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RESEARCH ARTICLE

Possible Implications of Endogenous Cannabinoid Receptors and Transient Receptor Potential Vannaloid-1 (trpv1) Receptors in Alcoholic Neuropathic Induced Pain in Wishtar Rat.

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Abstract:

Alcoholic neuropathy is caused due to long term consumption of alcohol which leads to degeneration of neurons consequently, leading to neuropathic pain. Trpv1 is a ligand gated channel which gets activated in chronic alcoholism. The present research work . has been design to investigate the involvement of endocannabinoids in the activation of TRPV1 receptor in neuropathic pain during chronic alcoholism in wistar rat. The drugs capsazepine (1mg/kg i.p), a TRPV1 receptor antagonist, tetrahydrolipstatin (12mg/kg i.p), endogenous cannabinoid biosynthesis inhibitor, were given alone as well as in combination, one week before the peak nociception alcoholic rats. Capsaicin (0.1mg/kg i.p), TRPV1receptor agonist were administered for one week after the administration of capsazepine (1mg/kg i.p) in alcoholic rats. Pain were assessed with the help of Eddy's hot plate, Tail flick method and Tail immersion method. After applied found to be decreasing in alcoholing control group as compared to normal control group. Decrease in pain threshold was obtained in animals which were administered alcohol twice daily.

KeyWords: Alcoholic Neuropathic Pain, TRPV1 Receptor Capsaicin, Capsazepine and Tetrahydrolipstatin.

INTRODUCTION:

The term "ALCOHOLIC NEUROPATHY" (Synonym: alcoholic neuritis, neuropathic beri beri, neuritic beri beri) refers to the ultimate damage suffered by nerves due to long term consumption of alcohol.[1] It has been noted that the chronic use of large quantity of alcohol leads to neuropathic pain that is due to insults of central and peripheral nervous system. The main pathological change in Alcoholic Neuropathy is axonal degeneration involving both myelinated and unmyelinated fibres.[2]

The axonal injury subsequent to alcohol consumption lead to alteration of phenotype of directly affected neurons leading to increased sensitivity and pain sensation.[3] Chronic alcoholism may cause neuropathy by creating spinal cord injury.[4]

TRP channels participate in several processes of paramount importance for the physiology of cells and organisms including intracellular calcium homeostasis, cellular chemotaxis, neuronal guidance and neurite extension, and calcium transport, cell proliferation and differentiation, the cellular immune response and several others. [5,6,7,8]

It has been reported that persistent intake of alcohol for longer span of time period increases level of capsaicin and thereby activating the receptor TRPV1. In chronic alcoholism of TRPV1 receptor gets activated and its activation contribute to pain.[9] Thus it has been well documented that endocannabinoids have the affinity for both the receptors i.e.CB1 receptors and TRPV1. However activation of both the receptors are responsible for opposite action of pain and signaling pathway i.e. activation of CB1 receptors increases the pain threshold and activation of TRPV1 decreases the pain threshold. During the chronic alcoholism CB1 receptors get downregulated whereas TRPV1 is reported to get upregulated while the level of endocannabinoids gets elevated.

MATERIAL AND METHOD

EXPERIMENTAL ANIMALS

In the behavioural paradigm for Alcoholic Neuropathic Pain (ANP) Study, Wistar rats of either sex, weighing 200-250 gm were used. They were housed in Central Animal House facility of the institute, in group of three, in polypropylene cages with husk bedding under standard conditions of light and dark cycle with food and water *ad libitum.* Animals were acclimatized to

laboratory conditions before the test. All the behavioural assessments were carried between 8:00 and 16:00 hrs. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and was carried out in accordance with the guidelines of the Indian National Science Academy (INSA) for the use and care of experimental animals.

EXPERIMENTAL PROTOCOL

GROUP I: Normal Control Group comprised of animals given distilled water in place of ethanol by oral gavage.

GROUP II: Alcohol control Group which were administered ethanol (35%v/v) by oral route for 9 weeks.

GROUP III: Alcohol treated animal received capsazepine (1mg/kg *i.p*), TRPV1 receptor antagonists (1 week before peak nociception).

GROUP 1V: Low dose of Capsazepine (0.1mg/kg *i.p*) was given half an hour before capsaicin(1mg/kg *i.p*) to alcohol treated groups (1 week before the peak nociception).

GROUP V: Tetrahydrolipstatin (12mg/kg *i.p*) was given to alcohol treated animals. (1 week before the peak nociception).

GROUP VI: Capsazepine (1mg/kg *i.p*) and tetrahydrolipstatin (12 mg/kg *i.p*) both were given altogether to the alcoholic treated group (1 week before the peak nociception).

INDUCTION OF ALCOHOLIC NEUROPATHIC PAIN

Alcoholic Neuropathic pain was induced by administration of 10g/kg of 35% v/v ethanol.

