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**Research Article**

# Alterations in glutamate metabolism in rat brain by tramadol analgesia during non-induction of pain

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**Abstract:** The objective of this study was to evaluate the effects of tramadol, a centrally acting synthetic opioid analgesic, on glutamate metabolism, without inducing pain. Male adult Wistar rats weighing 150 ± 20 g were used in the study. An effective dose of tramadol was injected subcutaneously into the rats at 0, 24, and 48 hours, and the changes in the levels of activities of glutamate dehydrogenase (GDH), glutamine synthetase (GS), glutaminase, aspartate (AAT) and alanine (AlAT) aminotransferases, and glutamine content, were recorded at 3, 6, 12, 24, 48 and 72 hours in different areas of the rat brain, viz. cerebral cortex, cerebellum, pons-medulla, hippocampus and hypothalamus. Aminotransferase activities were examined in serum also. Following the first administration of tramadol at zero hours, GDH activity showed positive deviations in all areas except pons-medulla, GS activity showed positive deviations in all areas, glutaminase activity showed negative deviations in all areas except pons-medulla, and glutamine content showed positive deviations in all areas except hippocampus, up to 12 hours. Aminotransferase activities showed differential deviations, with increases in some areas and decreases in the others. Peak deviations in all cases occurred either at 3 or at 6 hours. All parameters reverted towards near control levels by 24 hours. Following the second and third injections of tramadol at 24 and 48 hours respectively, the parameters recorded deviations at 48 and 72 hours that were slightly reverting from those at 24 hours. The results indicate differential tissue responses from different areas of the brain to the administered analgesic. Further, while the administration of opioids could affect the glutamate release *vis-à-vis* reuptake and the activation of N-methyl-D-aspartate (NMDA) receptors, these changes could presumably be associated with alterations in the levels of other parameters related to glutamate metabolism. This could be another facet of the analgesic effects of tramadol.

**Keywords:** Tramadol, glutamate metabolism, rat brain, non-induction of pain

## INTRODUCTION

Opioid analgesia has long been thought to be mediated exclusively within the central nervous system (CNS). These analgesic effects are particularly prominent in painful inflammatory conditions in both animals and humans [1,2]. Opioids are well established in the treatment of chronic pain and are increasingly used in non-malignant pain conditions. Opioid analgesics have been shown to be effective and well tolerated in selected patients with chronic and malignant pain [3, 4, 5].

Pain perception is modulated by a variety of analgesics including opioids, as well as by neurotransmitters [6]. Although it has not been studied systematically, opiates appear to have a rather poor effect on clinical central pain [7]. Different mechanisms of pain can be associated with different opioid receptor subtypes [8, 9], so that variable responses to opioids might occur [10]. Also, pain-related factors are involved in the response variability to analgesics, like poor responsiveness of neuropathic pain to opioids compared with nociceptive pain [10, 11]. The study of analgesics still poses some problems; even several years after Beecher first described methods of measuring pain and pain-relief [12].

The metabolic pathways of opioids can be determinant of analgesia [13]. Numerous studies have demonstrated that many experimental pain models are sensitive analgesic assays [14,15]). Despite this, the artificial nature of experimentally induced pain can make it difficult to extrapolate findings from laboratory studies to clinical pain treatment.

Studies on analgesia and glutamate-related aspects have been concentrated on the synthesis, release and uptake mechanisms, receptor functions and others [16-32]. The complex compartmentalization of glutamate metabolism in the brain was first noted by Waelsch and coworkers [33]. Other scientists pointed out its function in the detoxification of ammonia in

brain [34]. The action is widespread and effective, and several studies have provided support for the concept that glutamate is a transmitter in the brain [35-39]. Surveys of different regions of brain [40-42] show that glutamate is present everywhere but in high concentration (10 µmol/g) in the forebrain and cerebellum. In parts of brainstem, such as the medulla and pons, the concentration is about 50% less.

Glutamic acid, like most of the other free amino acids including aspartate, GABA and glycine, is widely distributed in neuronal cytoplasm [43,44], particularly bound to synaptic vesicles [45]. The large compartmentalization contains 85-98% of the total glutamate pool in the brain [46]. However, further subdivision of two compartments is necessary to accommodate the several functions and turnover rates of glutamate [37,47].

Several different precursors have been discussed for the synthesis of transmitter glutamate, but it is not known to what extent the transmitter glutamate pool is well differentiated from the metabolic pool [48]. Glutamine synthetase activity was high in cerebral and cerebellar cortices and lowest in medulla oblongata, corpus callosum and pons [49,50]. In intact brains, this enzyme is predominantly localized in glial cells where glutamate is metabolized to form glutamine. Compared with the other enzymes involved in glutamate metabolism, glutamate dehydrogenase (GDH) is less active than aspartate aminotransferase (AAT) but more active than glutaminase, glutamine synthetase and glutamic acid decarboxylase [51,52].

