IJPSR (2012), Vol. 3, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 August, 2012; received in revised form 11 September, 2012; accepted 24 November, 2012

THE ROLE OF TRANSIENT RECEPTOR POTENTIAL VANILLOID 1 RECEPTOR IN DESIPRAMINE INDUCED ANALGESIC EFFECT IN DIABETIC MICE

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Keywords:
Hyperalgesia, TRPV1, Nociceptive threshold, Desipramine, Diabetes mellitus, Mice

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QUICK RESPONSE CODE

IJPSR:
ICV (2011)- 5.07

Website:
www.ijpsr.com

ABSTRACT

Hyperalgesia is one of the debilitating complications of diabetes. The thermal allodynia and hyperalgesia in diabetic mice may be due to the hyperactivity of C-fiber in the spinal cord. Transient receptor potential vanilloid type 1 (TRPV1) present in spinal cord and activation of C-fibre may involve in hyperalgesia in diabetic mice. Desipramine is one of the tricyclic antidepressants, effective in diabetic neuropathy. The intravenous administration of desipramine depresses the C-fibre reflex that will involve in activation of convergent neurons of the spinal cord. Thus, the present study was carried out to find out the role of TRPV1 in desipramine induced analgesic effect in diabetic hyperalgesia. Mice were administered capsaicin (1 mg kg^{-1}), capsazepine (15 mg kg^{-1}), desipramine (10 mg kg^{-1}) from day 4 to day 11 after induction of diabetes and the nociceptive threshold was measured in terms of reaction time, tail flick latency and tail withdrawal latency. The nociceptive threshold was significantly ($p < 0.05$) lower in diabetic mice as compared with control group. Capsaicin produced a significant ($p < 0.05$) decrease in reaction time, tail flick latency and tail withdrawal latency as compare to diabetic group. Desipramine caused a significant ($p < 0.05$) increase in nociceptive threshold in diabetic group as well as capsaicin treated diabetic group. It was concluded that desipramine produced analgesic effect in diabetic hyperalgesia and TRPV1 might be involved in desipramine induced analgesic effect.

INTRODUCTION: Antidepressants are widely used in chronic pain states including both inflammatory and neuropathic pain conditions. It has been suggested that antidepressant drugs have specific analgesic properties, and various clinical [1, 2] and experimental [3-5] types of evidence together demonstrate that the analgesic effect may be independent of the antidepressant effects. Interactions of antidepressants with biogenic amines, opioid systems, excitatory amino acid receptors, substance P and calcium and sodium channels have been considered to be involved pain relieving properties of antidepressants [6].

Desipramine (**Figure 1**) is one of the tricyclic antidepressants, which block the uptake of amines by nerve terminals, by competition for the binding site of the amine transporters. Oral desipramine enhances the analgesic effects of morphine for post-operative pain [7], reduces pain intensity in post-herpetic neuralgia [8], and produces pain relief in diabetic neuropathy patients [9].

Acute administration of desipramine is antinociceptive in animal models of inflammatory pain [10] and attenuates thermal hyperalgesia in models of neuropathic pain [11, 12].

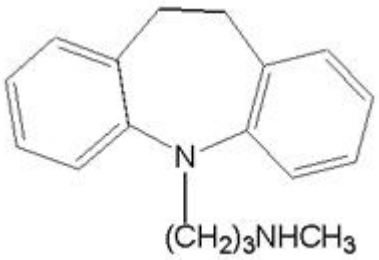

FIGURE 1: STRUCTURE OF DESIPRAMINE

TRPV1 is considered as key molecules of nociception and a correlation between TRPV1 expression and inflammatory thermal hyperalgesia has been established in rats [13]. TRPV1 may involve in transduction of chemical and thermal hyperalgesia in a model of diabetic neuropathy [14]. TRPV1 is a non-selective cation channel of TRP family located on sensory neurons. There are seven subfamily of TRP channel in which TRPV1 is associated with thermal hyperalgesia [15]. Hyperalgesia is one of the common complications of diabetes mellitus. Streptozotocin (STZ) induced diabetes neuropathy also demostrate thermal hyperalgesia and mechanical allodynia [16]. In experimental diabetic neuropathy, the development of hyperalgesia is due to increased expression of TRPV1 on neurons that do not normally express TRPV1 [17].

