_6$ receptor levels, using LC/MS/MS and Western blot analyses, respectively.

Streptozocin and SB-258585, a 5-$HT_6$ receptor antagonist, were purchased from Sigma-Aldrich (St Louis, MO, USA). SB-258585 was given in 20 % DMSO, 1 % Tween 80, 1 % ethanol, and 78 % saline. Doses and treatment times of STZ and SB-258585 were selected from previous studies [12, 14, 20, 24].

## LC/MS/MS

Frozen mice brain tissue samples were placed in a 1.5 mL polypropylene tube and an extraction solution (%1 formic acid in water, 10 dilution factor) was added. The mixture was vortexed for 1 min and sonicated for 10 min at 45 °C. After sonication the mixture was centrifuged for 5 min, at 9000 rpm. The supernatant was transferred to micro-vial and a 3 µL sample was injected into the LC–MS-MS system for analysis.

Chromatography was performed on an Agilent 1260 infinity LC system (Agilent Technology, Waldbronn, Germany), consisting of quaternary pumps, an autosampler, a vacuum degasser and a column compartment. Separation was on a reversed phase Agilent Zorbax C8 column (3.0 × 150 mm 3,5micron). The mobile phase A consisted of 5 mM ammonium formate and 0,1 % formic acid in water and mobile phase B consisted of %0,1 formic acid in acetonitrile. The flow rate was 0,5 mL $min^{-1}$ and the column temperatures was 35 °C. The injection volume was 3 µL. The analysis time was

Downloaded by: Trakya University. Copyrighted material.

11 min for each run. The elution strength usually increases with time, where the gradient starts at 3 %B and rise up 97 %B at 4 min and return 3 %B at 7 min. Mass spectra was obtained with an Agilent 6460 triple quadrupole mass spectrometer equipped with an ElectronSpray ionization (ESI) source, under the control of the MassHaunter software (version B.05.00). The mass spectrometer was operated in positive-ion, MRM mode. The source temperature of the sheath gas was adjusted to 400 °C and nebulizer rate was 45 psi. The gas flow was 10 L $min^{-1}$ and the capillary voltage was 2,500 V. Collision energy was 4 V for 160.1 and 30 V for 114.9. Singly charged precursor-product ion transitions were monitored at m/z 177.0 → 160.1 and 177.0 → 114.9 (serotonin). The dwell time was 100 ms for each product ion. The linearity fitted well over the range of 1.0–100 ng $mL^{-1}$ for 5 different concentration and serotonin LOD and LOQ values are 0,23 ng $mL^{-1}$ and 0.69 ng $mL^{-1}$, respectively. The linearity was $R^2$ 0,99931. The recoveries were carried out spiked real sample and 88.9 % was obtained for spiked 25 ng $mL^{-1}$ and 94.2 % for spiked 50 ng $mL^{-1}$.

### Western blot analysis

Western blot analyses were performed as described previously [30, 31] Tissue samples were homogenized in tissue extraction buffer (Santa cruz biotecnology, Finnell Street, Dallas, USA). Total protein concentration was measured using Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA). 20 µL samples were loaded into Nupage Novex 4–12 % Bis-Tris gel (Life Technologies, Invitrogen, Carlsbad, CA, USA) and subjected to vertical electrophoresis. Then, samples were transferred to nitrocellulose membrane using the iblot semi dry blotting system (The iBlotTM Gell Transfer Stack, Nitrocellulose, Invitrogen). The membrane was blocked with blocking solution for 30 min to reduce nonspecific binding (Western Breeze Chemiluminescent Western Blot Immunodetection Kit Antirabbit; Invitrogen). Afterwards, the membranes were incubated overnight at 4 °C with rabbit polyclonal 5-HT6 antibody (1 : 500 dilution in blocking solution, ab103016) and rabbit polyclonal anti-b-actin antibody (1 : 1000 dilution in blocking solution, Novus Biologicals). The membrane was washed 3 times for 10 min with wash buffer and then incubated with secondary antibody for 30 min (Western Breeze Chemiluminescent Western Blot Immunodetection Kit Antirabbit; Invitrogen). Following washing 3 times for 10 min with wash buffer, the protein was visualized by Chemidoc™ MP Imaging System (Universal Hood 3; Bio-Rad, Hercules, CA, USA) with enhanced chemiluminescence substrate (Invitrogen). Immunoblot bands for 5-$HT_6$ and b-actin were quantified using an ImageJ 1.48 v program (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). The ratio of the density of 5-$HT_6$ to b-actin was calculated for each sample.

