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Antinociceptive effect of intra-hippocampal CA1 and dentate gyrus injection of MK801 and AP5 in the formalin test in adult male rats

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Abstract

Previous research has shown that the hippocampus processes pain related-information, probably through hippocampal neurons that respond exclusively to painful stimulation. In the current experiments we tested whether blocking NMDA receptors in the hippocampal CA1 region and dentate gyrus could reduce nociceptive behaviors in rats. The competitive and noncompetitive NMDA receptor antagonists 2-amino-5-phosphonopentanoic acid (AP5; 3.75 μg/0.75 μl) and MK801 (1.5, 3, 6 μg/0.5 μl) were injected into the dentate gyrus and CA1 area of behaving rats 5 min before subcutaneous injection of formalin irritant. Pain behaviors in both acute and tonic phases of the formalin test were significantly reduced by AP5 (3.75 μg/0.75 μl) and MK801 (3 μg/0.5 μl, but not 1.5 and 6 μg/0.5 μl) injection to the dentate gyrus. In the CA1, injection of AP5 had no effect while injection of the effective dose of MK801 (3 μg/0.5 μl) had a significant antinociceptive effect. This effect was apparent only during the late phase of the formalin test. These results support the hypothesis that NMDA-sensitive mechanisms are involved in acute and persistent pain-related processing in the dentate gyrus and with tonic pain processing in the hippocampal CA1 region.

Keywords: NMDA receptor; Formalin test; Hippocampus; Nociception; (Rat)

1. Introduction

Several physiological, pharmacological and behavioral lines of evidence suggest that the hippocampal formation is involved in nociception (Delgado, 1955; Khanna and Sinclaire, 1992; McKenna and Melzack, 1992; Sinclair and Lo, 1986; Soleimannejad et al., 2006). For example, the pyramidal cells and interneurons in the dorsal hippocampal CA1 respond to persistent noxious activation (Khanna, 1997; Khanna and Zheng, 1999); Injection of local anaesthetic into the dentate gyrus of the hippocampal formation produces an analgesic effect in the formalin test (McKenna and Melzack, 1992); Hippocampal lesions can cause avoidance task impairments in both animals (Nadel, 1968; Olton and Issacson, 1968) and humans; Partial hippocampectomy has been used (with moderate success) as a treatment for chronic pain (Gol and Faibish, 1967), whereas electrical stimulation of the hippocampal formation evokes painful sensations in humans (Delgado, 1955; Gloor et al., 1981; Halgren et al., 1978); Blocking neural transmission along the major afferent (McKenna and Melzack, 1992) or efferent (Vaccarino and Melzack, 1992) hippocampal pathways has reduced pain behaviors; Peripheral noxious stimulation alters the induction of Fos (Aloisi et al., 1997; Funahashi et al., 1999) and Egr1 (Pearse et al., 2001; Wei et al., 2000) in the hippocampal formation, Fos and Egr1 being transcription proteins that are expressed in neurons following synaptic excitation (Aloisi et al., 1997; Khanna et al., 2004); Finally, the hippocampus is also assumed to play an important role in the affective and motivational components of pain perception (Henke, 1982; Melzack and Casey, 1968).

NMDA (*N*-methyl-D-aspartate) receptors are localized both in supraspinal (hippocampus, cerebral cortex, thalamus,

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striatum, cerebellum, and brain stems) and spinal (substantia gelatinosa and spinal gray matter) structures (Kalb and Fox, 1997; Mugnaini et al., 1996; Roth et al., 1996). The NMDA receptor plays a key role in central pain transduction mechanisms (D'Amico et al., 1996; Hudespith, 1997). Numerous studies have focused on NMDA receptor activation in pain-related neuroplasticity, especially within the spinal cord (Coderre and van Empel, 1994a,b; Dickenson et al., 1997). The excitatory amino acids glutamate and aspartate are involved in the processing of nociceptive information in the spinal cord. In particular, they facilitate and enhance the excitability of nociceptive inputs from spinal cord neurons to the central nervous system during persistent pain (Hudespith, 1997; Maione et al., 1999, 2000). NMDA receptor antagonists attenuate pain behaviors in models of neuropathic (Seltzer et al., 1991; Walters, 1987) and tonic (Coderre and Melzack, 1992a,b; Coderre and van Empel, 1994a,b; Eisenberg et al., 1993; Klepstad et al., 1990) pain, when applied to the CNS. Clinical studies have also shown that various NMDA receptor antagonists are useful analgesics in the treatment of acute (Henderson et al., 1999; Schmid et al., 1999) and neuropathic (Enarson et al., 1999; Klepstad et al., 1990; Kristensen et al., 1992; Pud et al., 1998; Rabben et al., 1999) pain syndromes. Electrophysiological responses of nociceptive neurons in the ventrobasal thalamus can be blocked by NMDA antagonists (Eaton and Salt, 1987, 1990; Salt et al., 1988); likewise, behavioral pain responses have been blocked by AP5 (2-amino-5-phosphonovalerate) applied to the centromedial thalamus (McKenna and Melzack, 1994). Finally, microinjection of the NMDA receptor antagonist AP5 into the dentate gyrus region of the hippocampus attenuated pain behaviors in both the acute and tonic phases of the formalin test, but had no effect when administered into the hippocampal CA1 region, the cortex or the cerebellar ventricles (McKenna and Melzack, 2001).