Neurotransmitters like glutamate are supposed to play a role in the maintenance of tonic pain [29]. Glutamate is the predominant excitatory amino acid neurotransmitter in the mammalian CNS acting on N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), kainate, and metabotropic receptors [29,30]. Morphine is known to inhibit the enzymes producing aspartate and glutamate [16,17] from asparagine and glutamine respectively [21], resulting in decreased levels of excitatory amino acids. Other enzymes such as aminotransferases play a very important role in cerebral metabolism as they are associated with amino acid metabolism. Aspartate aminotransferase (AAT) and alanine aminotransferase (AlAT) function at a strategic point as a link between the carbohydrate and protein metabolism. AAT catalyzes the interconversion of aspartic acid and α-ketoglutarate to oxaloacetate and glutamate, while the interconversion of alanine and α-ketoglutarate to pyruvate and glutamate is catalyzed by AlAT [53,54]. These two enzymes regulate the balance between protein and carbohydrate metabolism by providing keto-acids for the Krebs cycle and gluconeogenesis. Glutamate metabolism in the brain is studied by a variety of techniques. The most common approach to study the cerebral glutamate metabolism is the basic biochemistry involving the measurement of enzyme activities and levels of other metabolites [55].

Notwithstanding the available studies on glutamate and related metabolites and enzymes under analgesia, in the light of the importance of this segment of neurotransmitter system, the present study has been attempted to examine the changes in certain parameters related glutamate metabolism in healthy rats without induction of pain, in order to assess whether the changes differ from those during pain and analgesia, and during preemptive analgesia.

## MATERIALS AND METHODS

### Procurement and maintenance of experimental animals

Male adult Wistar rats weighing 150 ± 20 g were used as the experimental animals for the study. The rats were purchased from the Indian Institute of Science (I.I.Sc.), Bangalore, India, and were maintained in the animal house of S.V. University in polypropylene cages under laboratory conditions of 28 ± 2ºC temperature, LD 12:12 photoperiod and 75% relative humidity. The rats were fed with standard pellet diet and water *ad libitum*, and were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA 438/01/a/cpcsea/dt 17.07.2001 in its resolution No: 9/IAEC/SVU/2008/dt. 04.03.2008.

### Selection of the drug

The synthetic opioid analgesic drug tramadol was selected for the present study. It was obtained as a commercial grade chemical from Apollo Pharmacy, Hyderabad.

### Dosage for administration

After the rats were acclimated to the laboratory conditions, they were divided into groups depending on the dosage and time for sacrificing the animals. Five groups of six rats each were housed in separate cages. Tramadol was administered at 0, 24 and 48 hours. The time periods chosen for assays of the parameters were 3, 6, 12, 24, 48 and 72 hours.

All doses were given in the morning between 9 and 10 h, keeping in view the altered activity of rats during the nights compared to the daytime. Controls were maintained individually for each group.

Tramadol dosage was administered according to $ED_{50}$ value obtained in rats at 31 mg/kg in hotplate test conducted by Giusti *et al.* [56]. In pharmacology, "effective dose" is the minimal dose that produces the desired effect of the drug. The dosage that produces a desired effect in half of the test population is referred to as the $ED_{50}$, for "Effective Dose, 50%".

**Isolation of tissues**

The present study was carried out on different areas of the brain, viz. cerebral cortex (CC), cerebellum (CB), pons-medulla (PM), hippocampus (HI) and hypothalamus (HY). The animals were sacrificed at the chosen time periods mentioned above. The brain was isolated immediately and placed on a chilled glass plate. The brain areas were separated and immediately frozen in liquid nitrogen (-180ºC) and then stored at -70ºC until further use.

**Assay of glutamate dehydrogenase (GDH) (L-Glutamate; NAD Oxidoreductase; E.C. 1.4.1.3.) activity**

GDH activity was assayed by the method of Lee and Lardy [57]. Tissue homogenates (5%) were prepared in 0.25 M ice-cold sucrose solution, and the contents were centrifuged at 1000 x g for 15 minutes. The supernatant was used as the enzyme source.

The reaction mixture in a total volume of 2.0 ml contained 100 µmoles of phosphate buffer (pH 7.4), 40 µmoles of sodium glutamate, 0.1 µmoles of NAD, 2 µmoles of INT and 0.1ml of the enzyme source. The reaction mixture was incubated at 37ºC for 30 minutes. The reaction was stopped by adding 5 ml of glacial acetic acid and the formazan formed was extracted overnight into 5 ml of toluene. The intensity of the color was read at 495 nm against a toluene blank. The enzyme activity was expressed as µmoles of formazan formed/mg protein/h.

**Assay of glutaminase (L-glutamine aminohydrolase; E.C. 3.5.1.2) activity**

Glutaminase activity was assayed by the method of Meister [58]. Tissue homogenates (5%) were prepared in cold double-distilled water and centrifuged at 2000 x g for 15 minutes. The supernatants were collected, which served as the enzyme source. The reaction mixture contained 50 µmoles of L-glutamine freshly prepared in sodium acetate buffer (pH 4.9) and 0.3ml of enzyme source**.** The contents were incubated at 37ºC for 30 minutes. The reaction was stopped by adding 1.0 ml of 10% TCA. The contents were centrifuged at 1000 x g for 5 minutes. To the supernatant, 1.0 ml of 15% NaOH and 1.0 ml of Nessler's reagent were added. The intensity of color developed was read at 490 nm against a reagent blank. The enzyme activity was expressed as µmoles of ammonia/mg protein/h.

