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Effect of single and repeated administration of amitriptyline on neuropathic pain model in rats: Focus on glutamatergic and upstream nitrergic systems

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ABSTRACT

Aims: Few studies have compared the interaction of single and repeated administration of amitriptyline (amit) with the nitrergic system and glutamatergic system in the experimental model of neuropathic pain. We aimed to evaluate the antinociceptive effect of single and repeated administration of amit and to assess whether glutamate preceded inducible nitric oxide synthase (iNOS) inhibition as a mechanism of the analgesic effect of amit in the neuropathic model of pain.

Materials and methods: Male Wistar rats were subjected to left sciatic nerve ligation. The effect of single (25 mg kg^{-1}) and repeated (10 mg kg^{-1} daily for 3 weeks) administration of amit intraperitoneally (i.p.) alone or in combination with aminoguanidine (AG i.p., 100 mg kg^{-1} for 3 days, a selective iNOS inhibitor) and MK-801 (0.05 mg kg^{-1} i.p., NMDA antagonist) on resting paw posture and mechanical hyperalgesia were studied. Glutamate level and iNOS protein expression in hippocampus were detected.

Key findings: Single and repeated administration of amit alone or in combination with AG or MK-801 demonstrated a significant decrease in resting pain score and increase in the pain threshold. Both glutamate and nitrite levels decreased in the hippocampi of single and repeated amit + MK-801 groups. Immunohistochemistry showed a marked decrease in iNOS immunoreactivity in rats treated with single and repeated amit + MK-801.

Significance: Our results suggest that glutamate-dependent mechanisms are involved in the analgesic responses to amit administration. Importantly, glutamatergic system and its upstream nitrergic system play an important role in the antinociceptive action of amit.

1. Introduction

The tricyclic antidepressant, amitriptyline (amit), has been widely used in the treatment of chronic pain, regardless of the presence of depression [1]. It became evident, both clinically and experimentally, that antidepressants affecting both the serotoninergic and noradrenergic systems were more effective than those affecting only the noradrenergic system for pain control [2,3]. Amitriptyline is quite multimodal in its analgesic action and the mechanisms that underlie this analgesic action of AMI remain questionable [4]. The excitatory neurotransmitter, glutamate, is a key neurobiological target, with a significant role in nociceptive processing [5,6]. The ionotropic glutamate receptors; *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) and kainate as well as the metabotropic glutamate receptors, are well expressed in the central and peripheral nervous system and highly distributed in pain pathways [7].

Animal models of pain have added much to our understanding of the mechanisms underlying neuropathic pain. Despite intensive researches have been conducted on neuropathic pain, many mechanistic details are still lacking [8]. Previously, we demonstrated the analgesic effect of a single administration of amit in a rat model of chronic constriction injury (CCI) of the sciatic nerve [3]. Interestingly, Veldhuijzen et al. [9] reported that a single administration of 25 mg amit significantly impaired driving performance in patients with neuropathic pain in the form of increased reaction time on a memory test, while continued treatment for 2 weeks had no significant differences from placebo.

The time course and amount of released glutamate with hyperalgesia is inconsistent depending on the stimulus used to produce the injury. Several signalling cascades, including stimulation of nitric oxide

Abbreviations: Amit, amitriptyline; ANOVA, analysis of variance; CCI, chronic construction injury; CNS, central nervous system; iNOS, inducible nitric oxide synthase; NE, norepinephrine; nNOS, neuronal NOS; NO, nitric oxide; TCA, tricyclic antidepressant

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synthase (NOS) with a subsequent increase in NO production are activated with generation of free radicals [10]. Additionally, blocking of NO signalling results in a decrease of the expression of glutamate receptor subunits in the hippocampus [11]. Still, nNOS knocked down mice demonstrated reduced *S*-nitrosylation of cysteine that is needed for the oxidized glutamate to provide energy [12]. Whether the production of nitric oxide (NO) is an up- or downstream result of NMDA receptor activation is unclear. Thus, the present study aimed to determine the analgesic effect of single and repeated administration of amit on the CCI model of neuropathic pain and to study the potential involvement of either glutamate/NO or NO/glutamate in the analgesic action of amit.

2. Materials and methods

2.1. Animals

Male adult Wistar rats weighing 200–220 g were used in this study. The animals were purchased from the animal's house facility, Faculty of Medicine, Assuit University and were housed under standard conditions of light and temperature with free access to food and water. The experiments described in this study were approved by our institutional ethics committee in accordance with the ethical guidelines for pain research in conscious animals [13].