Absolute ethanol 99. 9 % (v/v).

Desired Concentration 35% (v/v)

35ml of absolute ethanol……..make upto 100 ml using Distilled Water.

CALCULATION OF DOSING VOLUME

Density of final mixture (35% v/v ethanol) = 0.97gm/ml.

Therefore,

$$\text{Administered Volume} = \frac{\text{Dose (g/kg) x Rat Body Weight}}{1000}$$

$$= \frac{10 \text{ x Rat Body Weight}}{1000x0.97}$$

TREATMENT SCHEDULE

All the animals were brought to laboratory environment for atleast 2 hour before starting the testing procedure. The animals were randomly divided into six experimental groups with 6 animals in each group. Control group was given distilled water (DW) in place of ethanol by oral gavage. Rats were administered alcohol for a total period of nine weeks. Treatment with Capsazepine (1mg/kg *i.p*), TRPV1 receptor antagonist, capsaicin (0.1mg/kg *i.p*), TRPV1receptor agonist and tetrahydrolipstatin (12mg/kg *i.p*) endocannabinoids biosynthesis inhibitor was started from seventh week i.e. 1 week before development of neuropathy to a peak level. All the behavioural parameters was assessed every week to study the changes with alcohol consumption as well as treatment drugs.

MEASUREMENT OF ANIMAL BODY WEIGHT

Rats were administered alcohol twice a day by oral gavage and the change in body weight with chronic consumption of alcohol was assessed every week.

MEASUREMENT OF THERMAL HYPERALGESIA

ASSESSMENT ON EDDY'S HOT PLATE

Hyperalgesia to thermal stimulation was determined using an Eddy's hot plate model as described by (Ferguson *et al.*, 1969).[10] Rats were placed individually on the hot plate of the apparatus which was maintained at the temperature of 55 ± 2^0 C. During the time, the rats initially demonstrated exploratory behavior, but subsequently stopped exploring and started licking their paw as soon as the heat of the hot plate was sensed and their jumping response was taken as their latency period. A cut-off latency of 15-20s was imposed to avoid tissue damage.[11] The response

latency was determined using a timer linked to it. Individual reading of all the rats were calculated and their mean was analysed.

ASSESSMENT ON TAIL WATER IMMERSION

Response to hot water was assessed with the help of test called tail water immersion test. In this test water was maintained at a temperature of 47^0 C and the tail of the rat was immersed into it in order to measure the latency period of rat and thereby assessing its nociceptive criteria. The cut off was taken as 10 sec in order to avoid any tissue damage.[11] While taking the basal reading the set up was validated and animals were made habitual to the environment and then the final readings were taken. The response latency was determined using a timer linked to it. Individual reading of all the rats were calculated and their mean was analysed.

ASSESSMENT ON TAIL FLICK ANALGESIOMETER

The analgesic activity was determined by radiant heat Tail-flick method in mice. Tail-flick latency was assessed by the analgesiometer. The strength of the current passing through the naked nichrome wire was kept constant at 0.3 mA . The distance between heat source and the tail was 1.5 cm and the application site of the heat on the tail was within 2 cm, measured from the root of the tail. Cut-off reaction time was 10s to avoid any tissue injury during the process. And hence the nociception was assessed along with the development of neuropathic state in the tails. The response latency was determined using a timer linked to it. Individual reading of all the rats were calculated and their mean was analysed.

STATISTICAL ANALYSIS

The results are expressed as Mean ± SD. The behavioral data were analysed using two-way analysis of variance (ANOVA) followed by Graphical Prism Software for multiple comparison. The p value < 0.05 was considered to be statistically significant.

RESULTS

ALCOHOL ADMINISTRATION AND INDUCTION OF NEUROPATHIC PAIN

Alcohol was administered by oral gavage for a period of nine weeks. The neuropathic pain was developed to a peak level at the seventh week as evidenced by lowered threshold of nociception.

The test drugs were administered 1 week before the peak nociception for the observing the improvement in neuropathic state.

EFFECT ON BODY WEIGHT

Body weight has been found to significantly increased in normal control rats as compared to alcohol control rats. The administration of capsaicin and capsazepine does not cause any change in the body weight as compared with the alcohol control rats

EFFECTS ON THERMAL HYPERALGESIA USING EDDY'S HOT PLATE

Alcohol control animals showed a significant decline in jumping or paw licking from the Eddy's hot plate maintained at 55± 2^0 C, in comparison to the age-matched normal control rats indicating development of hyperalgesia in neuropathic state. However one week treatment of animals with capsazepine (1 mg/kg *i.p*) significantly decreases the appearance of paw licking as compared to alcohol control group. Moreover combination of tetrahydrolipstatin (12mg/kg *i.p*), endocannabinoids biosynthesis inhibitor, and capsazepine (1mg/kg *i.p*) showed markedly improvement in hyperalgesic state as compared to alcohol control groups.

