Indian J Physiol Pharmacol 1999; 44 (1): 33–42

EFFECT OF GLUTAMIC ACID AND KETAMINE ON MORPHINE–INDUCED ANALGESIC RESPONSE IN RATS

PREM SUMAN*, ANITA TALWAR AND OM P. TANDON

Department of Physiology,
University College of Medical Sciences and G.T.B. Hospital,
Shahadara, Delhi – 110 095

(Received on April 15, 1999)

Abstract : Rats were treated with intraperitoneal injections of morphine (10 mg/kg) followed by glutamic acid (20 mg/kg.) and ketamine. (5 mg/kg). Pain thresholds were recorded as tail flick latencies for a period of 23 days and the mean area under curves calculated. Glutamic acid and ketamine, partially blocked the analgesic effects of morphine. Two types of effects were observed. In 4 animals, there was a partial blockade of the response, and in 2 animals there was a complete blockade followed by reversal in both the groups. It is suggested that two different mechanisms one excitatory and one inhibitory may be operating for the interaction of NMDA receptors with the opioid analgesic systems for modulating nociceptive responses.

Key words : glutamic acid, ketamine, morphine, NMDA-receptor, tail flick latency

INTRODUCTION

Recent studies have suggested an involvement of excitatory amino-acids especially N-Methyl-D-Aspartate (NMDA) in the antinociceptive action of opiates (1). Opiate drugs such as morphine are widely used in the clinical management of pain. Chronic administration of morphine to the rat by pellet implantation resulted in the development of high degree of tolerance to the analgesic effects of morphine, (2, 3, 4, 5, 6) and physical dependence. Tolerance to morphine has been shown to be associated with alteration of several receptors. Many studies have implicated the role of the NMDA receptors in producing morphine tolerance (5, 7, 8). There are a few studies to suggest the role of glycine and glutamate in NMDA receptor activity and binding in various regions of the brain and spinal cord (9, 10). However more studies are needed to elaborate the role of NMDA receptors in the chronic action of opiates. Our studies are aimed at further elucidating their role by use of effects of L-glutamic acid and ketamine on morphine-treated rats.

METHODS

Animals :

Experiments were conducted on 24 albino (Wistar) rats weighing between 300–350 gms. The animals were housed in the animal house until the commencement of the experiment, and fed, food and water

*Corresponding Author

ad libidum. They were maintained on a 12-hr. light and dark cycle, and temperature of 22°C. These animals were brought to the laboratory for one or two days to adjust to environmental conditions prior to the experiment.

Recordings of pain thresholds using Tail Flick latencies

The pain thresholds were recorded using the tail flick latencies, by the method of Coeddere and Melzack (11). During the tail flick test, the rat was held in one hand, and the distal 5 min. of the tail was immersed in a cup containing water maintained at 55°C. Time taken for the rat to flick its tail from the water was noted. If the rat did not remove the tail within 15 seconds, (cut-off latency) the trial was terminated to avoid tissue damage. During recording, the animals were restrained in tube containers, which were held by the observer while conducting the test.

Recording procedures

Morphine treatment: Control readings for pain threshold were monitored, without any drug (day 0). Recordings of tail flick latency were made at an interval of every 5 min for a period of 30 min to establish a base line latency. Subsequently, intraperitoneal injection of morphine (10 mg/kg) was started on (day 1), twice daily at 9.00 A.M. and 4.00 P.M. for a period of 10 days. On day 17, microinjections of glutamic acid (20 mg/kg) and ketamine (5 mg/kg) were given, intraperitoneally and recordings made until day 23. The NMDA modulators were given in a single dose daily at 10.00 A.M. intraperitoneally, for a period of 4 days and recordings started from day 17 onwards, till day 23.

Method of analysis

Statistical analysis: Graphic plots of tail flick latencies against time were made and the area under these curves calculated a two way ANOVA and a Tukey's (19) test were used to find out the statistical significances (Table I). The following equation was used to calculate the mean area under curve, (AUC) for every 5 mins.

AUC = [½ x base x (sum of heights)]

RESULTS

Experimental groups:

Control readings of tail flick latencies were taken on day zero, every 5 min, till a base line latency established. Six of these rats received peripheral intraperitoneal injections of glutamic acid (20 mg/kg), and 6 received ketamine (5 mg/kg). The other 12 rats were treated with morphine for 9 days, followed by injections of NMDA modulators. All recordings were taken till day 23. Six Rats also received intraperitoneal injections of 1 ml of 0.9% of saline.

Effects of glutamic acid on morphine induced in tail flick latency: In 6 animals studied, morphine (10 mg/kg) produced an increase in the tail flick latencies in 5 animals resulting in an analgesic state, as observed by an increase in the mean area under curve (AUC), whereas in 1 animal, it did not produce any significant change. Glutamic acid (20 mg/kg) produced a partial block of the response in 4 of these animals, out of which, in 2 animals the response was significantly blocked, and in the other 2, the response was blocked alongwith a reversal. In the other 2 animals, there was no change. (Fig. 1a & Fig. 1b).