Rats were kept in the laboratory for one week adaptation before the intervention. Animals were randomly divided into the following groups (n = 8 per group):

(1) sham group,
(2) saline-treated CCI group,
(3) single dose amit (25 mg kg^{-1})-treated CCI group,
(4) repeated doses amit (10 mg kg^{-1} daily for 3 weeks)-treated CCI group,
(5) single dose amit (25 mg kg^{-1}) + aminoguanidine (100 mg kg^{-1})-treated CCI group,
(6) repeated doses amit (10 mg kg^{-1} daily for 3 weeks) + aminoguanidine (100 mg kg^{-1})-treated CCI group,
(7) single dose amit (25 mg kg^{-1}) + MK-801 (0.05 mg kg^{-1})-treated CCI group
(8) repeated doses amit (10 mg kg^{-1} daily for 3 weeks) + MK-801 (0.05 mg kg^{-1})-treated CCI group.

Drug administration, and the decapitation of animals were carried out during the interval between 9:00 a.m. and 5:00 p.m. Before and after drug administration, the animals were subjected to motor coordination, behavioral pain score recording and mechanical hyperalgesia test.

2.2. Drugs and chemicals

Amitriptyline hydrochloride and MK-801 were purchased from Sigma, USA and aminoguanidine was obtained from Merck, Germany. All drugs were dissolved in saline and were administered i.p. in weight-related doses. Amitriptyline was administered in a single (25 mg kg^{-1}) and repeated (10 mg kg^{-1} daily for 3 weeks) doses alone or in combination with aminoguanidine (AG, a selective iNOS inhibitor) and MK-801 (NMDA antagonist). Rats were treated with aminoguanidine (100 mg kg^{-1}) daily for 3 days with the last injection 1 h before their sacrifaction. MK-801 (0.05 mg kg^{-1}) was administered 1 h before the test. All doses were chosen according to previous studies [14–16]. These doses have no toxic or motor impairment effect.

2.3. Surgical procedure

We used the peripheral neuropathy model described by Bennett and Xie [17]. Briefly, rats were anesthetized with i.p. sodium pentobarbital (50 mg/kg). A 7–10 mm length of the common left sciatic nerve was exposed and carefully cleared from underlying tissue using blunt dissection. Four 4.0 braided silk ligatures were tied loosely around the sciatic nerve at 1 mm intervals. The muscle groups moved towards each other and the skin incision is sealed with silk suture and was then covered with iodine. In sham-operated animals, the same dissection was performed in the left paw without placing ligatures on the sciatic nerve. All surgical procedures were carried under sterile condition. We evaluated the resting paw posture and response to mechanical stimulation 8 days after CCI and after drug administration.

2.4. Assessments of motor coordination (Rotarod test)

Motor coordination was assessed using an accelerating rat RotaRod apparatus (Ugo Basile, Italy). Before drug administration, the animals were trained daily for 3 days. The latency to drop from a rotating rod at a speed of 16 r.p.m. is recorded in all studied groups. Rats that remained on the rotarod bar for 180 s (cutoff time) were included in the study [18].

2.5. Resting paw posture

Scoring of the resting paw posture of each hind paw without an intervention was recorded on the 8th day after the operation prior to and at 3 h after amit administration as an indication of spontaneous pain. Each rat was placed in a rectangular plexiglass cage and allowed to accommodate for 15 min. Various paw positions were recorded for 2 min every 16 min for 48-min period. As detailed by Paulson et al. [19] the paw positions were plotted on a 5-point scale starting from 0, which represents the normal position of the hind limb (no pain). Pain will force the limb to take abnormal positions starting with light pressing on the floor with ventroflexed toes (score 1); only the internal edge of hind limb pressing on the floor (score 2); only heel pressing on the floor (score 3); the whole limb is elevated (score 4); animal licks hind paw, which represents severe pain (score 5).

2.6. Mechanical hyperalgesia

The nociceptive withdrawal threshold after CCI of the sciatic nerve was measured by using an analgesymeter (Ugo Basile, Italy) according to the Randall-Selitto test [20]. Before beginning the test, we should allow 5 min of proper handling of each rat to get used to manipulation. The animal was placed into a soft cotton cloth and immobilized carefully with the same hand used to hold the tested paw. The test was performed on the 8th day after CCI prior to and at 3 h after drugs administration. The test consisted of the application of an increasing mechanical force, in which the tip of the device was applied between the 3rd and 4th metatarsals until a withdrawal response resulted. The point of the application was marked with ink in order to maintain the location over repeated trials. The maximum force applied was limited to 200 g to avoid skin damage.

2.7. Biochemical measurements

At the end of these experiments, animals were anesthetized with 50 mg kg^{-1} pentobarbital sodium. After the decapitation, collected blood samples were centrifuged at 3000 r.p.m. for 10 min and serum stored at −20 °C for further assessment of NO.