EFFECTS ON TAIL WATER IMMERSION

Alcohol control groups showed a marked decline in tail withdrawal from the hot water maintained at a temperature of 47±3^0 C in comparison to the same age matched control rats indicating development of neuropathic state peripherally. However one week treatment of animals with capsazepine (1mg/kg *i.p*) significantly decreases the appearance of tail wthdrawal as compared to alcohol control group. Moreover combination of tetrahydrolipstatin (12mg/kg *i.p*), endocannabinoids biosynthesis inhibitor, and capsazepine (1mg/kg *i.p*) showed markedly improvement in hyperalgesic state as compared to alcohol control groups

EFFECT ON TAIL FLICK ON ANALGESIOMETER

Alcohol control groups showed a marked decline in tail withdrawal at analgesiometer which was having a red hot wire set at passing current of 0.3 mA, in comparison to the same age matched control rats indicating development of neuropathic state peripherally. . However one week treatment of animals with capsazepine (1mg/kg *i.p*) significantly decreases the appearance of tail withdrawal as compared to alcohol control group. Moreover combination of tetrahydrolipstatin

(12mg/kg *i.p*), endocannabinoids biosynthesis inhibitor, and capsazepine (1mg/kg *i.p*) showed markedly improvement in hyperalgesic state as compared to alcohol control groups.

DISCUSSION

Chronic excessive intake of alcohol produces a complex cascade of adaptive neurochemical events in the CNS leading to the development of tolerance and dependence on alcohol. Studies in experimental animal models, such as the alcohol induced neuropathic model have helped to define the series of constellating changes that occurs with the chronic consumption of alcohol being assessed by mechanical allodynia and thermal hyperalgesia. Mechanical allodynia, thermal hyperalgesia, mechanical hyperalgesia have been well documented to be index for alcoholic neuropathy.[12]

The TRPV1 is a calcium-permeable channel expressed mainly on nociceptive neurons. The TRPV1 can be activated by a number of stimuli, including heat and protons as well as numerous chemical agents, including capsaicin.[13] The TRPV1 appears to play an important role in the development of several painful conditions including inflammatory and neuropathic pain.[14] Moreover it has been documented that during chronic alcoholism TRPV1 receptor mediated action get sensitized and contribute in neuropathic pain.[9]

Endogenous TRPV1 agonists i.e capsaicin and modulators such as protons, anandamide and products of the arachidonic acid metabolism can be released or up-regulated by inflammation and tissue damage.[15,16,17] Preclinically, disruption of the TRPV1 gene suggests that TRPV1 receptors are essential for inflammatory hyperalgesia.[14] These and other published studies have suggested a potential therapeutic utility of TRPV1 antagonists in pain.[16,17,18] Hence TRPV1 is an important target for developing novel analgesics.

In present study treatment of capsazepine (1mg/kg i.p), a selective antagonist of TRPV1, one week before the peak nociception significantly increases the pain threshold measured by Eddy's hot plate, Tail immersion and Tail flick methods in alcoholic rats. It reflects the involvement of TRPV1 receptor in alcoholism induced neuropathic pain in rats. Capsazepine is very specific to capsaicin because the findings of the present study reveals that TRPV1 in case of chronic alcoholism gets activated only through the increament in the level of capsaicin but not with the help of its other modulators like lipooxygenase, heat, pH or any other factor. This is

said because the result obtained in administration of capsazepine alone and on the other side administration of capsazepine along with capsaicin produced almost the same result in alcoholic rats. Our result is supported by findings of different laboratories.[18,19,20,21]

Treatment of capsazepine (1mg/kg *i.p*), a selective antagonist of TRPV1, along with tetrahydrolipstatn (12mg/kg *i.p),* (Peng *et al.,* 2010) an inhibitor of endogenous cannabinoid biosynthesis inhibitor potentiate the antinociceptive properties of capsazepine in alcoholic rats. Therefore it can be suggested that endocannabinoids may be responsible for neuropathic pain during chronic alcoholism in rat by activation of TRPV1 receptor. Our linings are also supported by the researchers i.e endocannabinoids are also endogenous ligand for TRPV1.[15,16,17] Moreover CB1 gets downregulated and its antinociceptive properties during chronic alcoholism gets impaired.[22]

On the basis of the above discussion it may be concluded that TRPV1 receptor is involved in development of alcoholic neuropathic pain in rats, and endocannabinnoids may be responsible for its activation.

6. CONCLUSION

On the basis of above study it may be concluded that capsazepine, a TRPV1 antagonist, and tetrahydrolipstatin, endogenous cannabinoid biosynthesis inhibitors has a beneficial role in attenuation of Alcoholic Neuropathy.Hence using the them together may offer a novel approach in abrogation of Alcoholic Neuropathy.

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