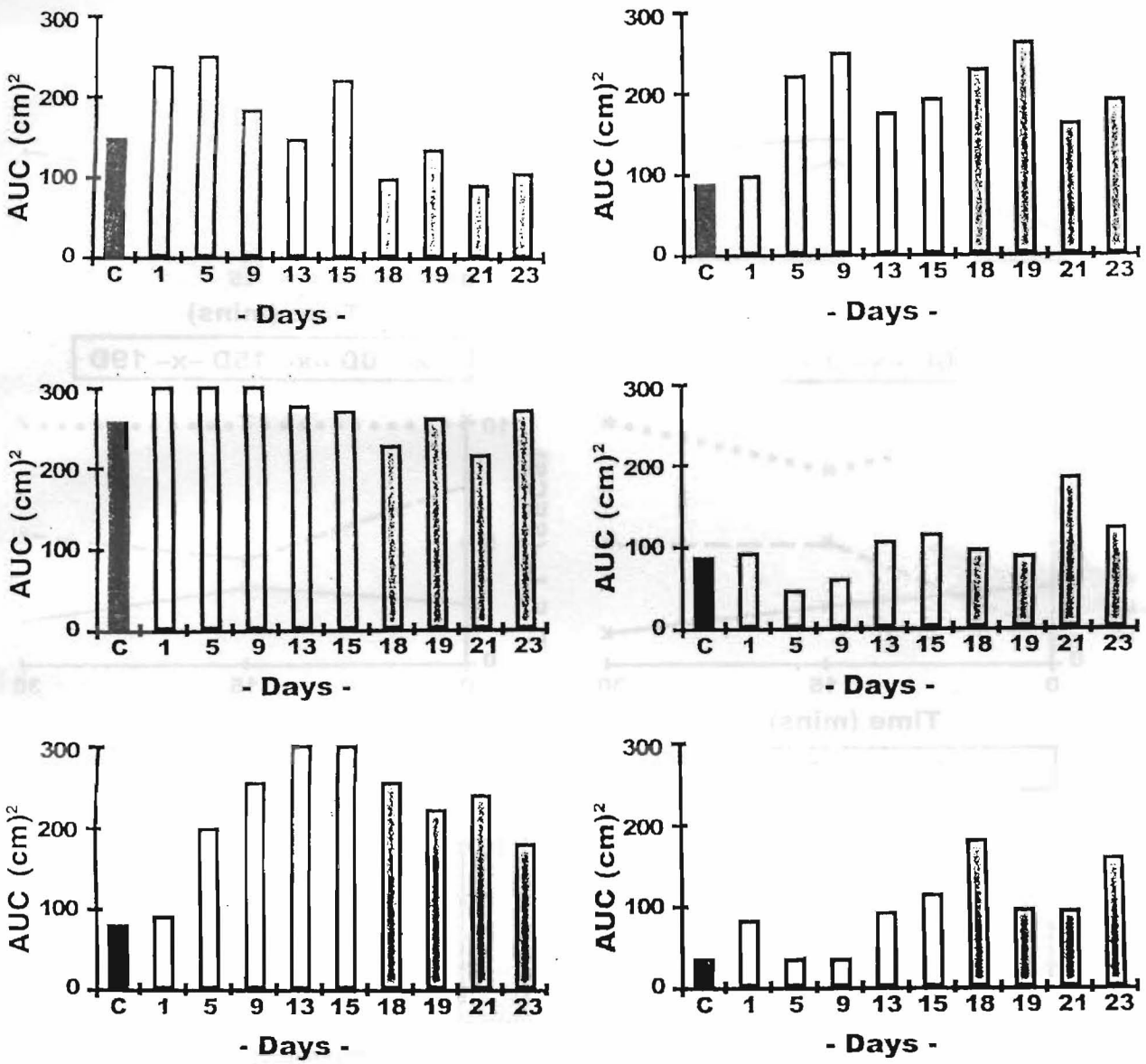

Fig. 1 a : Mean Areas under curve for effects of glutamic acid on morphine induced analgesia. ■ – Control, □ – After morphine 1–15 days, ▒ – After glutamic acid 18–23 days.

Effects of ketamine on morphine-induced changes in tail flick latencies: Fig. 2a & 2b shows the effects of Ketamine (5 mg/kg) on morphine induced changes in tail flick latencies, and mean area under curve. Ketamine produced a significant partial block of the analgesic response as observed after morphine in 4 of these animals, whereas in the other 2 rats it was seen that there was a partial blockade of the response followed by a reversal of the response.