The right and left hippocampi alongside with the cerebellum of the brain of each rat were separated and put in an ice plate; rinsed in ice-cold saline and weighed. The right hippocampus was homogenized in phosphate buffer with pH 7.4. An equal volume of 1 mol/l perchloric acid was added to the homogenate and well mixed. At room temperature, the mixture was allowed to stand for 5 min then, the sample was centrifuged for 10 min. The supernatant was collected and used for determination of glutamate directly or stored at −20 °C till assay. The

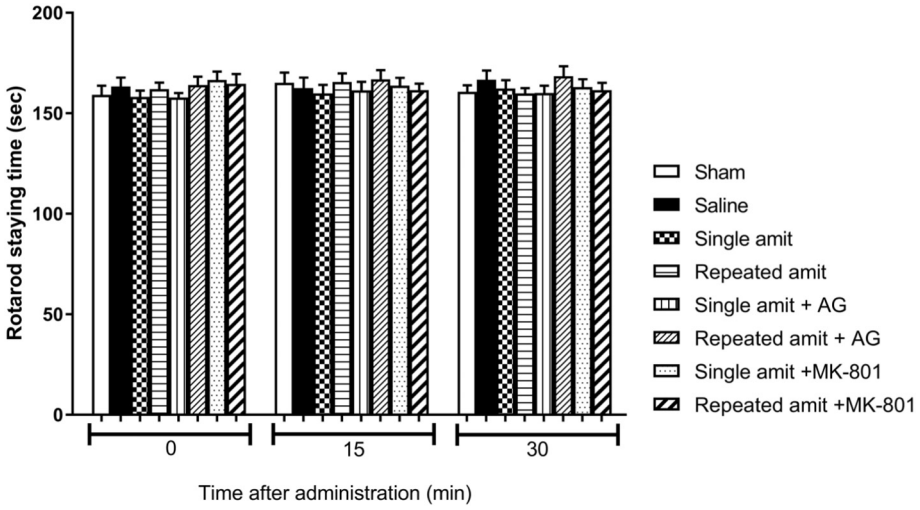

Fig. 1. The effect of single and repeated administration of amitriptyline (amit) alone and in combination with aminoguanidine (AG) or MK-801on locomotor coordination. Data are expressed as means ± s.e.m. for n = 8 animals per group. Rota rod staying time was recorded 8 days after CCI and at 0 time, at 15 min and at 30 min after drug treatment. Data were analyzed by a two-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc tests.

left hippocampus and cerebellum were submerged in 10% formalin for immunohistochemistry examination.

2.7.1. Assessment of NO

The formation of nitric oxide was measured in the serum by assaying nitrite, one of the stable end-products of NO oxidation. Serum nitrite concentration was assayed spectrophotometrically using Griess reagents [1% sulfanilamide in 5% phosphoric acid (sulfanilamide solution) and 0.1% *N*-1-naphthylethylenediamine dihydrochloride in bidistilled H2O (NED solution)] as described by Miranda et al. [21]. A standard curve was run simultaneously with each set of samples.

2.7.2. Assessment of glutamate

The hippocampal level of glutamate was determined in the supernatant fluid after its adjustment to pH 9 with phosphate solution [1.93 mol/l tripotassium phosphate (K3PO4·3H2O) solution]. The glutamate content was measured spectrophotometrically according to the method of Lund [22] via its enzymatic dehydrogenation by glutamate dehydrogenase with conversion of β-nicotinamide adenine dinucleotide (NAD+, oxidized form), to β-nicotinamide adenine dinucleotide (NADH, reduced form). The increase in NADH, as measured by the extinction change at 340 nm, is proportional to the amount of glutamate. A standard reference curve was prepared for each assay.

2.8. Immunostaining of iNOS

In brief, paraffin sections of 3–5 μm thickness were dewaxed and rehydrated in descending concentration of ethanol. The slides were washed twice with phosphate buffer (pH 7.2). The slides were placed in the microwaveable vessel containing antigen retrieval buffer (sodium citrate, pH 6.0) and boiled for 15 min. The slides were then removed from the microwave and left to cool at room temperature. The slides were subsequently incubated with a primary rabbit polyclonal antibody directed against iNOS (1:100; thermo scientific, South San Francisco, California, USA). To each slide, 50 μl of the primary antibodies were applied and the slides were incubated at 4 °C for 24 h. Detection System anti-polyvalent HRP/DAB kit (Thermo Fisher scientific, USA) was used. Slides were counterstained with Mayer's hematoxylin (blue) and cover slipped using a permanent mounting medium (DPX). Examination of slides was done using an Olympus CX41 optical microscope equipped with an Olympus U-CMAD3 digital camera interfaced to a computer. Negative control slides were done by staining of tissue sections with the omission of the primary antibody to exclude non-specific binding. Sections of rat lung were stained as a positive control for immunohistochemical staining specificity.