### Statistical analysis

Data from the hot-plate experiment were evaluated using 2-way analysis of variance (ANOVA), followed by Bonferroni t test. Differences in lumbar dorsal spinal cord 5-HT levels and 5-$HT_6$ receptor expression were compared using unpaired t-test. $P<0.05$ was considered to be significant for all experiments. All data are expressed as mean ± SEM.

## Results

### Effects of the 5-$HT_6$ antagonist on thermal hyperalgesia in diabetic mice

Starting from day 5 after induction of diabetes, significant thermal hyperalgesia was observed (2-way ANOVA followed by Bonferroni t test, * $P<0.0001$, ▶ **Fig. 1**). On day 15, systemic administration of the 5-$HT_6$ receptor antagonist SB-258585 (3, 10, 30 mg/kg, i.p.) decreased thermal hyperalgesia in diabetic mice (2-way ANOVA followed by Bonferroni t test, ▶ **Fig. 2a**). 3 mg/kg and 10 mg/kg doses of SB-258585 was effective at 60, 90 and 120$^{th}$ mins, whereas treatment with 30 mg/kg of SB-258585 was efficacious at 30, 60 and 90 mins. SB-258585 (0.01, 0.1, 1 nmol/mouse) had no effect on thermal hyperalgesia when administered intrathecally (2-way ANOVA followed by Bonferroni t test, ▶ **Fig. 2b**).

### Serotonin levels and 5-$HT_6$ receptor expression in the lumbar spinal cord

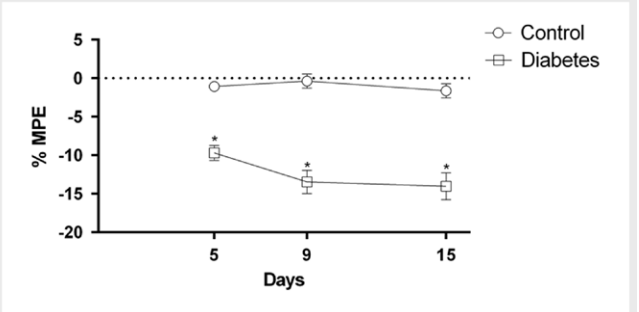
Spinal serotonin levels did not change in diabetic mice; it is worth mentioning that there was a modest but statistically insignificant increase on day 15 of diabetes (▶ **Fig. 3**, unpaired t-test). Lumbar spinal 5-$HT_6$ receptor levels were significantly decreased both in 7$^{th}$ (unpaired t-test, * $P<0.05$) and 15$^{th}$ (unpaired t-test, * * $P<0.0001$) days after STZ injection (▶ **Fig. 4**).

### Effects of diabetes and intrathecal injections on locomotor activity

Neither diabetes nor i.t. injection had any effect on motor coordination (data not shown).