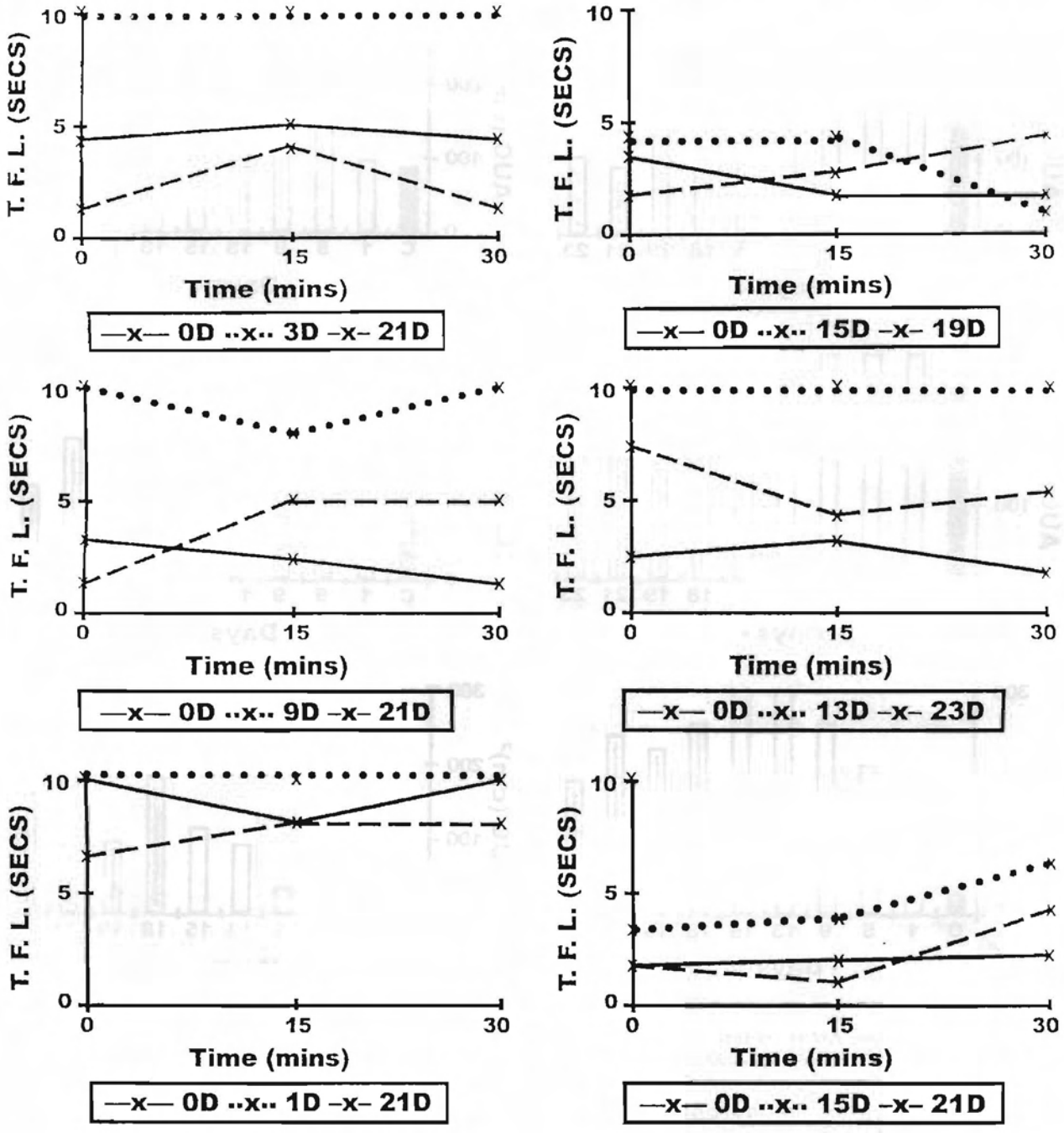

Fig. 1b : Tail flick latencies in rats treated with morphine followed by glutamic acid. ____*____ Control, ____*____ maximum analgesic effect after morphine .....*..... maximum analgesic effect after glutamic acid D-days on which the effects have been shown.