2.9. Statistical analysis

Data are represented as the group means ± s.e.m. The significance of differences between groups was analyzed using paired Student's *t*-test, one way or two way analysis of variance (ANOVA) followed by the post hoc Dunnett's test and Bonferroni respectively for multiple comparisons as appropriate. Differences were considered significant at $P < 0.05$. The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology [23]. All statistical analyses were calculated with Prism software (Graph-Pad Software, version 7, La Jolla, CA, USA).

3. Results

3.1. The effect of single and repeated administration of amitriptyline (amit) alone and in combination with aminoguanidine (AG) or MK-801 on motor coordination (Rotarod test) in rats

We performed the rotarod test to exclude the possible influence of motor deficits on the pain behavioral tests. After eight days of surgery, baseline values were established, then different drugs were injected and the test was repeated at 15 and 30 min following injection. Two-way ANOVA (Treatment × Time after drug administration) on 0, 15 min and 30 min after treatment showed that single and repeated administration of amit and the combination of amit with either AG or Mk-801 no significant on latency to fall [$F(14, 120) = 0.33$; $P = 0.99$] or time [$F(2, 120) = 0.21$; $P = 0.81$] after drug administration (Fig. 1).

3.2. The effect of single and repeated administration of amitriptyline (amit) alone and in combination with aminoguanidine (AG) or MK-801 on resting paw posture and hyperalgesia in rats

In the current study, we demonstrate that the sham-operated rats and un-operated right hind paw press normally on the floor showing a resting pain score of 0 and while CCI rats exhibit a resting pain score of 3–4, which is a hallmark of chronic pain. Mechanical hyperalgesia test confirmed the development of neuropathic pain after CCI. CCI of the sciatic nerve caused a significant increase in resting pain score (3.67 ± 0.21, $P < 0.001$; Fig. 2) and a significant decrease in pain threshold (63.33 ± 3.80, $P < 0.001$; Fig. 3) compared to sham group values (0.00 ± 0.00 and 116.7 ± 2.79, respectively). A single administration of amit alone demonstrated a significant decrease in resting pain score compared with saline-treated group [$F(2, 15) = 52.5$; $P < 0.01$; Fig. 2] or in combination with AG [$F(2, 15) = 52.5$; $P < 0.01$; Fig. 2] or MK-801[$F(2, 15) = 7.38$; $P = 0.006$; Fig. 2] compared with a single dose of amit. In the present study, prior

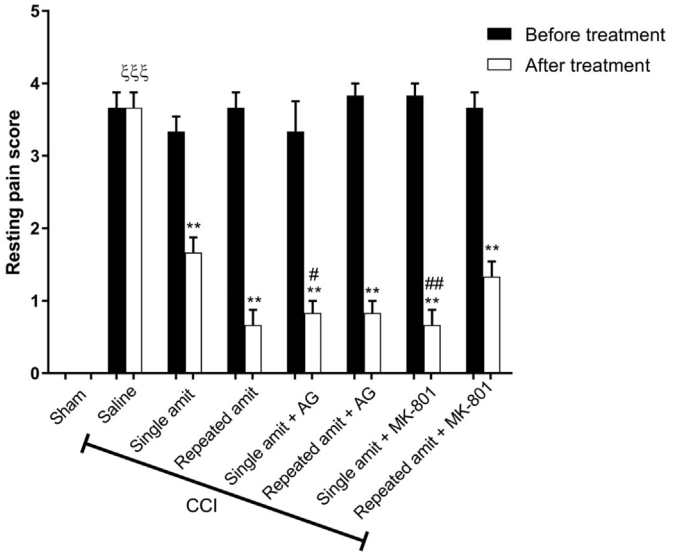

Fig. 2. The effect of single and repeated administration of amitriptyline (amit) alone and in combination with aminoguanidine (AG) or MK-801 on resting paw posture in rats.

Values are means ± s.e.m. for n = 8 rats per group. Resting pain score was recorded 8 days after CCI. Data were analyzed by a one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc tests.

ξξξ Significant difference at P < 0.001 compared to sham-operated group.

** Significant difference at P < 0.01 compared to after treatment value of saline-treated group.

Significant difference at P < 0.05 compared to single amit alone group.

Significant difference at P < 0.01 compared to single amit alone group.

