

## Intraventricular gabapentin is antinociceptive and enhances systemic morphine antinociception in rat tail flick test

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### ABSTRACT

**Background:** Gabapentin has been recently considered as an analgesic in neuropathic pain through spinal site of action. In addition co-administration of low dose of morphine with gabapentin, is proposed not only to reduce side effects, tolerance, and dependency of morphine but also has some analgesic effects.

In this study, the analgesic effect of intracerebroventricular (ICV) gabapentin and its effect on morphine antinociception were investigated in tail-flick test.

**Methods:** An intraventricular cannula was surgically inserted into ventricle space of rat brain. The latency time was measured after microinjection of 100,300,600 and 1000 µg of gabapentin or normal saline (sham). After determination of subanalgesic dose of gabapentin (300µg), the combinational groups received subanalgesic and low dose of morphine (2 and 7 mg /kg) intraperitoneally, thirty minutes prior to gabapentin administration. Time response curve and Area Under the Curve (AUC), as antinociceptive index, were compared among the groups.

**Results:** Intraventricular gabapentin showed analgesic effects at 600 µg (ICV) . The combination of subanalgesic doses of gabapentin (300 µg ICV) and morphine (2 mg /kg i.p) increased significantly time-response curve and AUC compared to other groups. In addition, the analgesic response following co-administration of gabapentin (300 µg ICV) and analgesic dose of morphine was increased significantly compared to the sham and gabapentin group.

**Conclusion:** The results demonstrated that intraventricular gabapentin has analgesic effect in transient model of pain and enhances morphine antinociception through cerebral site of action.

**Keywords:** Intraventricular, Gabapentin, Morphine, Tail- flick, Antinociception.

### INTRODUCTION

Gabapentin, a new anticonvulsant drug, is useful in treatment of other neurologic and psychiatric conditions as spasticity, anxiety and pain(1, 2) and its efficacy has been demonstrated in neuropathic (3), inflammatory (4) and post-operative pain (5). The wide spectrum of analgesic effects of gabapentin may be attributed to certain neuronal changes which explains its efficacy in both neuropathic and post- tissue injury pain. Many recent studies have demonstrated that gabapentin reduced selectively pain transmission in a sensitized but not in a normal nervous system (5-8). Therefore in formalin test, as a persistent model of pain, gabapentin has no effect on pain behavior during initial acute phase but in the second phase which is characterized as neuronal changes, gabapentin decreased the number of flinching in rats (7, 9). Also, gabapentin has been effective in post-operative model of acute pain by

establishment of central sensitization and movement evoked pain (6, 10). Limited studies have demonstrated the efficacy of gabapentin in hot-plate and tail-flick models of acute pain (7, 11). Therefore the efficacy of gabapentin in transient model of pain remains the subject of further investigations.

Although the antinociceptive mechanisms of gabapentin and its site of action are not well understood, gabapentin has been proposed as coanalgesic with opioids. In fact, many studies have demonstrated that gabapentin enhances the analgesic effect of morphine in different model of pain (11-13). Based on these findings, most authors believe that spinal site of action is involved in enhancement of morphine antinociceptive effect (13-15). This combination has found important implications in clinical treatment of pain to avoid undesirable side effects like development of dependency and tolerance of morphine because of using low doses of each

drug. Since the efficacy of combination of low doses of morphine with gabapentin is proved, it would be necessary to elucidate the underlying site of action of gabapentin. In this study, the antinociceptive effect of intraventricular gabapentin administration in tail flick test and also its interaction with intraperitoneal morphine injection was investigated in order to establish whether gabapentin has analgesic effects through supraspinal level in transient model of pain or it affects the analgesic properties of systemic use of morphine.

## MATERIAL AND METHODS

### Animals

Male wistar rats (200 – 300 g) were housed three or four per cage at controlled temperature ( $23 \pm 1$  °C) at 12-h light/dark cycle. Food and water were available continuously. Experiments were performed at the same time on light cycle in all groups. Each animal was used only once and killed under anesthesia. The protocol was approved by research committee of Kerman Neurosciences Research Center, Kerman, Iran.