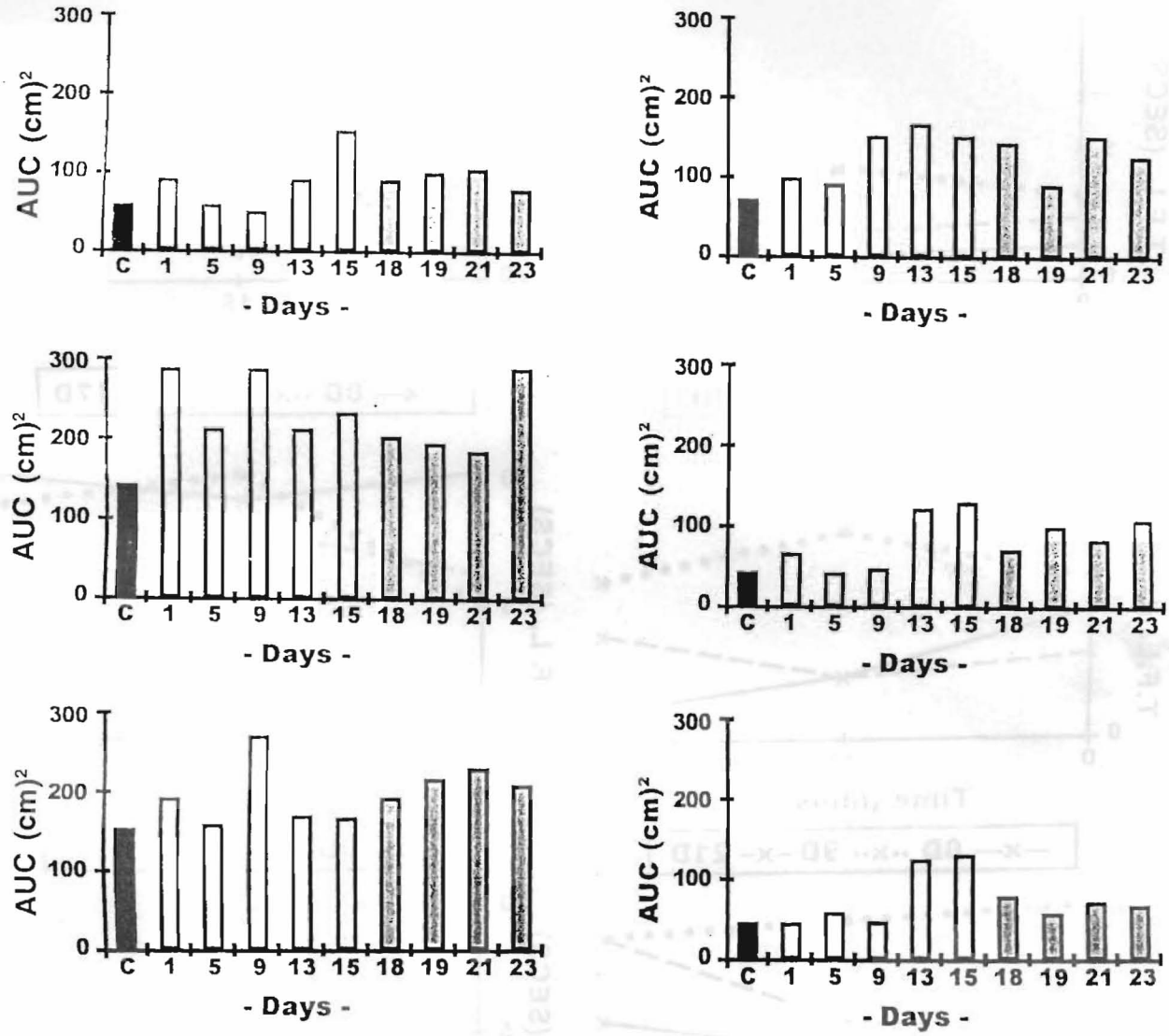

Fig. 2a : Mean areas under curve for effect of Ketamine on morphine induced analgesia [■ – Control, □ – after morphine (1–15 days) ▒ – after ketamine (18–23 days].

Effects of peripheral injections of glutamic acid and ketamine on tail flick latencies: Six rats were treated with glutamic acid (20 mg/kg) and six with ketamine (5 mg/kg) given intraperitoneally. No morphine injections were given in these animals. Figs. (3a & 3b) shows the effects of peripheral microinfusions of ketamine and glutamic acid, on the mean area under curve and the flick responses.

Glutamic acid in all the rats produced an increase in the MAUC, and the tail flick latencies, whereas ketamine also induced, an increase in mean area under curve, and an increase in the tail-flick latencies.