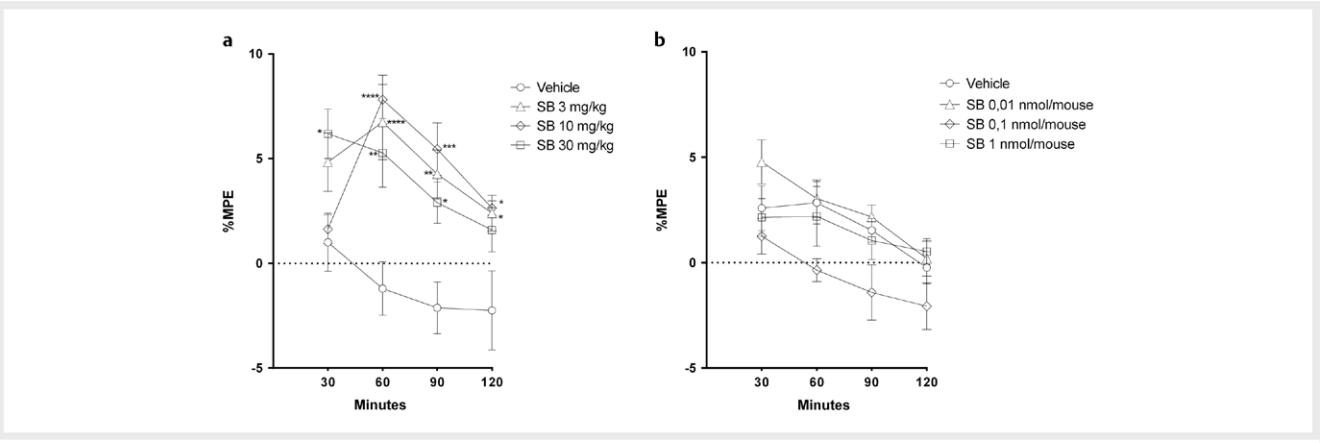
## Discussion

The 5-$HT_6$ receptor is a 7-transmembrane receptor positively coupled to adenylate cyclase. Its activation leads to stimulation of adenylyl cyclase and may produce an excitatory effect on neuronal activity [18]. When released, 5-HT may exert opposite (pronociceptive or antinociceptive) actions mostly depending upon the 5-HT receptor subtype. Although there is little research on the role 5-$HT_6$ receptors in nociception, these receptors appear to mediate pronociceptive effects both at the periphery and the spinal cord.

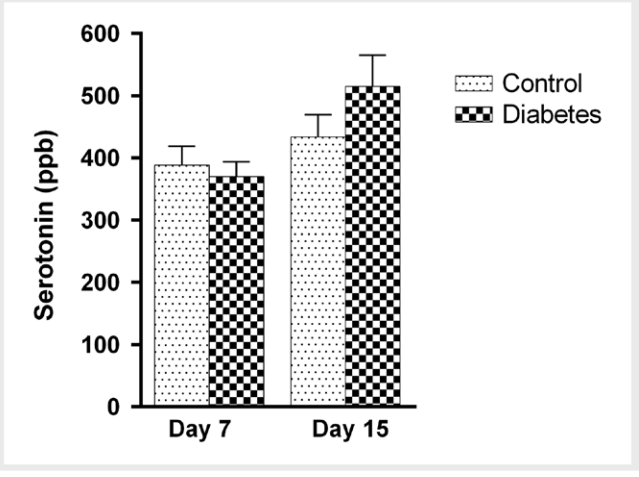



▶ **Fig. 1** Thermal hyperalgesia induced by diabetes. Thermal thresholds for the hot plate tests were determined on days 5, 9 and 15 after streptozocin injection (150 mg/kg, i.p.; 2-way analysis of variance followed by Bonferroni *t* test, * $P<0.0001$, vs. control group).

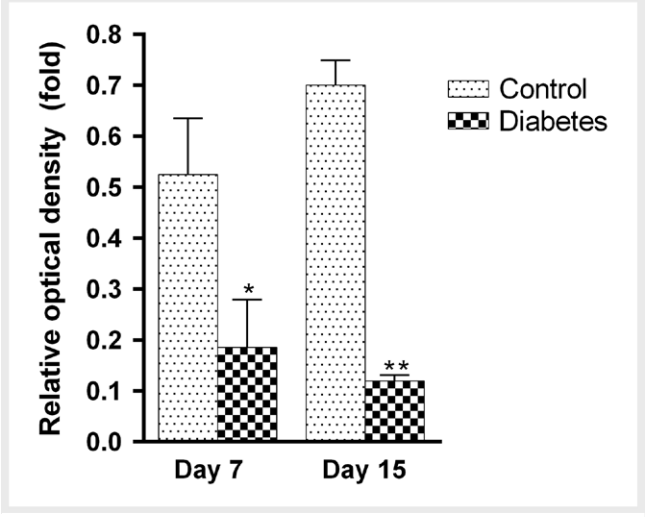
Downloaded by: Trakya University. Copyrighted material.




▶ **Fig. 2** Time course of the effects of systemic (3, 10, 30 mg/kg) and intrathecal (0.01, 0.1, 1 nmol/mouse) administrations of the 5-$HT_6$ receptor antagonist SB-258585 on thermal hyperalgesia in diabetic mice on day 15 after streptozocin injection (2-way analysis of variance followed by Bonferroni *t* test, * P<0.05, * * P<0.01, * * * P<0.001, * * * * P<0.0001, vs. control group.