### Drugs

The following drugs were used: Gabapentin (Park Davis Company, Italy), Morphine sulfate (Temad Co., Iran). The drugs were dissolved in freshly prepared saline.

To inject gabapentin in ventricle, animals were anaesthetized with ketamin (40-60 mg /kg) and xylazine 2% (5mg /kg). Then a stainless steel, thin-walled guide cannula was inserted into left ventricle according to Paxinos and Watson characteristics ( $A_p = -0.5$  mm,  $L = 1.0$  mm,  $D = 3$  mm) using stereotaxic apparatus(16). The animals were allowed to recover from surgery for 5-7 days prior to initiation of experimental protocol. Microinjection of gabapentin and normal saline in volume of 10 $\mu$ l Hamilton syringe were administered continuously. As morphine increases latency time in tail flick test approximately after 30 minutes(11), it was injected intraperitoneally 30 minutes before intraventricular injection of gabapentin or saline (ICV). Following experimental sessions, animals were deeply anesthetized and then 1 $\mu$ l of dye was microinjected through cannula and brains were removed. Animal brains were fixed in formalin solution (10%) for subsequent histological observation after 72 hours and from obtained data those were used that the insertion of cannula into ventricles had been verified.

### Antinociception measurement

The tail – flick test was used to assess the antinociceptive effect of drug groups. Radiant

heat was applied to the tail from 5 – 8 cm of the tip using a tail flick apparatus (PANLAB 7160, Spain). Tail flick latency time was measured as the time from the onset of the heat exposure to the time of withdrawal of the tail. The intensity of radiant heat was adjusted to establish the baseline latencies for 3-5 seconds. The heat stimulus was discontinued after 15 seconds to avoid tissue damages. (Cut off point = 15 s).

For each animal, baseline latency was obtained as the mean of three measurements before administration of any drug and then the latency times were determined at every 15 minutes intervals for 75 minutes from the time of gabapentin or saline injection. Tail flick latency time was measured in control group which did not receive any drug, sham group which received microinjection of normal saline (ICV), morphine groups which received injection of 2mg /kg (as subanalgesic dose) and 7 mg /kg (as analgesic dose) of morphine intraperitoneally and at last gabapentin groups which received microinjection of 100,300,600,1000  $\mu$ g of gabapentin (ICV). The group which received 1000  $\mu$ g of gabapentin was excluded from comparisons because of high mortality. After determination of subanalgesic dose of intraventricular gabapentin (300  $\mu$ g, ICV), the latency time was measured in combination groups which received subanalgesic and analgesic doses of morphine 30 minutes before administration of 300  $\mu$ g of gabapentin (groups; m2, m7, gbm2 and gbm7).

Antinociception was quantified as either tail-flick latency time or the area under curve (AUC) which includes both maximum effects and duration of action (11). The AUC was calculated by consideration latency time from 15 to 75 post-injection based on Trapezoid rules as follows:  
 $AUC = 15 \times TLF [(MIN 15) + (MIN 30) + (MIN 45) + (MIN 60) \dots + (MIN 75)/2]$ .

### Statistical analysis

One way and repeated measure of ANOVA models were used to assess the effects of time and drug. In these models, the dependent variable was latency time. Also one way ANOVA was used to compare (AUC) of each drug- treated group. As post – hoc, Dunnet test was used for a pair wise comparison between sham and each drug - treated group. Data were expressed as Mean  $\pm$  SE of at least six rats.

To deal with multiple comparison effects and to improve the statistical power of the tests, the sample size in sham group (n=10) was 40% greater than in other drug treated groups. The value of  $p < 0.05$  was considered statistically significant.

## RESULTS

### *Determination of subanalgesic dose of intraventricular injection of gabapentin*

Since no difference in latency time or AUC was observed between sham and control groups, the comparisons were made with sham group.