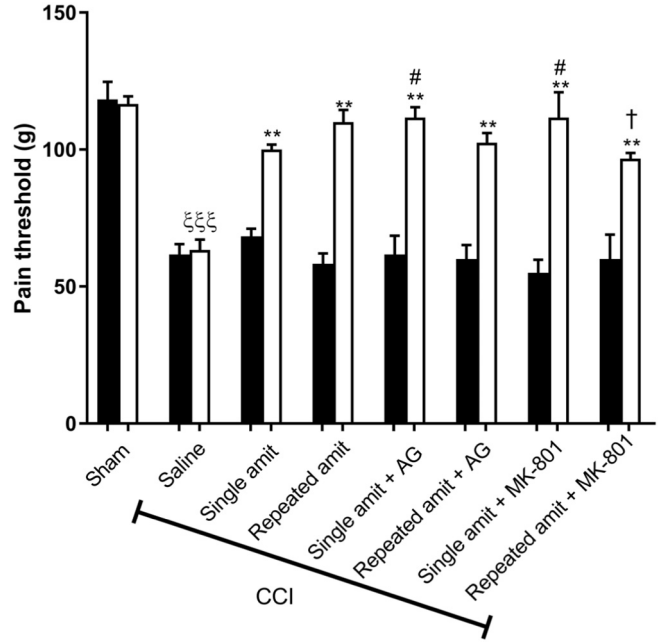

Fig. 3. The effect of single and repeated administration of amitriptyline (amit) alone and in combination with aminoguanidine (AG) or MK-801 on pain threshold in rats.

Values are means ± s.e.m. for n = 8 rats per group.

ξξξ Significant difference at P < 0.001 compared to sham-operated group.

** Significant difference at P < 0.01 compared to after treatment value of saline-treated group.

Significant difference at P < 0.05 compared to single amit alone group.

† Significant difference at P < 0.05 compared to repeated amit alone group.

injection of glutamate antagonist augmented the acute antinociceptive effects of amit while the analgesic effect of repeated amit was partially antagonized by prior administration of MK-801. On continued treatment with amit, the resting pain score decreased, particularly when combined with MK-801 [F (2, 15) = 3.095; P = 0.07; Fig. 2].

Fig. 3 indicates that there is a decrease in the pain threshold of saline-treated group (63.33 ± 3.80) compared with sham operated group (116.7 ± 2.79) (P < 0.001; Fig. 3). Administration of amit increased the pain threshold compared with pre-treatment values (68.33 ± 2.79) to (100.00 ± 1.83) for single amit administration and (58.33 ± 3.80) to (110.00 ± 4.47) for repeated amit administration (P < 0.05; Fig. 3). Concomitant administration of single amit and AG or MK-801 significantly increased the pain threshold [F (2, 15) = 4.224; P = 0.03; Fig. 3]. Again, data presented in Fig. 3 showed a significant increase when repeated doses of amit were combined with MK-801[F (2, 15) = 3.587; P = 0.05; Fig. 3].

3.3. The effect of single and repeated administration of amitriptyline (amit) alone and in combination with aminoguanidine (AG) or MK-801 on hippocampal glutamate level in rats

Because of the significant improvement in resting pain score and pain threshold when single and repeated amit were concomitantly administered with the NMDA antagonist i.e. MK-801, we studied whether the glutamate level in the hippocampus might explain these behavioral changes. No elevation in glutamate concentrations in the hippocampus of sham-operated animals was observed. Our results revealed that both single and repeated amit significantly decreased glutamate level compared with saline treated group [(F (2, 15) = 20.11, P < 0.001; Fig. 4)] which suggested that decreased glutamate in the hippocampus contributes to the antinociceptive effect of amit in CCI-induced neuropathic pain. Unexpectedly, post-CCI treatment of the rats with single dose amit + AG produced a significant increase in glutamate level [F (2,

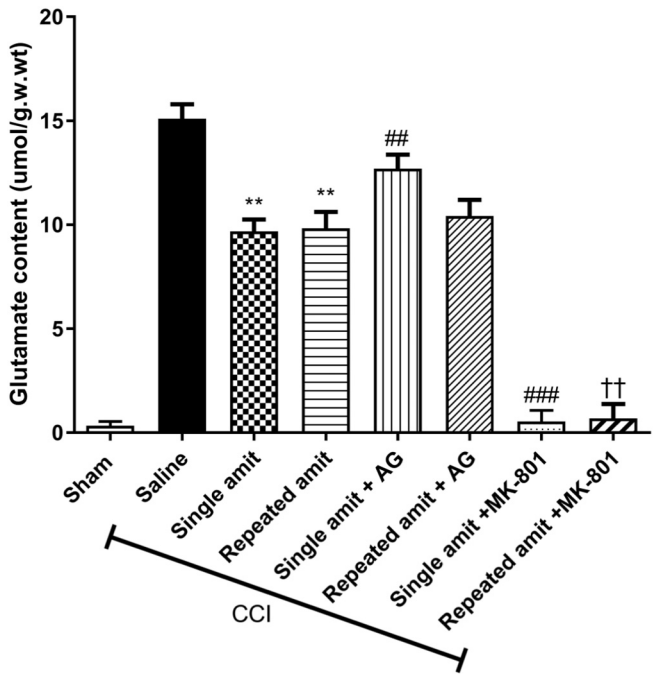

Fig. 4. The effect of single and repeated administration of amitriptyline (amit) alone and in combination with aminoguanidine (AG) or MK-801 on hippocampal glutamate level of rats.