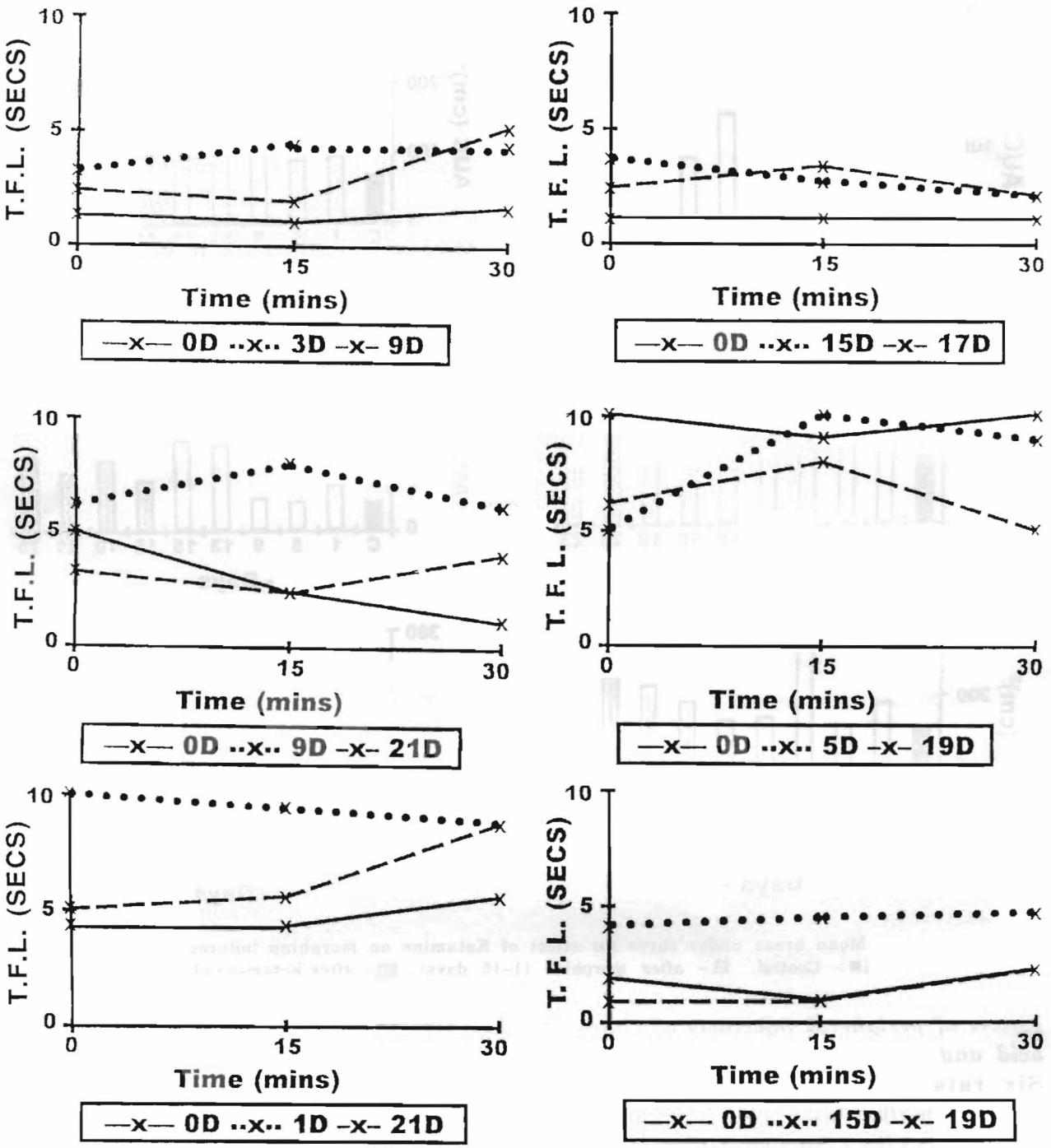

Fig. 2b : Tail flick latencies in rats treated with morphine followed by ketamine ____*____ control, ...*..... maximum analgesic effect after morphine, -------*------maximum analgesic effect after ketamine. D-signifies days on which the effects have been shown.

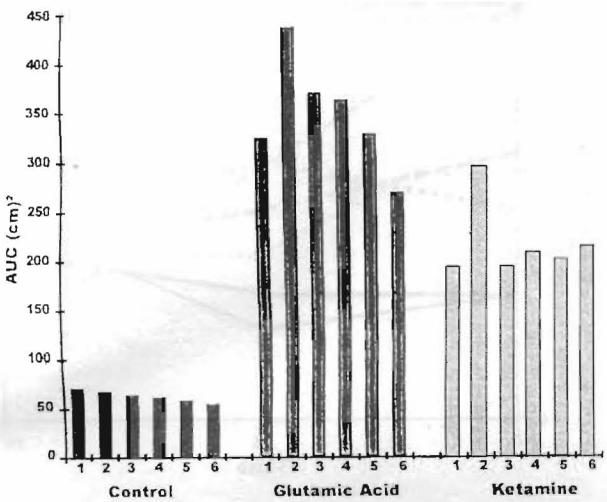

Fig. 3a : The effect of peripheral micro-injection of glutamic acid and ketamine on the mean area under curve in rats untreated with morphine (■ – control, ■ – glutamic acid, ■ – ketamine)

These observations show that the peripheral effects of glutamic acid and ketamine differ in their response on morphine-induced analgesia (Fig. 2a, b), as compared to those animals in which no morphine was given (Fig. 3a, b). This indicates that the two modes of nociception may exist. When glutamic acid was given to the morphine treated rats, the analgesic effect of morphine was markedly reduced, giving an inhibition of the inhibitory responses. Glutamic acid and ketamine when given peripherally produced an analgesic effect, which by itself was much greater than the effects on the morphine induced responses. Glutamic acid effects were also mainly of 2 types, those which resulted in a blockade, and those which produced a partial reversal of the response.