**Assay of glutamine synthetase (L-glutamine ATP ammonia ligase; E.C. 6.3.1.2) activity**

Glutamine synthetase activity was assayed by the method of Wu [59]. Brain homogenates (10%) were prepared in cold double-distilled water and centrifuged at 1000 x g for 10 minutes. The supernatant was used as the enzyme source. The reaction mixture in a total volume of 1.0 ml contained 80 µmoles of imidazole buffer (pH 7.2), 20 µmoles of magnesium chloride, 0.1 ml of 2-mercapto-ethanol, 50 µmoles of L-glutamate (pH 7.2), 10 µmoles of ATP, 100 µmoles of hydroxylamine (pH 7.2), and 0.1 ml of enzyme source. The reaction mixture was incubated at 37ºC for 15 minutes. The reaction was stopped by adding 1.5 ml of ferric chloride (0.37 M)**.** The contents were centrifuged at 1000 x g for 15 minutes. The clear supernatant was read at 535 nm against a reagent blank. The glutamine synthetase activity was expressed as µmoles of γ-glutamyl hydroximate formed/mg protein/h.

**Estimation of glutamine content**

Glutamine content was estimated by acid hydrolysis method as described by Colowick and Kaplan [60]. Homogenates (10%) of the tissue were prepared in cold double-distilled water and centrifuged at 1000 x g for 15 minutes. To 0.1 ml of the supernatant 0.2 ml of 10% $H_2SO_4$ was added, and the tubes were kept in boiling water bath for 15 minutes and then cooled. The contents were centrifuged, and to the supernatant 0.3 ml of 10% sodium hydroxide was added and the mixture was made up to 2 ml with distilled water. Ammonia was estimated by nesslerization. The glutamine content was expressed as µmoles of $NH_3$/g wet wt of the tissue.

**Assay of aspartate aminotransferase (AAT) ((L-aspartate-2-oxoglutarate aminotransferase; EC. 2.6.1.1) activity**

AAT activity was estimated by the method of Reitman and Frankel [61]. Tissue homogenates (5%) were prepared in 0.25 M ice-cold sucrose solution and centrifuged at 1000 x g for 15 minutes. The supernatant was used as the enzyme source. The reaction mixture in a total volume of 2 ml contained 100 µmoles of phosphate buffer (pH 7.2), 40 µmoles of L-aspartic acid, 2 µmoles of α-ketoglutarate and 0.3 ml of the enzyme source. After incubating the reaction mixture at 37ºC for 30 minutes, the reaction was stopped by the addition of 1 ml of 0.001 N 2, 4-dinitrophenyl-hydrazine and allowed to stand at room temperature for 20 minutes. The color developed by the addition of 10 ml 0.4 N sodium hydroxide was read at 545 nm against a reagent blank. The enzyme activity was expressed as µmoles of pyruvate formed/mg protein/h.

**Assay of alanine aminotransferase (AlAT) (DL–alanine–2-oxoglutarate aminotransferase; E.C. 26.1.2) activity**

AlAT activity was assayed by the method of Reitman and Frankel [61]. Tissue homogenates (5%) were prepared in 0.25 M ice-cold sucrose and centrifuged at 1000 x g for 15 minutes. The supernatant was used as the enzyme source. The reaction mixture in a total volume of 2 ml contained 100 µmoles of phosphate buffer (pH 7.2), 60 µmoles of L-alanine, 2 µmoles moles of α-ketoglutarate and 0.3 ml of the enzyme source. Rest of the procedure was the same as described for AAT. The enzyme activity was expressed as µmoles of pyruvate formed/mg protein/h.

For serum AAT and AlAT, the abdomen was cut open and the blood was collected into a vial, allowed to stand for a certain period of time and centrifuged to get a clear straw-colored serum. 0.2 ml of serum was used as the enzyme source. Rest of the procedure was the same as for the estimation of AAT and AlAT described above.

**Statistical treatment of data**

All the assays were carried out with six separate replicates from each group. The mean and standard deviation (SD) were worked out using INSTAT statistical software, and Analysis of Variance (ANOVA) was done using SPSS statistical software using Basic Programming techniques on IBM compatible personal computer for different parameters. Difference between control and experimental assays was considered significant at $P < 0.05$.

**RESULTS**

The results on the effects of administration of effective doses of tramadol at 0, 24 and 48 hours on the levels of parameters related to glutamate metabolism at 3, 6, 12, 24, 48 and 72 hours are presented in Tables 1 to 7.

The glutamate dehydrogenase (GDH) activity in the brain of control rats was highest in the cerebral cortex (CC), followed by the cerebellum (CB), hypothalamus (HY), and pons-medulla (PM) and hippocampus (HI). Following the injection of initial effective dose of tramadol at zero hours, the GDH activity showed an increase in all the areas of the brain, except in PM where it recorded a decrease, with the peak deviation occurring at 3 or 6 h in different areas after the initial dose. Following this, the activity showed a recovery towards the control levels in all the areas by 24 h (Table 1).