Values are means ± s.e.m. for n = 8 rats per group.

*** Significant difference at P < 0.001 compared to saline-treated group.

Significant difference at P < 0.01 compared to single amit-treated group.

†† Significant difference at P < 0.01 compared to repeated amit-treated group.

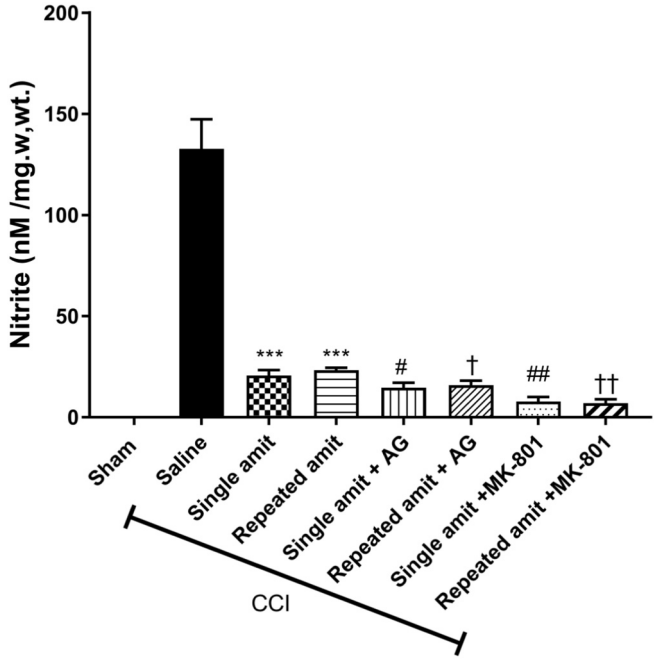

Fig. 5. The effect of single and repeated administration of amitriptyline (amit) alone and in combination with aminoguanidine (AG) or MK-801 on the level of nitrite in the hippocampus in rats. Values are means ± s.e.m. for n = 8 rats per group.
*** Significant difference at P < 0.0001 compared to saline- treated group.
# Significant difference at P < 0.05 compared to single amit-treated group.
Significant difference at P < 0.01 compared to single amit-treated group.
† Significant difference at P < 0.05 compared to repeated amit treated group.
†† Significant difference at P < 0.01 compared to repeated amit-treated group.

15) = 112.7, P = 0.005; Fig. 4] while treatment of the rats with single dose amit +AG produced significant decrease in glutamate level [F (2, 15) = 112.7, P = 0.0001; Fig. 4]. As shown in Fig. 4, 8 days after CCI of a 21-day schedule with 10 mg kg^{-1} amit once daily the basal extracellular concentrations of glutamate in the hippocampus were not significantly different from the single amit treatment (P = 0.88; Fig. 4). Repeated amit + MK-801 significantly reduced glutamate level in the hippocampus of rats (P = 0.0001, Fig. 4) while no significant difference in the effect of AG was found when added to repeated amit group (P = 0.8, Fig. 4).

3.4. The effect of single and repeated administration of amitriptyline (amit) alone and in combination with aminoguanidine (AG) or MK-801 on hippocampal nitrite level in rats

Given that, the use of AG in combination with repeated amit did not affect the glutamate level in the hippocampus and moreover, single amit +AG increased glutamate level, we measured nitrite level in the hippocampusFig. 5 shows the effect of single and repeated amit administration compared with saline-treated group or their combination with AG or MK-801. Both single and repeated amit treatments significantly reduced nitrite production [F (2, 15) = 328, P < 0.0001; Fig. 5]. Cotreatment with either iNOS blocker i.e. AG or NMDA antagonist i.e. MK-801 decreased glutamate level after single amit [F (2, 15) = 39.54, P < 0.01; Fig. 5] and after repeated amit [F (2, 15) = 115.7, p < 0.01; Fig. 5].

3.5. The effect of single and repeated administration of amitriptyline (amit) alone and in combination with aminoguanidine (AG) or MK-801 on immunohistochemical expression of iNOS in (I) cerebellum and (II) hippocampus

In the current study, the immunohistochemical analysis of brain tissues obtained from rats treated saline shows diffuse, intense iNOS positivity in Purkinje cells of cerebellum and the hippocampus. To further investigate the antihyperalgesic mechanism of single and repeated administration of amit, we questioned whether they alter the expression of iNOS in the hippocampus and the cerebellum from CCI rats. iNOS immunoreactivity was still evident as focal and mild iNOS immunostaining in the cerebellum and hippocampus of CCI rats, but not in sections from either single or repeated amit plus AG or K-801 (Fig. 6).