Fig. 4 shows the effects of saline infusion on the mean area under curve and tail flick latencies.

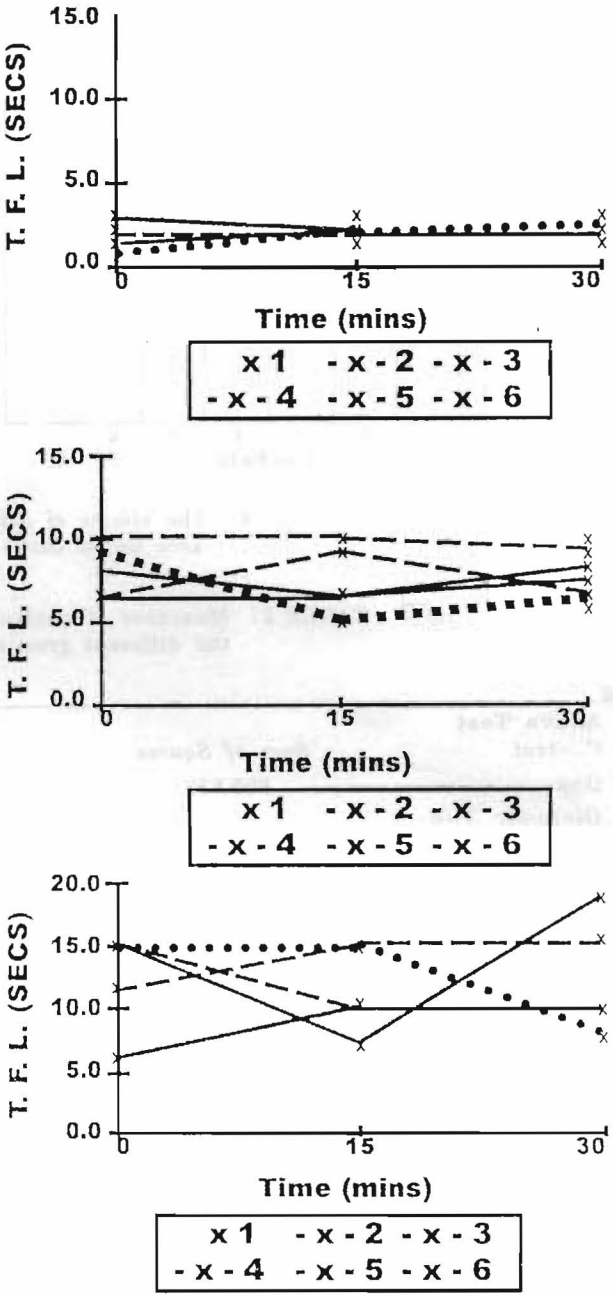

Fig. 3b : The effects of peripheral injection of glutamic acid and ketamine on the tail flick latencies in rats untreated with morphine. A–Control, B–after glutamic acid and C–after ketamine.

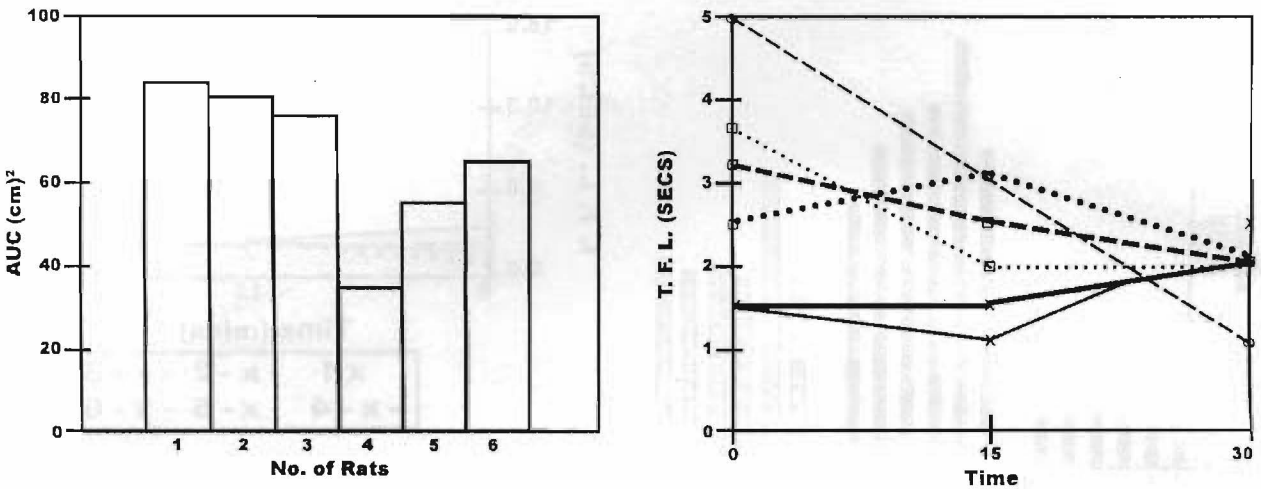

Fig. 4 : The effects of saline microinfusion on the mean area under curve and tail flick latency (n = 6).