It was suggested that the thermal allodynia and hyperalgesia in diabetic mice may be due to the hyperactivity of C-fiber in the spinal cord. The TRPV1 receptor is expressed predominantly by primary sensory neurons in the spinal cord, probably in unmyelinated C-fibers and sensitization of TRPV1 receptors might be involved in the mechanism of thermal hyperalgesia and allodynia seen in diabetic mice [17, 18]. Desipramine, inhibit the spinal processing of C inputs by acting directly at the spinal cord level [19].

In light of above facts, the present study was designed to find out the role of TRPV1 in desipramine induced analgesic effect in STZ induced diabetic mice model.

MATERIALS AND METHODS:

Animals: Healthy albino mice of either sex, weighing 25–30 g were procured from Zydus Research Centre, India. The animals were housed and maintained at 298 K, 50 ± 15 % RH for 12 hour light-dark cycles, in polypropylene cages with free access to food and water *ad libitum*.

The experimental protocol (KBIPER/2011/247) was approved by the Institutional (K.B. Institute of Pharmaceutical Education and Research) Animal Ethics Committee (IAEC) under the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guideline, before carrying out the project.

Chemicals: Streptozotocin, desipramine and capsaicin were obtained from Sigma-Aldrich, USA while capsazepine obtained from Cayman chemical, China. Dimethylsulphoxide (DMSO) was purchased from Sujan Chemials, India.

Desipramine was dissolved in saline. Capsaicin and capsazepine was dissolved in 30% DMSO to prepare stock solution and it was diluted further in saline to prepare working solutions.

Induction of type 1 diabetes: Diabetes mellitus was induced in mice using STZ. Streptozotocin was freshly prepared by dissolving 1 g STZ in 10 mL 0.1 N cold citrate buffer (1.45 g citric acid and 1.05 g sodium citrate dissolved in 100 mL distilled water) at a pH of 4.5. Single dose of STZ (25 mg kg^{-1}, *i.p.*) was administration for induction of diabetes and 10% W/V oral glucose was given to all animals after 6 h of STZ administration to prevent the hypoglycemic shock. Control animals received an equivalent volume of saline. Confirmation of hyperglycemia was made 3 days later by measurement of the fasting blood glucose (FBG) level. Mice with FBG level >14 mmol L^{-1} were considered diabetic and were included for further study [20].

Estimation of blood glucose: Blood glucose levels were estimated spectrophotometrically by glucose-oxidase methodusing a commercially available enzymatic kit (Span Diagnostics Ltd., India) [21].

Experimental design:

The mice were divided into the following groups:

- GROUP I (Non-Diabetic control group)
- GROUP II (STZ-induced diabetic group) to serve as diabetic control animals.
- GROUP III (Capsaicin treated diabetic group: 1 mg kg^{-1}, i.p)

- GROUP IV (Capsazepine treated diabetic group:15 mg kg^{-1}, i.p)
- GROUP V (Desipramine treated diabetic group: 10 mg $kg^{-}1$, i.p)
- GROUP VI (Capsaicin (1 mg kg^{-1}, i.p) + Desipramine (10 mg kg^{-1}, i.p)
- GROUP VII (Capsaicin (1 mg kg^{-1}, i.p) $_+$ Capsazepine (15 mg kg^{-1}, i.p)

Mice of group I were administered normal saline solution to serve as a non-diabetic group. Desipramine, capsaicin and capsazepine were administered to their respected groups, daily for 8 days, starting on day 3 after STZ administration. In case of group VI and group VII, desipramine and capsazepine were administered 30 min before capsaicin administration. Nociceptive threshold was noted on day 4, 8 and 11 in all groups.