4. Discussion

Neuropathic pain is one of the widespread and least treatable forms of chronic pain. Nociceptive sensitization is perceived as a decreased threshold to both noxious and innocuous stimulation, called hyperalgesia and allodynia, respectively. Taking into account the involvement of glutamate and NO in pain processing, the present study investigated whether or not downregulation of iNOS upstream the decrease of glutamate level in the hippocampus of amit-treated CCI model of neuropathic pain in rats. An addition goal to compare the utility of single against repeated administration of amit in neuropathic pain.

Earlier, we demonstrated an antinociceptive effect after a single treatment with amit in a CCI model of neuropathic pain [3]. Thus, in the current study, we evaluated the analgesic effect of single or repeated administration of amit in CCI model in rats. We also demonstrated that the analgesic effect of amit is essentially through reduction of iNOS with a secondary decrease of glutamate content in the hippocampus. So, we concluded that the analgesic effect of the unit maybe mediated via inhibition of iNOS which precedes inhibition of glutamate. Inhibitor of iNOS activity has also provided evidence for the role of NO in pain sensitization.

At a dose similar to the dose used in the current study, Burke et al. [1] reported that repeated amit administration (10 mg kg^{-1}/day, i.p) for 24 days before L5-L6 spinal nerve ligation as a model of neuropathic pain and for 20 days thereafter alters cold allodynia and heat hyperalgesia in male Sprague-Dawley rats, an effect which may involve reduction of increased expression of IL-10 and enhanced the expression of TNFα, in the prefrontal cortex. However, clinical data showed that a single administration of 25 mg amit increased reaction times, but was not recorded after 2 weeks daily administration of amit when used in the treatment of patients with neuropathic pain [9]. One can argue that long-term use of amit is limited by side effects. Thus, our results of the effectiveness of single dose amit allowed on demand use. Although, Veldhuijzen et al. [24] studied that amit increased reaction times after single, but not after repeated administration for 2 weeks. But they added that tolerance develops to the sedative side effects after repeated administration of amit. These results support the use of single and repeated amit in neuropathic pain depending on the type of nociceptive stimulus.

Neuropathic pain can be maintained by a state of central sensitization with the excitatory neurotransmitter glutamate playing an essential role initiating a cascade of downstream events, such as the release of NO [25,26]. There are several hypotheses that may explain the difference in the antinociceptive action of single and repeated administration of amit. One possibility may arise from the differential interaction of each single and repeated administration of amit with monoaminergic or non-monoaminergic receptors. There is a role of several intracellular second messengers such as NO that coupled excitatory amino acid to central sensitization and persistent nociception following