When the effective dose of tramadol was administered for the 2nd and 3rd times at 24 and 48 h respectively, the GDH activity showed higher levels at 48 and 72 h compared to the activity level at 24 h in CC, CB, HI and HY. The enzyme activity in PM decreased further at 48 and 72 h compared to the level at 24 h (Table 1).

In control rats, the activity of glutamine synthetase (GS) was found to be highest in CC and lowest in HI, with intermediate levels in the other areas. With initial effective dose of tramadol, the GS activity showed an increase in all areas of the brain, with the highest increase of 49.09% at 6 h in HI. Following this, the activity reverted towards the control levels in all the areas by 24 h (Table 2).

Following the administration of effective dose of tramadol for the 2nd and 3rd times at 24 and 48 h respectively, the GS activity showed elevated levels at 48 and 72 h in all areas of the brain compared to the activity level at 24 h (Table 2).

Glutaminase activity in the brain of control rats was highest in HI, and least in PM, with the other areas showing activity levels intermediate between those of HI and PM. Following the administration of initial effective dose of tramadol, the activity showed a decrease in all areas of the brain except in pons-medulla where it showed a moderate increase. The highest decrease was 26.32% in cerebellum at 6 h. The enzyme activity recovered towards the control levels by 24 h in all areas (Table 3).

For the administration of effective dose of tramadol for the 2nd and 3rd times at 24 and 48 h respectively, the glutaminase activity showed a decrease at 48 and 72 h in all areas of the brain except in CB and PM, where it showed an increase (Table 3).

In control rats the glutamine content was found to be highest in PM and lowest in CB, with intermediate levels in other areas. Following the administration of initial effective dose of tramadol, the glutamine content showed an increase in all areas of the brain, except in HI where it showed a decrease. The highest increase was 43.09% in CC at 6 h. Following the highest increase or decrease, the glutamine content returned towards control levels by 24 h in all areas of the brain (Table 4).

When the effective dose of tramadol was administered for the 2nd and 3rd times at 24 and 48 h respectively, the glutamine content showed further elevation from the level at 24 h in all areas of the brain, except in HI where it showed further decrease from the level at 24 h (Table 4).

In control rats, maximal activity of aspartate aminotransferase (AAT) was recorded in CB and minimal activity was recorded in HI. The remaining areas showed intermediate levels of activity. With the injection of initial effective dose of tramadol, the AAT activity showed an elevation in CB and HI, while a decrease in activity was recorded in other areas. Following the highest increases or decreases, the enzyme activity reverted back towards the control levels by 24 h (Table 5).

Following the administration of effective dose of tramadol for the 2nd and 3rd times at 24 and 48 h respectively, the AAT activity showed slight elevations at 48 and 72 h in all areas of the brain compared to the levels at 24 h, except in CB where the activity showed a decrease from the level at 24 h, although still maintaining positive deviations (Table 5).

In the control rats, maximal activity of alanine aminotransferase (AlAT) was recorded in CB and minimal activity was recorded in HY, with intermediate

levels in other areas. Following the administration of initial effective dose of tramadol, the AAT activity recorded differential changes, with an elevation in CB, PM and HI and a decrease in CC and HY. Following this, the enzyme activity reverted back towards the control levels in all areas by 24 h (Table 6).

For the administration of effective dose of tramadol for the 2nd and 3rd times at 24 and 48 h respectively, the AlAT activity showed slight increases at 48 and 72 h in all areas of the brain compared to the levels at 24 h (Table 6).

In the serum of control rats, AAT activity recorded a higher level than AlAT activity. Both AAT and AlAT showed a decrease in activity upon the administration of initial effective dose of tramadol. Following this, the activities of both the enzymes reverted back towards the control levels by 24 h (Table 7).

When the effective dose of tramadol was administered for the 2nd and 3rd times at 24 and 48 h respectively, the AAT and AlAT activities in serum showed positive deviations at 48 and 72 h compared to the levels at 24 h (Table 7).

**Table-1: Changes in glutamate dehydrogenase (GDH) activity levels in different brain regions of rats at different time periods after administration of effective dose of tramadol at 0, 24 and 48 hours. The values are expressed as µ moles formazan formed/mg protein/h.**