Measurement of Nociceptive Threshold: For the measurement of nociceptive threshold, the hot plate, tail immersion test and the tail flick test were carried out at 0, 30, 60 and 120 minutes intervals.

1. **Hot plate method:** The mice were placed individually on a hot plate (Max enterprise/ HP99M, India) maintained at 328 K and the time taken by the animal for the reaction either by licking the paw or jumping or raising the limbs which ever was observe first taken as the end point. Reaction time was note down before and at 0, 30, 60 and 120 min after the drug or saline administration in each animal. Cut off time of 60 s was followed to avoid any thermal injury to the paws [22].
2. **Radiant heat tail-flick method:** Tail-flick latency was assessed by the analgesiometer (Instruments manufacturing corporation -model/MC-102413, India). The strength of the current passing through the naked nichrome wire was kept constant at 2 A. The distance between heat source and the tail was 1.5 cm and the application site of the heat on the tail was maintained within 2 cm, measured from the root of the tail. Cut-off reaction time was 30 s to avoid any tissue injury during the process. Tail-flick latency was measured before and 0, 30, 60 and 120 min after the drug administration. The time taken by mice to withdraw (flick) the tail was taken as the reaction time. The animals were subjected to the same test procedure at 0, 30, 60, 120, and 180 min after the administration of drug [22].
3. **Tail immersion test:** Mice were held in position in a suitable restrainer with the tail extending out. 3-4 cm area of the tail was marked and immersed in the water bath thermo-statistically maintained at 328 K. The withdrawal time of the tail from hot water was noted as the reaction time or tail flick latency. The maximum cut off time for immersion was 30 s to avoid the injury of the tissues of tail [22].

Statistical analysis: All the values were expressed as Mean±SEM. The data were analyzed by One-way Analysis of Variance (ANOVA) followed by Tukey's multiple range test. The level of significance was expressed at $p < 0.05$.

RESULTS:

Effect of STZ on Fasting blood glucose level: FBG level was significantly ($p < 0.05$) increase in STZ treated mice as compare with control mice (**Figure 2**).

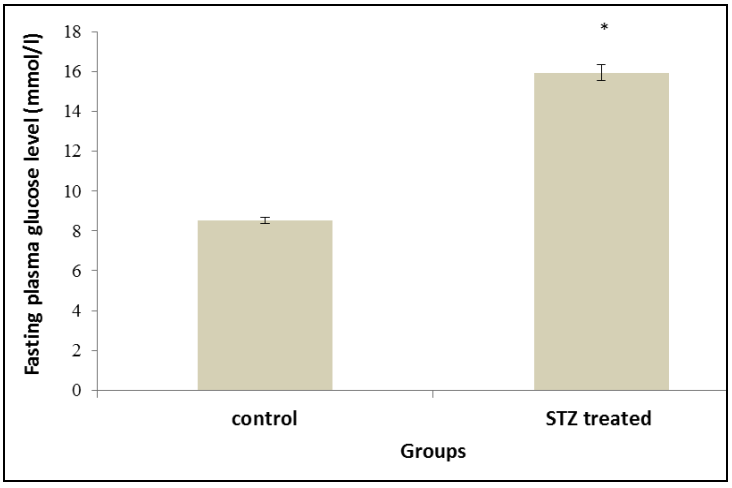

FIGURE 2: FASTING BLOOD GLUCOSE LEVEL IN CONTROL AND STZ TREATED GROUPS Each bar are expressed as mean ± SEM. (n=6); * $p<0.05$ as compared with control group; (One way ANOVA followed by Tukey's Test)

Measurement of Nociceptive Threshold: The diabetic group showed a significant ($p < 0.05$) decrease in reaction time, tail- flick latency, tail withdrawal latency as compared to control group on day 11. Reaction time, tail- flick latency, tail withdrawal latency was significantly ($p < 0.05$) decrease in capsaicin treated group than diabetic mice on day 11.