Repeated ANOVA model showed significant temporal variations by different doses of gabapentin ( $F= 4.463$ ,  $P=0.001$ ), but the pattern of time- effect curve was similar in all gabapentin groups. Also repeated measurement showed that intraventricular gabapentin (600  $\mu\text{g}$ ) increased the time- effect curve compared to the sham group ( $F= 7.443$ ,  $P=0.002$ ). The latency time of this group (animals that received 600  $\mu\text{g}$  of gabapentin) at time point of 30 minutes was increased significantly not only in comparison to the sham group but also compared to all other treated groups. Comparison of (AUC) of gabapentin groups showed significant differences between gabapentin at the dose of 600  $\mu\text{g}$  and other groups ( $F= 6.044$ ,  $P=0.005$ ). (fig 1).

Based on these results, gabapentin at the dose of 600  $\mu\text{g}$  (ICV) was antinociceptive, so the dose of 300  $\mu\text{g}$  (ICV) was considered as subanalgesic dose.

### *Effects of gabapentin on antinociceptive effects of morphine*

Comparison of time- response curves of subanalgesic dose of morphine (2 mg /kg i.p) or gabapentin (300 $\mu\text{g}$  ICV) and their combinations showed no significant difference in temporal variation or following similar pattern. However a significant difference in latency time was observed at 45th minute between the combination (gbm2) and other groups. ( $F= 5.49$ ,  $P= 0.004$ ). Significant differences between sham and combined groups were found in AUC; which means in the presence of both drugs the latency time and AUC increased. The analysis of variance (ANOVA) of AUC, post hoc Dunnett, showed a significant increase by combinational group (but not by subanalgesic doses of gabapentin or morphine) compared to the sham group ( $P<0.05$ ) (fig. 2).

When morphine at analgesic dose (7 mg /kg i.p) was co-administrated with subanalgesic dose of gabapentin (300  $\mu\text{g}$  ICV) the time response curve showed significant differences ( $F= 3.178$ ,  $P=0.012$ ) compared with other groups. The latency time after injection of morphine (7 mg /kg i.p) with 300  $\mu\text{g}$  of gabapentin significantly increased compared to other groups, especially 30 minutes after gabapentin injection ( $P<0.05$ ).

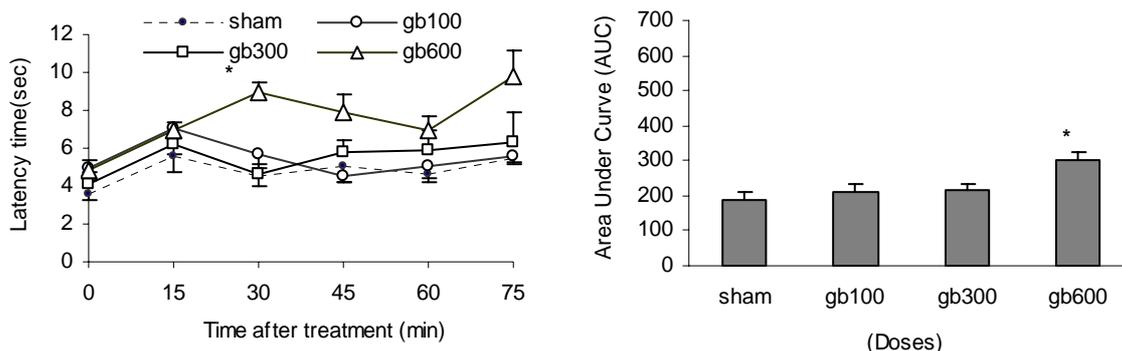
The AUC of groups in figure 3 was significantly different in ANOVA test ( $F=4.599$ ,  $P=0.018$ ). The post hoc Dunnett showed that the AUC increased significantly after injection of 7 mg /kg of morphine (i.p) and also after administration of

300 $\mu\text{g}$  of both morphine and gabapentin (ICV) ( $P=0.029$ , 0.036) but not after administration of gabapentin alone.