| Brain Area | Indices | Control | 3 Hours | 6 Hours | 12 Hours | 24 Hours | 48 Hours | 72 Hours |
|---|---|---|---|---|---|---|---|---|
| Cerebral Cortex | Mean | 1.52 | 1.89 | 1.78 | 1.65 | 1.58 | 1.61 | 1.63 |
| | SD (±) | 0.09 | 0.14 | 0.11 | 0.13 | 0.10 | 0.09 | 0.12 |
| | % Change | | +24.34* | +17.10* | +8.55 | +3.95 | +5.92 | +7.24 |
| Cerebellum | Mean | 1.40 | 1.58 | 1.61 | 1.52 | 1.45 | 1.47 | 1.50 |
| | SD (±) | 0.09 | 0.07 | 0.10 | 0.07 | 0.06 | 0.08 | 0.08 |
| | % Change | | +12.86* | +15.00* | +8.57* | +3.57 | +5.00 | +7.14 |
| Pons-Medulla | Mean | 0.54 | 0.35 | 0.36 | 0.41 | 0.48 | 0.45 | 0.44 |
| | SD (±) | 0.09 | 0.06 | 0.08 | 0.07 | 0.06 | 0.05 | 0.07 |
| | % Change | | -35.19* | -33.34* | -24.08* | -11.11 | -16.66* | -18.52* |
| Hippocampus | Mean | 0.54 | 0.71 | 0.65 | 0.61 | 0.59 | 0.60 | 0.62 |
| | SD (±) | 0.07 | 0.10 | 0.08 | 0.09 | 0.07 | 0.06 | 0.07 |
| | % Change | | +31.48* | +20.37* | +12.96 | +9.26 | +11.11 | +14.81* |
| Hypothalamus | Mean | 0.96 | 1.30 | 1.33 | 1.18 | 1.09 | 1.12 | 1.15 |
| | SD (±) | 0.06 | 0.09 | 0.09 | 0.08 | 0.11 | 0.07 | 0.12 |
| | % Change | | +35.42* | +38.54* | +22.92* | +13.54* | +16.66* | +19.79* |

Each value is the mean ± standard deviation (S.D.) of observations from six separate experiments. *The values are significant at least at P < 0.05 in SNK test.

**Table-2: Changes in glutamine synthetase (GS) activity levels in different brain regions of rats at different time periods after administration of effective dose of tramadol at 0, 24 and 48 hours. The values are expressed as µ moles of γ- glutamyl hydroxymate formed/mg protein/h.**

| Brain Area | Indices | Control | 3 Hours | 6 Hours | 12 Hours | 24 Hours | 48 Hours | 72 Hours |
|---|---|---|---|---|---|---|---|---|
| Cerebral Cortex | Mean | 1.33 | 1.84 | 1.86 | 1.57 | 1.45 | 1.48 | 1.51 |
| | SD (±) | 0.13 | 0.15 | 0.18 | 0.13 | 0.14 | 0.16 | 0.17 |
| | % Change | | +38.34* | +39.85* | +18.04* | +9.02 | +11.28 | +13.53 |
| Cerebellum | Mean | 0.91 | 1.09 | 1.11 | 0.98 | 0.90 | 0.95 | 0.97 |
| | SD (±) | 0.06 | 0.08 | 0.10 | 0.09 | 0.08 | 0.07 | 0.09 |
| | % Change | | +19.78* | +21.98* | +7.69 | -1.1 | +4.39 | +6.59 |
| Pons-Medulla | Mean | 0.72 | 0.98 | 0.93 | 0.84 | 0.76 | 0.78 | 0.81 |
| | SD (±) | 0.08 | 0.09 | 0.09 | 0.06 | 0.07 | 0.08 | 0.07 |
| | % Change | | +36.11* | +29.17* | +16.67* | +5.56 | +8.33 | +12.50* |
| Hippocampus | Mean | 0.55 | 0.78 | 0.82 | 0.72 | 0.58 | 0.61 | 0.63 |
| | SD (±) | 0.06 | 0.08 | 0.06 | 0.08 | 0.09 | 0.07 | 0.09 |
| | % Change | | +41.81* | +49.09* | +30.91* | +5.45 | +10.90 | +14.54* |
| Hypothalamus | Mean | 1.24 | 1.71 | 1.74 | 1.44 | 1.31 | 1.35 | 1.38 |
| | SD (±) | 0.10 | 0.12 | 0.08 | 0.13 | 0.11 | 0.12 | 0.11 |
| | % Change | | +37.90* | +40.32* | +16.13* | +5.64 | +8.87 | +11.29* |

Each value is the mean ± standard deviation (S.D.) of observations from six separate experiments. *The values are significant at least at P < 0.05 in SNK test.

**Table-3: Changes in the glutaminase activity levels in different brain regions of rats at different time periods after administration of effective dose of tramadol at 0, 24 and 48 hours. The values are expressed as µ moles of ammonia formed/mg protein/h.**