▶ **Fig. 3** LC/MS/MS analysis of lumbar dorsal spinal cord for serotonin content in control and diabetic mice on days 7 and 15 after respective vehicle or streptozocin injections. No significant change was observed (unpaired t-test).




▶ **Fig. 4** Western blot analysis of lumbar dorsal spinal cord for 5-$HT_6$ expression in control and diabetic mice on days 7 and 15 after respective vehicle or streptozocin injections (unpaired t-test, * P<0.05, * * P<0.0001 vs. control group).

First studies on this subject indicated that pharmacological blockade of 5-$HT_6$ receptors reduce formalin-induced nociceptive behavior [21], and that both local peripheral and spinal 5-$HT_6$ receptors play a pronociceptive role in formalin-induce pain [20]. Then, both peripheral and spinal 5-$HT_6$ receptors have been shown to play roles in development and maintenance of formalin-induced secondary mechanical allodynia and hyperalgesia [12, 14]. The same group also demonstrated that 5-$HT_6$ receptors takes part in peripheral pronociceptive but not in spinal antinociceptive effects of fluoxetine, a selective serotonin re-uptake inhibitor [11].

All of the above-mentioned observations point to the pronociceptive feature of 5-$HT_6$ receptors both at the periphery and the spinal cord; however, in case of neuropathic pain there are contradictory findings. In the rat spinal nerve ligation model of neuropathic pain, oral administration of compounds that exert potent 5-$HT_6$ antagonistic effects exhibited stronger potency than gabapentin against cold allodynia, whereas a diminished pain inhibitory effect was observed against mechanical allodynia [22]. Similar oral compounds showing good 5-$HT_6$ inhibitory activity confirmed these findings displaying anti-allodynic effects in spinal nerve ligated neuropathic rats, but this time mechanical allodynia- alleviating effect was superior to cold allodynia [23]. On the contrary, 5-$HT_6$ antagonists had no effect on mechanical allodynia in neuropathic rats, but only improved analgesic effects of gabapentinoids, such as gabapentin and pregabalin [25]. Different from these oral administrations, intrathecal injection of 5-$HT_6$ antagonists decreased tactile allodynia in a dose-dependent manner and 5-$HT_6$ receptors have been indicated to contribute to maintenance of neuropathic pain in rat [24]. Other than these researches on allodynia, we show for the first time that systemic, but not intrathecal, 5-$HT_6$ antagonist reduces ther-

Downloaded by: Trakya University. Copyrighted material.

mal hyperalgesia in diabetic neuropathy. It should be taken into consideration that peripheral sensitization and central changes contribute to generation and maintenance of different subtypes of allodynia and hyperalgesia with separate mechanisms [3]; moreover, allodynia and hyperalgesia of nerve injury-induced neuropathic animals may not share the same etiology with allodynia and hyperalgesia of STZ-induced diabetic animals [32, 33].

Pain stimuli activates descending serotonergic pathways and in turn release of 5-HT at the spinal cord, which is pivotal for spinal nociceptive transmission [9, 34]. Then, 5-HT can exert facilitatory or inhibitory effects depending basically on the spinal 5-HT receptor subtype and several other reasons. Both nerve injury and diabetes have been shown to increase 5-HT content at the spinal cord, which may account for enhanced pain during these neuropathic pain states [9, 34, 35]. Our results indicating that spinal 5-HT levels do not change during development of hyperalgesia appear to be contradictory to findings of Morgado et al. [35], but they demonstrated higher levels of 5-HT 4 weeks after induction of diabetes; our results also point to a slight increase in spinal 5-HT levels on day 15 which may be an indication of higher 5-HT levels after 4 weeks.

Spinal nerve injury leads to a loss of descending serotonergic neurons [36], whereas it did not modify expression of spinal 5-$HT_6$ receptors [35]. Unlike nerve injury-induced neuropathy, we found that expression of 5-$HT_6$ receptors were significantly attenuated in diabetes-induced neuropathy; this can be the reason of inadequate efficacy of the intrathecally administered 5-$HT_6$ antagonist against thermal hyperalgesia in diabetic rats. In conclusion, our data demonstrate that systemic, but not spinal, blockade of 5-$HT_6$ receptors attenuate thermal hyperalgesia in diabetic mice and propose that 5-$HT_6$ receptor antagonists may have utility in the pharmacotherapy of diabetic neuropathy.