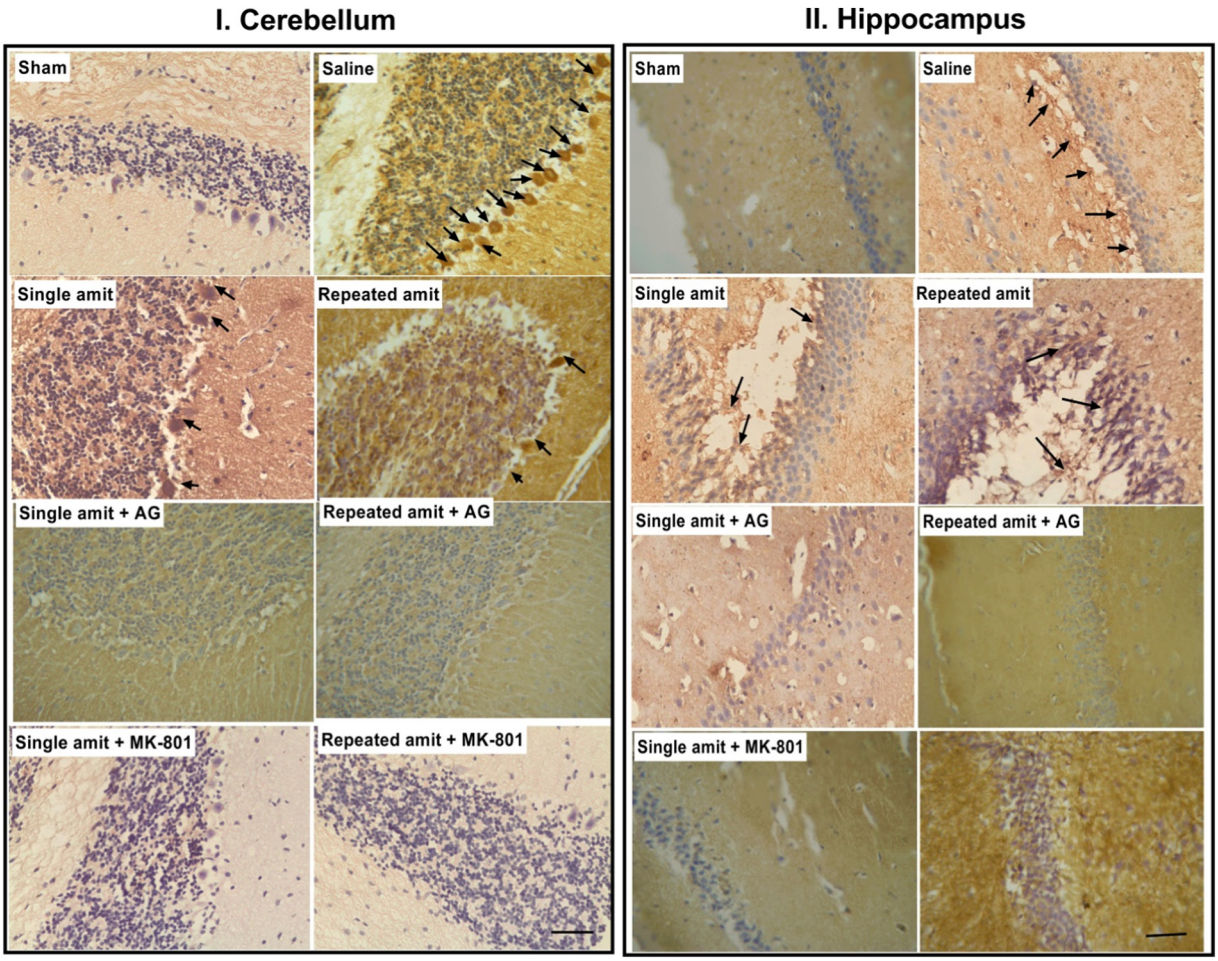

Fig. 6. Photomicrographs of (I) cerebellum and (II) hippocampus sections from rats treated with single and repeated amit alone and in combination with aminoguanidine (AG) or MK-801 on the protein expression of iNOS. Focal iNOS immunoreactivity was clearly evident (arrows) in Purkinje cells of cerebellum and hippocampus of saline–treated group. N = 3 per group. The microscope used was an Olympus CX41 optical microscope equipped with an Olympus U-CMAD3 digital camera interfaced to a computer. Magnifications for all panels, X 40 Scale bar, 100 μm. The sections were counterstained with hematoxylin. Positive staining indicated by the brown color (black arrows). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

nerve injury [16,27]. Despite all these positive studies, some studies failed to unveil antinociceptive effects of amit in neuropathic pain with cancer [28].

It has been found that amit reversed inflammatory hyperalgesia in rats by a mechanism unrelated to norepinephrine/serotonin reuptake inhibition and possibly due to NMDA receptor antagonism [29]. Moreover, amit inhibited NMDA receptor mediated-induced neuroplasticity in hippocampal formation in vivo [30]. In the same line, treatments with NMDA receptor blockers [31] or with glutamate release inhibitors extensively reduce the hyperalgesia induced in experimental rodent models of neuropathic pain [32].

The hippocampus is a vital structure implicated in memory, learning, and emotional function. Various stress-related pathological conditions are able to change the hippocampal cell renewal process [33]. Sciatic nerve injury, signs of pathological inflammation emerge in the dentate gyrus of the hippocampus, leading to decreased neurogenesis accompanied by memory disruptions [34,35]. In line with our results, Tai et al. [36] showed that co-administration of amitriptyline attenuated the development of morphine tolerance to pain and suppressed the morphine-evoked increase in glutamate levels in the CSF dialysates of chronic morphine-infused rats at least in part, by enhancing NF-κB transcription. Conversely, a recent study by Gawel et al. [37] demonstrated that glutamate agonists inhibited the development of tolerance to the antinociceptive effect of morphine. Gawel et al. [37] added that MK-801 and AMN082 (both are NMDA antagonist) did not alter the tolerance to the analgesic effect of morphine, they explained that these results are due to the use of noneffective dose of NMDA antagonists. The data of Gawel et al. [37] are surprising because glutamate has been shown to contribute to chronic treatment with amit in neuropathic pain [38].

Our results showed decreased glutamate level after single administration of Amit whereas, after repeated administration of Amit the level of glutamate was high. These observations demonstrate different effects of different modes of administration. Firstly, the effect of different time scale, i.e. faster onset of analgesic action after single administration of amit with modulation of other neurotransmitters after repeated administration [39]. Secondary, our finding could support the adaptive changes in iNOS gene expression in neuropathic pain [40]. Also, in the current study, the finding that only AG but not MK-801, potentiated the antinociceptive activity of amit suggested that NO affected other pathway(s) that was independent of glutamate [39].

5. Conclusion

The effect of amit on glutamate may represent an essential site of action for amit to decrease glutamatergic neurotransmission involved during neuropathic pain. Inducible NOS may be also involved in the analgesic effect of amit.

Declaration of competing interest

None.

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