| Brain Area | Indices | Control | 3 Hours | 6 Hours | 12 Hours | 24 Hours | 48 Hours | 72 Hours |
|---|---|---|---|---|---|---|---|---|
| Cerebral Cortex | Mean | 0.22 | 0.19 | 0.18 | 0.20 | 0.21 | 0.22 | 0.23 |
| | SD (±) | 0.04 | 0.03 | 0.04 | 0.05 | 0.05 | 0.03 | 0.03 |
| | % Change | | -13.64 | -18.18 | -9.09 | -4.55 | 0.00 | +4.54 |
| Cerebellum | Mean | 0.19 | 0.15 | 0.14 | 0.17 | 0.18 | 0.18 | 0.17 |
| | SD (±) | 0.03 | 0.03 | 0.04 | 0.04 | 0.05 | 0.03 | 0.05 |
| | % Change | | -21.05* | -26.32* | -10.53 | -5.26 | -5.26 | -10.53 |
| Pons-Medulla | Mean | 0.17 | 0.20 | 0.19 | 0.18 | 0.16 | 0.18 | 0.19 |
| | SD (±) | 0.03 | 0.03 | 0.03 | 0.05 | 0.05 | 0.04 | 0.04 |
| | % Change | | +17.64 | +11.76 | +5.88 | -5.82 | +5.88 | +11.76 |
| Hippocampus | Mean | 0.27 | 0.20 | 0.21 | 0.23 | 0.26 | 0.27 | 0.26 |
| | SD (±) | 0.03 | 0.03 | 0.04 | 0.03 | 0.04 | 0.04 | 0.05 |
| | % Change | | -25.93* | -22.22* | -14.82* | -3.70 | 0.00 | -3.70 |
| Hypothalamus | Mean | 0.24 | 0.19 | 0.21 | 0.22 | 0.25 | 0.24 | 0.23 |
| | SD (±) | 0.03 | 0.04 | 0.04 | 0.05 | 0.05 | 0.03 | 0.03 |
| | % Change | | -20.83* | -12.50 | -8.33 | +4.17 | 0.00 | -4.17 |

Each value is the mean ± standard deviation (S.D.) of observations from six separate experiments. *The values are significant at least at $P < 0.05$ in SNK test.

**Table-4: Changes in glutamine content in different brain regions of rats at different time periods after administration of effective dose of tramadol at 0, 24 and 48 hours. The values are expressed as µg of glutamine/g wet wt of tissue.**

| Brain Area | Indices | Control | 3 Hours | 6 Hours | 12 Hours | 24 Hours | 48 Hours | 72 Hours |
|---|---|---|---|---|---|---|---|---|
| Cerebral Cortex | Mean | 1.23 | 1.67 | 1.76 | 1.59 | 1.39 | 1.43 | 1.47 |
| | SD (±) | 0.09 | 0.07 | 0.12 | 0.14 | 0.14 | 0.16 | 0.13 |
| | % Change | | +35.77* | +43.09* | +29.27* | +13.01* | +16.26* | +19.51* |
| Cerebellum | Mean | 0.53 | 0.73 | 0.70 | 0.66 | 0.61 | 0.63 | 0.64 |
| | SD (±) | 0.09 | 0.07 | 0.08 | 0.11 | 0.08 | 0.06 | 0.07 |
| | % Change | | +37.73* | +32.07* | +24.53* | +15.09 | +18.86* | +20.75* |
| Pons-Medulla | Mean | 4.40 | 4.88 | 4.86 | 4.67 | 4.59 | 4.68 | 4.71 |
| | SD (±) | 0.12 | 0.10 | 0.09 | 0.13 | 0.16 | 0.12 | 0.14 |
| | % Change | | +10.90* | +10.45* | +6.14 | +4.32 | +6.36 | +7.04 |
| Hippocampus | Mean | 2.96 | 2.31 | 2.25 | 2.44 | 2.75 | 2.69 | 2.61 |
| | SD (±) | 0.13 | 0.09 | 0.09 | 0.13 | 0.12 | 0.14 | 0.15 |
| | % Change | | -21.96* | -23.99* | -17.57* | -7.09 | -9.12 | -11.82* |
| Hypothalamus | Mean | 0.65 | 0.89 | 0.95 | 0.78 | 0.71 | 0.73 | 0.76 |
| | SD (±) | 0.08 | 0.07 | 0.09 | 0.08 | 0.11 | 0.12 | 0.13 |
| | % Change | | +36.92* | +46.15* | +20.00* | +9.23 | +12.31 | +16.92 |

Each value is the mean ± standard deviation (S.D.) of observations from six separate experiments. *The values are significant at least at $P < 0.05$ in SNK test.

**Table-5: Changes in aspartate aminotransferase (AAT) activity levels in different brain regions of rats at different time periods after administration of effective dose of tramadol at 0, 24 and 48 hours. The values are expressed as µ moles of pyruvate formed/mg protein/h.**