## Acknowledgement

This work was supported by a grant from Trakya University Research Council (TUBAP-2016/43). We thank K. Duvan-Aydemir for her technical support for behavioral & western blot analysis.

### Conflict of Interest

The authors have no conflicts of interests to report.

### References

[1] Colloca L, Ludman T, Bouhassira D et al. Neuropathic pain. Nat Rev Dis Primers. 2017; 3: 17002

[2] Colvin LA, Dougherty PM. Peripheral neuropathic pain: signs, symptoms, mechanisms, and causes: Are they linked? Brit J Anaesth 2015; 114: 361–363

[3] Jensen TS, Finnerup NB. Allodynia and hyperalgesia in neuropathic pain: Clinical manifestations and mechanisms. Lancet Neurol 2014; 13: 924–935

[4] Ulugol A, Aslantas A, Karadag HC et al. The effect of combined systemic administration of morphine and L-name, a nitric oxide synthase inhibitor, on behavioral signs of neuropathic pain in rats. Neurosci Res Commun 2002; 30: 143–153

[5] Finnerup NB, Attal N, Haroutounian S et al. Pharmacotherapy for neuropathic pain in adults: A systematic review and meta-analysis. Lancet Neurol 2015; 14: 162–173

[6] Dworkin RH, O'Connor AB, Kent J et al. Interventional management of neuropathic pain: NeuPSIG recommendations. Pain 2013; 154: 2249–2261

[7] Gunduz O, Oltulu C, Guven R et al. Pharmacological and behavioral characterization of the saphenous chronic constriction injury model of neuropathic pain in rats. Neurol Sci 2011; 32: 1135–1142

[8] Bardin L. The complex role of serotonin and 5-HT receptors in chronic pain. Behavioural Pharmacology 2011; 22: 390–404

[9] Millan MJ. Descending control of pain. Prog Neurobiol 2002; 66: 355–474

[10] Viguier F, Michot B, Hamon M et al. Multiple roles of serotonin in pain control mechanisms -Implications of 5-HT7 and other 5-HT receptor types. European Journal of Pharmacology 2013; 716: 8–16

[11] Cervantes-Duran C, Rocha-Gonzalez HI, Granados-Soto V. Peripheral and spinal 5-HT receptors participate in the pronociceptive and antinociceptive effects of fluoxetine in rats. Neuroscience 2013; 252: 396–409

[12] Godinez-Chaparro B, Barragan-Iglesias P, Castaneda-Corral G et al. Role of peripheral 5-HT4, 5-HT6, and 5-HT7 receptors in development and maintenance of secondary mechanical allodynia and hyperalgesia. Pain 2011; 152: 687–697

[13] Sommer C. Serotonin in pain and analgesia: actions in the periphery. Mol Neurobiol 2004; 30: 117–125

[14] Godinez-Chaparro B, Lopez-Santillan FJ, Orduna P et al. Secondary mechanical allodynia and hyperalgesia depend on descending facilitation mediated by spinal 5-HT4, 5-HT6 and 5-HT7 receptors. Neuroscience 2012; 222: 379–391

[15] Oyama T, Ueda M, Kuraishi Y et al. Dual effect of serotonin on formalin-induced nociception in the rat spinal cord. Neuroscience Research 1996; 25: 129–135

[16] Aksu AG, Gunduz O, Ulugol A. Contribution of spinal 5-HT5A receptors to the antinociceptive effects of systemically administered cannabinoid agonist WIN 55,212-2 and morphine. Can J Physiol Pharmacol 2018; 96: 618–623

[17] Gencer A, Gunduz O, Ulugol A. Involvement of descending serotonergic and noradrenergic systems and their spinal receptor subtypes in the antinociceptive effect of dipyrone. Drug Res 2015; 65: 645–649

[18] Ruat M, Traiffort E, Arrang JM et al. A novel rat serotonin (5-HT6) receptor - molecular-cloning, localization and stimulation of cAMP accumulation. Biochem Bioph Res Co 1993; 193: 268–276

[19] Gerard C, ElMestikawy S, Lebrand C et al. Quantitative RT-PCR distribution of serotonin 5-HT6 receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. Synapse 1996; 23: 164–173