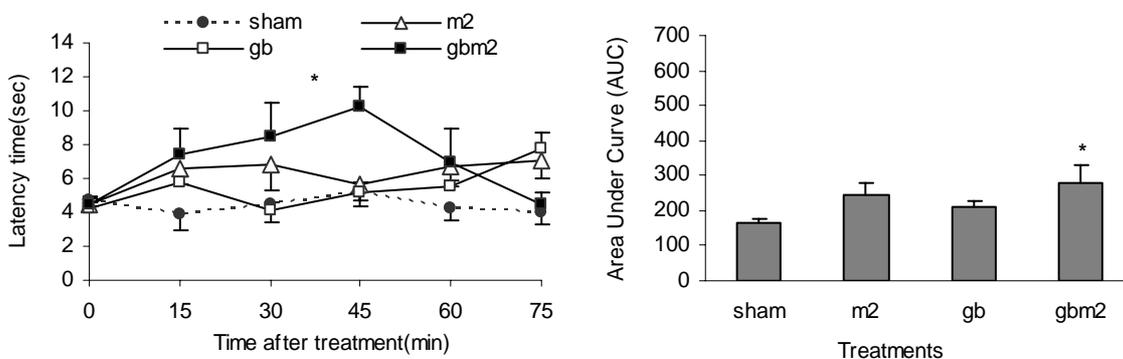
## DISCUSSION

Gabapentin, as a safe well-tolerated drug, is considered analgesic in limited forms of pain. Its antinociception in transient model of pain which is not considered pathological pain and is not due to central sensitization is under investigation. In the first part of this study, the intraventricular injection of gabapentin in an intact nervous system showed that gabapentin produced analgesic effects at cerebral level. The latency time, after injection of 600  $\mu\text{g}$  of gabapentin increased during 75 minutes of tail-flick test (AUC). Several studies have shown that gabapentin has antinociceptive effects at spinal level but its intraventricular effects on analgesic response has not been determined. For example; in acute herpetic pain in mice, intraventricular injection of gabapentin (10-100  $\mu\text{g}$ ) was not analgesic but intrathecal injection of the same doses relieved pain (17). The same analgesic effect was observed after administration of 25-200  $\mu\text{g}$  (i.t) of gabapentin which reduced tactile allodynia in a dose dependent manner (18). Also in nerve ligated rats, administration of 500  $\mu\text{g}$  of gabapentin (i.t) reduced tail-flick latency time while similar to results of this study administration 300  $\mu\text{g}$  was subanalgesic dose (13). Same results were obtained in pancreatic model of visceral pain where 300  $\mu\text{g}$  gabapentin did not reduce hind limb extension(14). It has been reported that electrophysiological activity of dorsal horn neurons response in spinal nerve-ligated rats was inhibited after systemic administration of gabapentin (10-20 mg /kg s.c.) similar to those which has been observed in behavioral pain assessment (19, 20). It has been suggested that gabapentin is not analgesic in transient model of pain because it does not change pain transmission nor it affects pain threshold in intact nervous system (2, 6, 21).

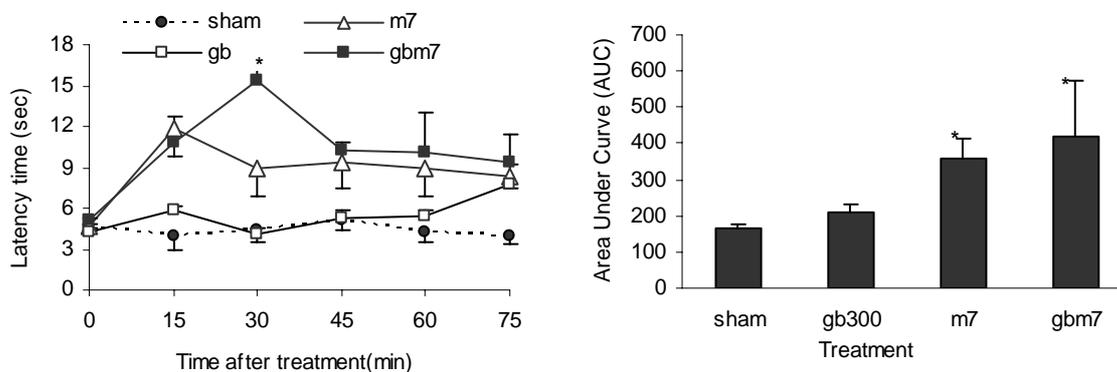
In the second part of this study, subanalgesic dose of gabapentin, administrated intraventricularly enhanced the antinociception of systemic administration of morphine. It means that although morphine or gabapentin alone had no analgesic effect, their combination was able to increase the latency time and the AUC compared to sham group. These results suggest a possible central interaction between these drugs. In accordance, the intrathecal co-administration of subanalgesic dose of gabapentin has been reported to have the same effects in rat tail flick test (13).