The formalin model of inflammatory pain is probably better than phasic mechanical or thermal stimuli tests in modeling human pain (Abbott et al., 1995; Tjolsen et al., 1992). During the late phase of the formalin test the spinal cord releases excitatory amino acids and NMDA receptor subtypes are activated (Coderre and van Empel, 1994a). Intrathecal injection of selective NMDA antagonists prevents the nociceptive behaviour of the late phase (Coderre and van Empel, 1994a; Eisenberg et al., 1993).

In the current study we investigated the role of NMDA-sensitive mechanisms at the hippocampal CA1 and dentate gyrus, using in the formalin model of persistent pain. For this purpose, intra-CA1 and intra-dentate gyrus injections of a competitive (AP5) and noncompetitive (MK801) antagonist NMDA were used.

2. Materials and methods

Experiments were approved by the Institute for Studies in Theoretical Physics and Mathematics in Teheran and conducted according to its regulations. Experiments adhered to the guidelines for animal experimentation of the International Association for the Study of Pain (Zimmermann, 1983).

2.1. Animals

Male albino rats of Wistar strain, weighing 250–280 g, were used as subjects. Rats were housed four per cage in a temperature and light-controlled room under a 12:12 h light: dark cycle with water and food provided ad libitum.

2.2. Surgery

Rats ($n=7$/group) were anesthetized with a mixture of ketamine and xylazine (100 mg/kg i.p.). Two guide cannulae (22-gauge) were implanted bilaterally into the CA1 region of the hippocampus at the following stereotaxic coordinates: −3.72 mm posterior to bregma, ±2.2 mm laterally and 2.4 mm dorsal-ventrally, ventral to the outer skull surface, or into the dentate gyrus region of the hippocampus at the following coordinates: −3.72 mm posterior to bregma, ±2.2 mm laterally and 3.4 mm dorsal-ventrally, ventral to outer skull surface. Cannulae were held in place with dental acrylic applied around them and two anchoring screws.

2.3. Microinjection procedure

Drugs and vehicle were administered into the CA1 region of the hippocampus or into the dentate gyrus through guide cannulae using injection needles (27-gauge) connected by polyethylene tubing to 10 μl Hamilton microsyringe. The injection needle was inserted 0.5 mm beyond the tip of the cannula and vehicle (saline) or various doses of the tested compounds (MK801 or AP5, Sigma co.) were injected over 3 min.

2.3.1. Experiment 1

The aim of this experiment was to investigate the role of NMDA receptors in the CA1 region of hippocampus on nociception. Rats with cannula aimed at the CA1 were divided into four groups and treated with either saline ($n=7$) or three doses of MK801 (1.5, 3, 6 μg/0.5 μl; Tucci et al., 1998), injected bilaterally into the CA1 region. Five minutes after intra-CA1 injection of 0.5 μl saline or MK801 the formalin test was performed.

2.3.2. Experiment 2

The aim of this experiment was to investigate the role of NMDA receptors in the CA1 region of hippocampus on nociception. Rats with cannula aimed at the CA1 were divided into two groups ($n=14$). Saline (0.75 μl) or AP5 (3.75 μg/0.75 μl; McKenna and Melzack, 2001) were injected bilaterally into the CA1 region. Five minutes later the formalin test was performed.

2.3.3. Experiment 3

The aim of this experiment was to investigate the role of NMDA receptors in the dentate gyrus on nociception. Rats with cannula aimed at the dentate gyrus were divided into four groups and treated with either saline ($n=7$) or three doses of MK801 (1.5, 3, 6 μg/0.5 μl; Tucci et al., 1998), injected

bilaterally into the dentate gyrus. Five minutes after intra-CA1 injection of 0.5 μl saline or MK801 the formalin test was performed.

2.3.4. Experiment 4

The aim of this experiment was to investigate the role of NMDA receptors in the dentate gyrus of hippocampus on nociception. Rats with cannula aimed at the CA1 were divided into two groups ($n=14$). Saline (0.75 μl) or AP5 (3.75 μg/0.75 μl; McKenna and Melzack, 2001) were injected bilaterally into the dentate gyrus. Five minutes later the formalin test was performed.