TABLE I : Measures of analysis of variance, comparison between the different groups and drug treatments.

Anova Test				
Control	*Sum of Squres*	*DF*	*F*	*Significance of F*
Day	898.532	1	0.904	0.344
Glutamic Acid				
Main effects	16906.165	7	0.525	0.814
Group	13922.533	2	1.514	0.244
Day	2618.405	5	0.114	0.989
Two way interaction				
Group	4180.059	8	0.114	0.999
Day	4180.059	8	0.114	0.999
Morphine				
Main Effects	30626.00	7	1.805	0.132
Group	24367.5	1	10.055	0.004
Day	6179.571	6	0.425	0.885
Two way interaction	4289.00	4	0.442	0.777
Ketamine				
Main effects	3373.00	7	0.112	0.997
Group	60.00	1	0.014	0.906
Day	2724.87	6	0.106	0.995
Two way interaction	4001.5	4	0.233	0.919

Tukey's Test			
Drug	*Mean AUC*	*SD*	*P value*
1. Morphine	133.29	75.51	0.002*
Glutamic Acid	113.66	58.86	5% level
2. Ketamine	105.62	58.7	0.225

DISCUSSION

Our observations confirm the involvement of NMDA receptors, and L-Glutamate receptors in nociceptive responses in rats. Morphine-induced analgesia was seen to be affected in two different ways by glutamic acid and ketamine, (i) in which there was a blockage and the other (ii) in which there was a blockage accompanied with a reversal of the response. Analysis suggests that the glutamic acid has an excitatory and inhibitory effect on the opioid system, which leads to reduction of the analgesic response, produced by morphine. The NMDA receptor activity may be modulated in the antinociceptive action of morphine by two types of activities of the glutamate receptors. Our observations are supported by the work of Bond and Lodge (12) who have suggested that the glutamate receptors involved in the antinociceptive action of morphine may be of two types. By using the method of micro-iontophoretic application in the spinal neurons, they have studied the activity of these two types of glutamate receptors, (Group 1 & 2), some which contribute to the excitation and others to the inhibition of the opioid systems. Several different types of metabotropic receptors have been shown to be present in other regions of the brain, such as thalamus, and hippocampus. Interactions between these cannot be ruled out (13). Various workers have also suggested that there is a regional variation in functional response of the glutamate receptors, in synaptic transmission mechanisms (14) and alterations may be because of the changes in pre-synaptic and post-synaptic conductances, and changes in Ca+influx. Dickenson (15) in an article on pharmacology of pain transmission and control gave some information on how opioids and other inhibitory transmitters may act to control these excitatory events. Stimulation of the NMDA receptors involved in C-fibre stimulation of the dorsal horn neurons, leads to a hypersensitive state, and a 'wind up' phenomenon. The activity of the dorsal horn neurons, increased dramatically despite the lack of change of input into the spinal cord. This response could be magnified about 20 fold increase continuing even after cessation of the peripheral input. The opioid systems may be acting in a similar manner. Considerable number of sensory fibres in dorsal root ganglion cells contain glutamate and aspartate and 90% of the substance P containing fibres in spinal cord also contain glutamate (16). The co-existence of more than one transmitter in a nerve fiber makes it highly unlikely that a noxious stimulus would induce a release of both peptides and excitatory amino-acids into the spinal cord. It is, therefore, suspected that these transmitters co-coperate to activate spinal neurons in nociceptive pathways. Dickenson (15), have also stated that opioid effectiveness at a certain dose will vary, and depends on the level of excitatory activity the opioids are controlling. Inhibition must balance excitation. The NMDA receptor for glutamate may be prime candidate for generation of this state and many NMDA dependant pains. When the NMDA mediated central events leading to hypersensitivity are active, there is a reduced sensitivity to opioids (17). The NMDA antagonist ketamine has the potential not to abolish pain but to prevent or block certain hypersensitive states (18). Thus it can be seen that the final effects are transmitter interactions, with the eventual activation of the NMDA receptors. They play a key event in spinal nociceptive processing. The relative importance of these transmitters can change ion different pain states. Not only can the excitatory events alter, but inhibitions; and

in particular opioid controls can vary. Plasticity is very much a part of pain. (15). Opioid effectiveness at a certain point of time will vary as a result of the level of the excitatory activity, and the inhibitory activity, and by the effects of the transmitter systems, in which the NMDA receptors may be playing an important role.