| Brain Area | Indices | Control | 3 Hours | 6 Hours | 12 Hours | 24 Hours | 48 Hours | 72 Hours |
|---|---|---|---|---|---|---|---|---|
| Cerebral Cortex | Mean | 0.79 | 0.59 | 0.54 | 0.71 | 0.75 | 0.81 | 0.84 |
| | SD (±) | 0.09 | 0.06 | 0.07 | 0.09 | 0.06 | 0.08 | 0.09 |
| | % Change | | -25.32* | -31.65* | -10.13* | -5.06 | +2.53 | +6.33 |
| Cerebellum | Mean | 0.91 | 1.17 | 1.12 | 1.05 | 1.01 | 0.99 | 0.95 |
| | SD (±) | 0.09 | 0.06 | 0.07 | 0.06 | 0.08 | 0.08 | 0.09 |
| | % Change | | +30.00* | +24.44* | +16.66* | +12.22* | +8.79 | +4.39 |
| Pons-Medulla | Mean | 0.62 | 0.41 | 0.45 | 0.52 | 0.57 | 0.64 | 0.63 |
| | SD (±) | 0.06 | 0.05 | 0.08 | 0.06 | 0.09 | 0.07 | 0.08 |
| | % Change | | -33.87* | -27.42* | -16.13* | -8.07 | +3.22 | +1.61 |
| Hippocampus | Mean | 0.51 | 0.65 | 0.67 | 0.59 | 0.56 | 0.55 | 0.53 |
| | SD (±) | 0.06 | 0.07 | 0.08 | 0.07 | 0.05 | 0.08 | 0.09 |
| | % Change | | +27.41* | +31.37* | +15.69* | +9.8 | +7.84 | +3.92 |
| Hypothalamus | Mean | 0.56 | 0.31 | 0.29 | 0.43 | 0.51 | 0.58 | 0.59 |
| | SD (±) | 0.07 | 0.06 | 0.08 | 0.06 | 0.09 | 0.07 | 0.262 |
| | % Change | | -44.64* | -48.22* | -23.22* | -8.93 | +3.57 | +5.36 |

Each value is the mean ± standard deviation (S.D.) of observations from six separate experiments. *The values are significant at least at $P < 0.05$ in SNK test.

**Table-6: Changes in alanine aminotransferase (AlAT) activity levels in different brain regions of rats at different time periods after administration of effective dose of tramadol at 0, 24 and 48 hours. The values are expressed as µ moles of pyruvate formed/mg protein/h.**

| Brain Area | Indices | Control | 3 Hours | 6 Hours | 12 Hours | 24 Hours | 48 Hours | 72 Hours |
|---|---|---|---|---|---|---|---|---|
| Cerebral Cortex | Mean | 0.66 | 0.52 | 0.55 | 0.63 | 0.75 | 0.71 | 0.68 |
| | SD (±) | 0.08 | 0.06 | 0.06 | 0.07 | 0.09 | 0.08 | 0.09 |
| | % Change | | -21.22* | -16.67* | -4.55 | +13.64* | +7.58 | +3.03 |
| Cerebellum | Mean | 0.86 | 1.19 | 1.15 | 0.99 | 0.91 | 0.93 | 0.94 |
| | SD (±) | 0.07 | 0.08 | 0.07 | 0.05 | 0.09 | 0.10 | 0.09 |
| | % Change | | +38.37* | +33.72* | +14.12* | +5.81 | +8.14 | +9.30 |
| Pons-Medulla | Mean | 0.65 | 0.81 | 0.78 | 0.72 | 0.67 | 0.69 | 0.71 |
| | SD (±) | 0.08 | 0.07 | 0.06 | 0.09 | 0.08 | 0.07 | 0.09 |
| | % Change | | +24.61* | +20.00* | +10.77 | +3.08 | +6.15 | +9.23 |
| Hippocampus | Mean | 0.59 | 0.77 | 0.80 | 0.71 | 0.66 | 0.61 | 0.63 |
| | SD (±) | 0.07 | 0.06 | 0.06 | 0.09 | 0.08 | 0.07 | 0.215 |
| | % Change | | +30.51* | +35.59* | +20.33* | +11.86 | +3.39 | +6.78 |
| Hypothalamus | Mean | 0.50 | 0.38 | 0.33 | 0.42 | 0.47 | 0.49 | 0.52 |
| | SD (±) | 0.05 | 0.06 | 0.05 | 0.08 | 0.07 | 0.08 | 0.09 |
| | % Change | | -24.00* | -34.00* | -16.00* | -6.00 | -2.00 | +4.00 |

Each value is the mean ± standard deviation (S.D.) of observations from six separate experiments. *The values are significant at least at $P < 0.05$ in SNK test.

**Table-7: Changes in the activity levels of serum aspartate (AAT) and alanine (AlAT) aminotransferases in rats at different time periods after administration of effective dose of tramadol at 0, 24 and 48 hours. The values are expressed as µ moles of pyruvate formed/mg protein/h.**

| Brain Area | Indices | Control | 3 Hours | 6 Hours | 12 Hours | 24 Hours | 48 Hours | 72 Hours |
|---|---|---|---|---|---|---|---|---|
| AAT | Mean | 40.48 | 22.63 | 27.94 | 35.18 | 38.28 | 41.51 | 41.99 |
| | SD (±) | 4.17 | 4.80 | 3.77 | 4.16 | 3.44 | 3.66 | 3.98 |
| | % Change | | -44.10* | -30.98* | -13.09 | -5.44 | +2.54 | +3.73 |
| ALAT | Mean | 32.13 | 20.85 | 25.64 | 35.47 | 36.19 | 35.88 | 36.51 |
| | SD (±) | 3.17 | 3.89 | 3.61 | 3.02 | 3.97 | 3.24 | 3.13 |
| | % Change | | -35.11* | -20.20* | +10.39 | +12.64 | +11.67 | +13.63 |

Each value is the mean ± standard deviation (S.D.) of observations from six separate experiments. *The values are significant at least at $P < 0.05$ in SNK test.