Nociceptive threshold was significantly ($p < 0.05$) increase in desipramine as well as capsazepine treated mice than diabetic group at 11 day. In animals pretreated with desipramine and capsazepine before capsaicin administration, there was a significant ($p < 0.05$) increase in reaction time, tail flick latency and tail withdrawal latency as compared with capsaicin treated diabetic mice (**Figure 3**).

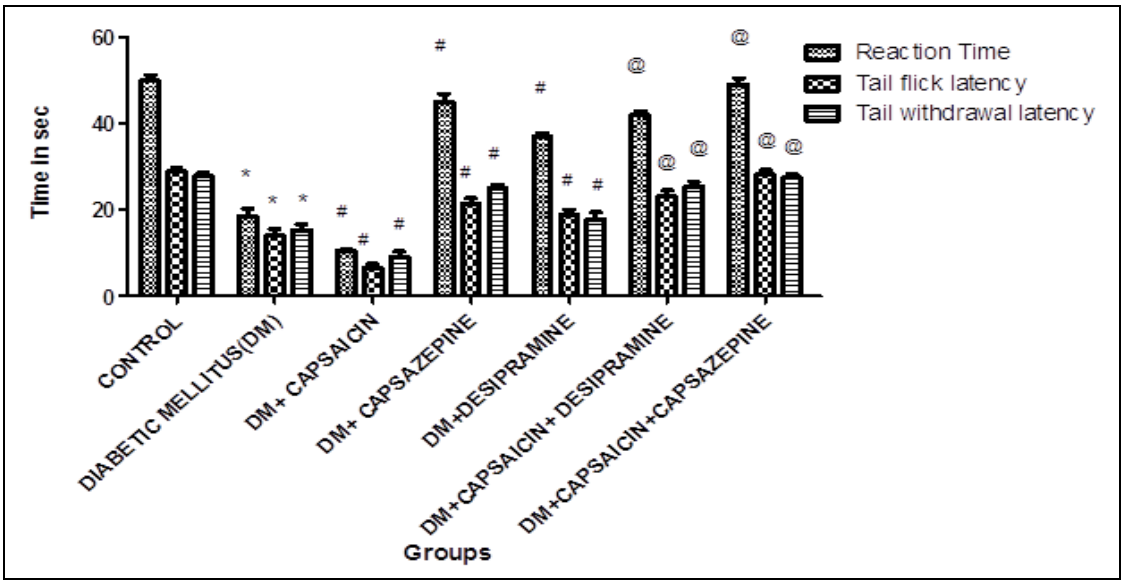

FIGURE 3: EFFECT OF CAPSAICIN, DESIPRAMINE AND CAPSAZEPINE ON REACTION TIME, TAIL FLICK LATENCY, TAIL WITHDRAWAL LATENCY AT 60 MIN ON DAY 11. Each bar are expressed as mean ± SEM. (n=6); * $p<0.05$ as compared with control group; # $p<0.05$ as compared with diabetic group; @ $p<0.05$ as compared with Capsaicin group; (One way ANOVA followed by Tukey's Test)

DISCUSSION: The present work aimed to study the involvement of TRPV1 in analgesic effect of desipramine in diabetic hyperalgesic mice.

The transient receptor potential vanilloid 1 (TRPV1) receptor, previously known as the vanilloid receptor 1 (VR1) or capsaicin receptor, is a ligand-gated, non-selective cation channel expressed predominately by primary nociceptive sensory neurons [14]. It activated by the pungent component of hot chilli peppers, capsaicin, as well as heat, protons and some endogenous substances known to be associated with tissue inflammation. TRPV1 has, therefore, been suggested to be a molecular integrator of chemical and physical stimuli that elicit pain [23]. It has been shown that TRPV1 play a significant role in nociception and in the pathogenesis of experimental diabetic hyperalgesia and the development of its complications [16].