The present findings suggest that the NMDA receptors may have a modulatory role in morphine-induced analgesia by alteration of "opioid" controls. These receptors may have two types of activities, for their actions of glutamatic acid and ketamine, ones which produce and inhibition and secondly those which produce an excitation followed by an inhibition or vice versa.

ACKNOWLEDGEMENTS

The financial assistance offered by the Indian Council of Medical Research is greatly acknowledged. The first author wish to express gratitude to Prof. K.N. Sharma, for his guidance from time to time.

REFERENCES

1. Krishnamurthy P, Gudehithlu, Poluru LR, Hemendra NB. Effect of morphine tolerance and abstinence on the binding of (H3) MK 801 to brain regions and spinal cord of the rat. *Brain Res* 1994; 639: 269–274.
2. Detweiler DJ, Dana S Rohde, Allan J Besbaum. The development of opioid tolerance in the formalin test in the rat. *Pain* 1995; 63: 251–254.
3. Trijillo KA, Akil H. Behavioural interactions between morphine and MK 801, analgesia tolerance dependence and lethality. *Soc Neuro Sci Abst* 1990; 16: 211.
4. Trijillo KA, Akil H. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist. MK 801. *Science* 1991; 251: 85–87.
5. Ben-Eliyahu Marek S, Vaccarino AC, Mogil JS, Steinberg WF, Liebeskind JC. The NMDA receptor antagoinst MK 801, prevents long lasting non-associative morphine tolerance in the rat. *Brain Res* 1992; 575: 304–308.
6. Lufty K, Diana EH, Eckerd W. Blockade of morphine-induced analgesia and tolerance in mice and MK-801. *Brain Res* 1993; 616: 83–88.
7. Tisco PJ, Intrussi CE. Attenuation and Reversal of Morphine, Tolerance, by the Competitive N-Methyl-D-Asparatate Receptor Antagonist. *J Pharmacol Exp Therap* 1993; 264: 1090–1096.
8. Tisco PJ, Jie Cheng, Gavhil WP, Charles EI. Modulation of morphione tolerance by the competitive N-Methyl D-Aspartate Receptor Antagonist, LY 274614. Assessment of opioid receptor changes. *J Pharmacol Exp. Ther* 1993; 268: 194–201.
9. Gutstein HB, Trijillo KA. MK 801, inhibits the development of morphine tolerance at spinal sites. *Brain Res* 1993; 626: 332–334.
10. Jenson TS, Taksh TL. Brainstem excitatory amino acid receptors, in nociception: microinjection, mapping and pharmacological characterisation of glutamate sensitive sites in the brainstem, associated with allogenic behaviour. *Neuro Science* 1992; 46(3): 535–547.
11. Coderre TJ, Melzack R. The contribution of excitatory amino-acids to central sensitization and persistent nociception, after formaline induced tissue injury. *J Neuro Sci* 1992; 12: 3665–3670.
12. Bond A, Lodge D. Pharmacology of metabotropic glutamate receptor, meditated enhancement of responses to excitatory and inhibitory amino acids in rat spinal neurons *in vivo*. *Neuropharmacology* 1995;3408 : 1015–1023.
13. Furuyaha T, Kiyama H, Sato K, Park HT, Maeno H, Takagi H, Tohyama M. Region specific expression of subunits of ionotropic gultamate receptors in the rat spinal cord with reference to nociception. *Brain Res* 1993; 18 : 141–51.
14. Manahan-Vaughan D, Reymana KG. Regional and developmental profile of modulation of hippo-campal synaptic transmission and LTP by AP4-sensitive MG LURs *in vivo*. *Neuropharmacology* 1995; 34–38:991–1001.
15. Dicknson AH. Pharmacology of pain transmission and control in Pain 1996; an updated review.
16. Battaglia G, Rustroni A. Co-existence of glutamate and substance P in the dorsal root ganglion cell of the rat and monkey. *J Com Neurol* 1988; 277: 302–312.
17. Dickenson AH. NMDA receptor antagonists as analgesics. In field H.L. and Liebesekind, JC. Phamacological approaches to the treatment of chronic pain. *Progress in Pain Research and Management*. 1994 Vol. 1. Seattle. IASP. 173–187.
18. Loscher W, Gabriele F, Martin G. Comparison of pharmacodynamic effects of the non-competitive NMDA receptor antagonosts MK 801 and Ketamine in pigs. *Eur J Pharmacol* 1991; 192 : 377–382.
19. Snedecor WG, Cochran WG. Statistical Methods. 1967 Published by Oxford and IBH Company, New Delhi.