**DISCUSSION**

The present investigation was carried out under the premise that the actions of analgesic drugs would be the same in the presence or absence of pain, and to explore the accompanying biochemical changes in different areas of the brain.

The present study was focused on examining different parameters related to glutamate metabolism, viz. activities of the enzymes glutamate dehydrogenase (GDH), glutamine synthetase (GS) glutaminase, aspartate (AAT) and alanine (AlAT) aminotransferases, and glutamine content in different areas of rat brain (aminotransferase activities were estimated in serum as well), under tramadol administration and without the induction of pain. Studies in this direction are sparse.

Glutamate is the predominant excitatory amino acid neurotransmitter in the mammalian central nervous system acting on N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and metabotropic receptors [29, 30]. Several studies have demonstrated that the excitatory amino acid (EAA) receptor system is involved in the process of opioid dependence. The chronic presence of opioid receptor agonists decreases the normal activation of NMDA receptors [22, 23]. Decreased levels of EAA result during morphine analgesia. Glutamatergic transmission may have a role in learning and memory, central pain transduction, and the pathophysiology of neuronal death after brain injury [29]. Inhibition of glutamate release or of glutamate receptors attenuates both acute and chronic pain in animal models [62]. The general increase in GDH activity found in all the brain areas in the present study following the initial administration of effective dose of tramadol points to this possibility.

Alterations in the activities of key enzymes of amino acid metabolism, like GDH, signify modulation in the turnover rates of amino acids, glutamate in particular. Endogenous production of glutamate through metabolic pathways is very important in the brain, since the transport of circulating glutamate to brain normally plays only a minor role in regulating the brain glutamate levels [63]. Glutamate can be utilized in the brain predominantly either as a metabolite in TCA cycle or for the synthesis of glutamine and GABA [64].

In the present study, the activity of glutamine synthetase recorded an increase in all areas of the brain following the initial injection of tramadol, with peaks at 3 or 6 hours. Elevation in the activity of glutamine synthetase in general depicts greater mobilization of glutamate for the synthesis of glutamine. Cohen and Brown [65] considered glutamine as a transport form of ammonia. Further support to these possibilities could be found in the general increase in glutamine content and decrease in glutaminase activity in different areas of the brain in the present study.

In the present study, AAT and AlAT activity levels showed differential pattern of changes in different brain regions following the initial injection of effective dose of tramadol. The activation or inactivation of these enzyme activities observed in the present study is an index of altered turnover of amino acids, particularly glutamate, in different brain areas. Morphine is known to inhibit the enzymes producing aspartic acid and glutamic acid from asparagine and glutamine, respectively [16, 17, 21], resulting in the decreased level of excitatory amino acids. While increased levels of lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) activities were noticed, alkaline phosphate (AP) and AlAT activities were unaffected [20]. As against this report, significant decreases have been recorded in serum AAT and AlAT activities in the present study after the initial injection of tramadol, with both the enzymes recording maximal decreases at 3 hours after injection, and then returning to about control levels by 24 hours. Notwithstanding that the nature of analgesia of tramadol is different from that of morphine, the changes recorded in aminotransferase activities are in general agreement with the observations of Koyuncuoglu *et al*. [16,17] and Bielarczyk *et al*. [21], pointing to decreased levels of excitatory amino acids.

The differential changes in AAT and AlAT activities in different areas of the brain reflect the overall decrease in their activities in the serum. Since the blood has no synthetic machinery of its own, changes in enzyme activities in serum reflect the changes in the tissues *vis-à-vis* leakages from the tissues into the blood. The tissues include not only the brain areas but those of different organs of the body. Thus, although the changes in AAT and AlAT activities in different tissues under tramadol administration are not examined in the present study, it may be surmised that tramadol causes differential changes of these activities in the tissues and a general decrease in the serum.

The present investigation demonstrates significant changes in the levels of different parameters related to glutamate metabolism in different areas of the rat brain following tramadol administration. Research in this line is necessary as the consumption of analgesic drugs is seen even when there are no actual signs or feel of pain. It is presumed from the present study that, while the administration of opioids could affect the glutamate release *vis-à-vis* reuptake and the activation NMDA receptors [22,23,29,30], these changes probably result from or at least associated with alterations in the levels of other parameters related to glutamate metabolism.

In the present study, repeated injection of tramadol at 24 and 48 h caused changes in the levels of different parameters at 48 and 72 h similar to those caused after the initial injection. Since the changes were

not recorded at short intervals after second and third dosing, as against the time schedules up to 24 hours, it could probably be surmised that the time-course of changes was similar after second and third administrations, as observed up to 24 hours for the initial administration. This indicates that tramadol could be safely administered over prolonged time periods, without adverse effects in analgesic treatment of nociceptive situations.

The above changes indicate possible alterations in glutamate and GABA levels as one of the facets of analgesia even in the absence of induction of pain, as in the present study. Despite the logical assumption that the actions of analgesic drugs would be same both in the presence or absence of pain, the biochemical actions on different areas of the brain during experimental induction of pain remain relatively unexplored.

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