[20] Castaneda-Corral G, Rocha-Gonzalez HI, Araiza-Saldana CI et al. Role of peripheral and spinal 5-HT(6) receptors according to the rat formalin test. Neuroscience 2009; 162: 444–452

[21] Finn DP, Forte KCF, Beckett SRG et al. The effects of pharmacological blockade of the 5-HT6 receptor on formalin-evoked nociceptive behaviour, locomotor activity and hypothalamo-pituitary-adrenal axis activity in rats. European Journal of Pharmacology 2007; 569: 59–63

[22] Devegowda VN, Hong JR, Cho S et al. Synthesis and the 5-HT6 receptor antagonistic effect of 3-arylsulfonylamino-5,6-dihydro-6-substituted pyrazolo[3,4]pyridinones for neuropathic pain treatment. Bioorg Med Chem Lett 2013; 23: 4696–4700

[23] Hong JR, Choo H, Nam G. Neuropathic pain-alleviating effects of pyrazole-conjugated arylsulfonamides as 5-HT6 receptor antagonists. Bioorg Med Chem Lett 2017; 27: 4146–4149

[24] Pineda-Farias JB, Barragan-Iglesias P, Valdivieso-Sanchez A et al. Spinal 5-HT4 and 5-HT6 receptors contribute to the maintenance of neuropathic pain in rats. Pharmacol Rep 2017; 69: 916–923

Downloaded by: Trakya University. Copyrighted material.

[25] Jayarajan P, Nirogi R, Shinde A et al. 5-HT6 receptor antagonist attenuates the memory deficits associated with neuropathic pain and improves the efficacy of gabapentinoids. Pharmacol Rep 2015; 67: 934–942

[26] Gunduz O, Karadag CH, Ulugol A. Modulatory role of the endogenous nitric oxide synthase inhibitor, asymmetric dimethylarginine (ADMA), in morphine tolerance and dependence in mice. J Neural Transm 2010; 117: 1027–1032

[27] Gunduz O, Topuz RD, Karadag CH et al. Analysis of the anti-allodynic effects of combination of a synthetic cannabinoid and a selective noradrenaline re-uptake inhibitor in nerve injury-induced neuropathic mice. European Journal of Pain 2016; 20: 465–471

[28] Ulugol A, Oltulu C, Gunduz O et al. 5-HT7 receptor activation attenuates thermal hyperalgesia in streptozocin-induced diabetic mice. Pharmacology Biochemistry and Behavior 2012; 102: 344–348

[29] Hylden JL, Wilcox GL. Intrathecal morphine in mice: a new technique. Eur J Pharmacol 1980; 67: 313–316

[30] Ulugol A, Topuz RD, Gunduz O et al. Changes in nociceptin/orphanin FQ levels in rat brain regions after acute and chronic cannabinoid treatment in conjunction with the development of antinociceptive tolerance. Fundam Clin Pharmacol 2016; 30: 537–548

[31] Kizilay G, Cakmak H, Yen CF et al. Expression and regulation of c-Jun N-terminal kinase (JNK) in endometrial cells in vivo and in vitro. Histochem Cell Biol 2008; 130: 761–771

[32] Calcutt NA, Chaplan SR. Spinal pharmacology of tactile allodynia in diabetic rats. Br J Pharmacol 1997; 122: 1478–1482

[33] Field MJ, McCleary S, Hughes J et al. Gabapentin and pregabalin, but not morphine and amitriptyline, block both static and dynamic components of mechanical allodynia induced by streptozocin in the rat. Pain 1999; 80: 391–398

[34] Suzuki R, Rygh LJ, Dickenson AH. Bad news from the brain: descending 5-HT pathways that control spinal pain processing. Trends in Pharmacological Sciences 2004; 25: 613–617

[35] Morgado C, Silva L, Pereira-Terra P et al. Changes in serotoninergic and noradrenergic descending pain pathways during painful diabetic neuropathy: The preventive action of IGF1. Neurobiology of Disease 2011; 43: 275–284

[36] Leong ML, Gu M, Speltz-Paiz R et al. Neuronal loss in the rostral ventromedial medulla in a rat model of neuropathic pain. Journal of Neuroscience 2011; 31: 17028–17039

Downloaded by: Trakya University. Copyrighted material.