**Figure 1.** The antinociceptive effects of intraventricular administration of gabapentin on tail-flick test. The animal received saline or 100, 300 and 600  $\mu$ g of gabapentin (sham; gb100; gb300; gb600) in ventricles. The latency time increased significantly at 30th minutes after injection of 600  $\mu$ g of gabapentin. The latency time curve and AUC increased significantly compared to other groups. Data are expressed as the mean  $\pm$  SE of at least six rats. \*  $P < 0.005$



**Figure 2.** The antinociceptive effect of subanalgesic dose of gabapentin and morphine in tail-flick test. Animals received normal saline (sham) or 300  $\mu$ g of gabapentin intraventricularly (gb) or morphine (2 mg /kg) intraperitoneally (m2) or morphine 30 minutes before administration of gabapentin (gbm2). The latency time increased at 45th minutes significantly in gbm2 compared to all other group. The latency time curve and AUC increased significantly compared to sham groups. Data are expressed as the mean  $\pm$  SE of at least six rats. \*  $P < 0.05$



**Figure 3.** The antinociception effects of subanalgesic dose of gabapentin and analgesic doses of morphine in tail-flick test. Animals received normal saline (sham) or 300  $\mu$ g of gabapentin (gb) intraventricularly or morphine (7 mg /kg) intraperitoneally (m7) or morphine 30 minutes before administration of gabapentin gbm7. The latency time increased at 30th minutes significantly in gbm7 compared to other groups. The AUC of m7 and gbm7 groups increased significantly compared to sham group. Data are expressed as the mean  $\pm$  SE of at least six rats. \*  $P < 0.05$

In pancreatic model of visceral pain and in reduction of second phase of nociception after orofacial formalin test, the effectiveness of coadministration of gabapentin and morphine have been reported (14, 22), suggesting the involvement of the spinal mu opioid receptors and primary afferent neuron endings in spinal cord (13, 22). In addition, patch clamp recording of the whole cells of rat spinal slices showed that gabapentin inhibit the release of excitory amino acids from presynaptic terminals(23) and after acetic acid induced writhing (24). Increased excitatory amino acids in morphine tolerated rats were also inhibited by gabapentin. Not only intrathecal, but also systemic administration of non- analgesic dose of gabapentin (subcutaneous) inhibited dorsal horn neuronal response in neuropathic pain following spinal nerve ligation (19).Based on the route of administration or method of assessment, these studies confirm the spinal site of interaction between morphine and gabapentin.

It has been shown recently that gabapentin did enhance the antinociception of morphine in transient model of pain in intact central nervous system (11) or in healthy volunteers where it enhanced morphine effect in cold pressure test (12). As a result other theories on this interaction such as changing morphine pharmacokinetics or reduction of movement evoked pain by gabapentin, have been suggested (5, 12).

Although each of these studies explain the mechanisms of interaction between morphine and

gabapentin to some extent, the highly specific gabapentin binding site in brain identified as  $\alpha 2\delta$  subunit of calcium channels should also be considered (25). In fact, the central interaction of gabapentin with morphine which was observed in this study, confirm the action on this binding site.

The reduction of excitatory amino acid induced by gabapentin (15, 23, 24) and activation of  $K^+$  channels and subsequent blockade of  $Ca^{+2}$  channels due to higher hyperpolarization are other possible mechanism of interaction(18, 21). Same mechanism is related to  $\mu$ -opioid G-protein coupled receptors involved in transient pain antinociception (19). Opioids like gabapentin release of spinal substance P release and central neurotransmitter (19, 26, 27). Therefore, morphine and gabapentin may interact by concomitant decrease in excitation and increase in inhibition of pain transmission.

In conclusion, findings of this study was not only the analgesic effect of gabapentin in transient model of pain in an intact central nervous system, but also it proved that gabapentin can enhance morphine antinociception through cerebral site of action. More differential investigations are required to explain the modality of this interaction.

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