Streptozotocin (STZ) is a glucosamine nitrosourea compound used for the induction of the diabetes mellitus in animals and also used to study the associated complications. The induction of diabetes by STZ is characterized by initial phase of hyperglycemia, then hypoglycemia and then it will cause hyperglycemic effect in animal. This effect will cause diabetic mellitus after 3 days of STZ administration [24].

In present study, the STZ treated mice showed significantly ($p<0.05$) increase in FBG level and became diabetic. The diabetogenic action of STZ was also accompanied by the development of persistent hyperalgesia by enhancing expression and function of TRPV1 receptor in DRG neurons [25].

The present study demonstrated that diabetic mice induced by a single injection of STZ developed significant hyperalgesia. The nociceptive threshold was significantly lower in diabetic mice as compared with the control group. Hyperalgesia was evident on Day 4 and the maximum decrease in pain threshold was observed on Day 11 after STZ injection.

The modulation of nociception by antidepressants is mainly centrally mediated and involved central serotonergic, noradrenergic, and opioidergic systems in the modulation of pain threshold caused by antidepressants.

Most pharmacological data so far revealed that facilitation of central serotonergic and noradrenergic transmission is potentially anti-nociceptive, whereas inhibition of serotonergic and noradrenergic activity increases the sensitivity to noxious stimuli [26, 27].

Tricyclic antidepressants exhibit anti-nociceptive properties in neuropathic, nociceptive and inflammatory models of pain [28] but this effect through the TRPV1 receptor is not clear. It has been demonstrated that desipramine is effective in diabetic neuropathy and can serve as a relatively selective adrenergic probe of analgesic mechanisms [9].

Desipramine, which is a powerful reuptake inhibitor of norepinephrine, has been found to have marked antinociceptive properties in some investigations [3, 29].

Oral desipramine reduces pain intensity in post-herpetic neuralgia [8] and produces pain relief in diabetic neuropathy patients [9]. The intravenous administration of desipramine depresses the C-fibre reflex that will involve in activation of convergent neurons of the spinal cord [28, 30, 31]. The thermal allodynia and hyperalgesia in diabetic mice may be due to the hyperactivity of C-fiber in the spinal cord [32, 33].

TRPV1 present in spinal cord and activation of C-fibre activity may involve in hyperalgesia in diabetic mice. The anti-nociceptive effect of desipramine was measured by hot plate method, tail flick method, tail immersion method. In present study, desipramine showed significant increase in reaction time, tail flick latency and tail withdrawal latency as compare to diabetic group. This suggests that desipramine gives analgesic effect in diabetes mellitus induced hyperalgesia.

Capsaicin, the active agent found in hot chilli peppers is powerful agonist of TRPV1. Capsaicin accomplishes its effect by evoking sharp burning pain sensation. Capsaicin induces influx of cations in nociceptors. Capsaicin activates TRPV1, that produce hyperalgesia [34]. Capsazepine is a powerful antagonist of TRPV1 receptor. Capsazepine, the competitive capsaicin antagonist significantly reduces inflammatory heat hyperalgesia [35, 36].

In present investigation, capsaicin produced a significant decrease in reaction time, tail flick latency and tail withdrawal latency as compare to diabetic group. Capsazepine antagonized the hyperalgesic effect of capsaicin. Likewise, desipramine caused a significant increase in reaction time, tail flick latency and tail withdrawal latency of capsaicin treated group compare to diabetic group. This suggests the involvement of TRPV1 in desipramine induced analgesic effect in diabetic hyperalgesia.

CONCLUSION: In conclusion, desipramine produced analgesic effect in diabetic hyperalgesia and TRPV1 might be involved in desipramine induced analgesic effect.

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How to cite this article:
Shaikh S, Deshpande S and Gohil P: The Role of Transient Receptor Potential Vanilloid 1 Receptor in Desipramine induced Analgesic Effect in Diabetic Mice. *Int J Pharm Sci Res*. 3(12); 4882-4887.