2.4. Formalin test

Seven days after surgery, each rat was placed in a transparent acrylic cage and was allowed to move freely for 15–20 min to habituate. A mirror was placed under the cage to allow an unobstructed view of the animal's paws to the behavioral observer. Five minutes after intra-hippocampal injections of either vehicle, MK801 or AP5, each rat was restrained and received a 50-μl subcutaneous injection of 5% buffered formalin acetate into the left hind-paw and placed in the observation box for 60 min. The pain response in the formalin test consists of an initial display of nociceptive behaviors (described below) that subsides after approximately 5 min and reappears after an additional 10–15 min; it then slowly diminishes over the subsequent 40–60 min. Subjects in this experiment were observed for 60 min following formalin injection and each 15 s were continuously rated by a 4-point scale:

A score of 0 denotes normal use of the injected paw (i.e., the plantar surface of the paw comes into full contact with the floor of the observation box and the animal's weight is evenly distributed between hind paws).

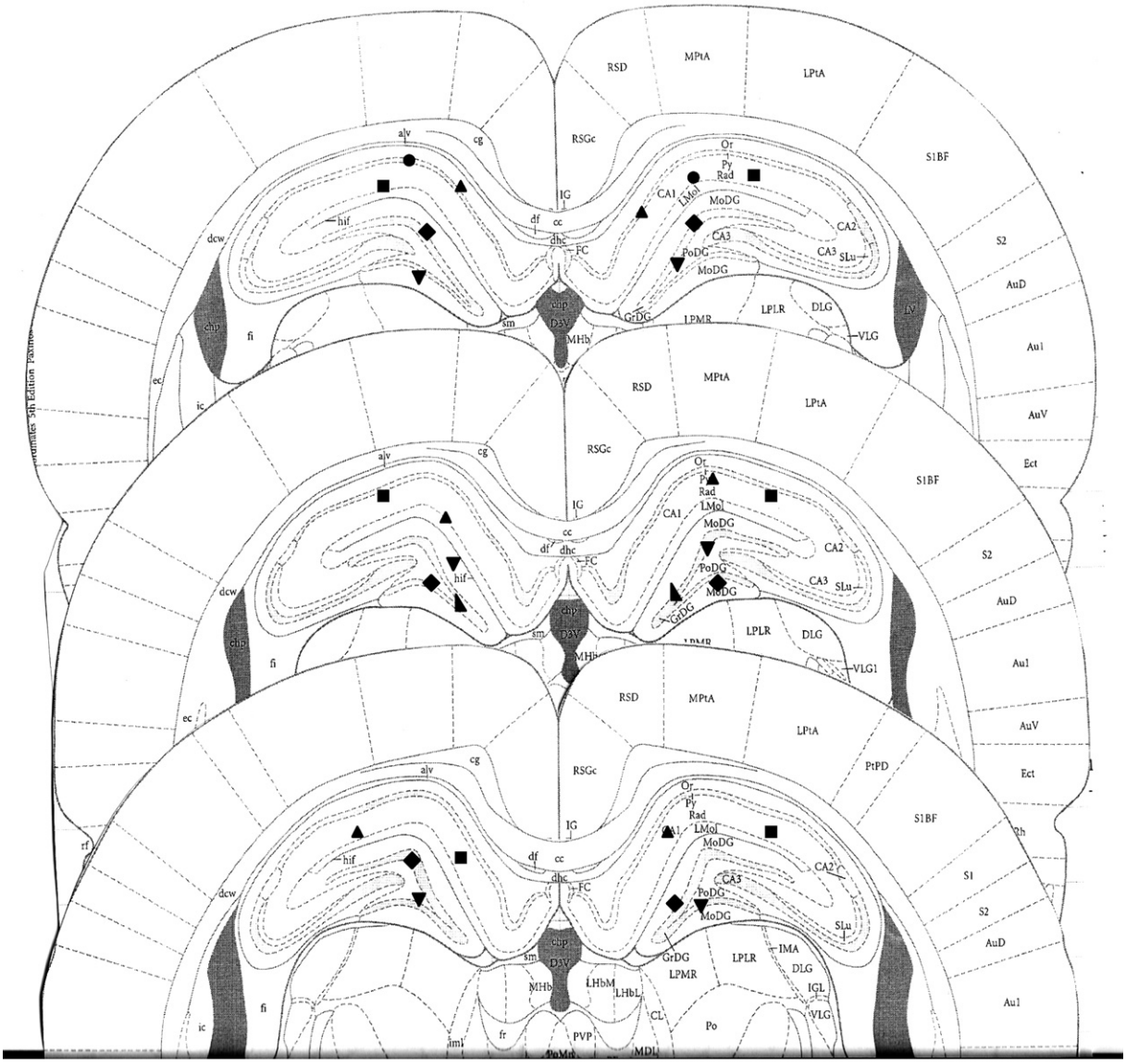

Fig. 1. Schematic picture of the injection sites. The injection of AP5 into the CA1 (7 sites; ▲, ● and ■ signs) and dentate gyrus (7 sites; ◆, ◣ and ▼ signs) in −3.60, −3.72 and −3.84 bregmas (from up to down) are shown.

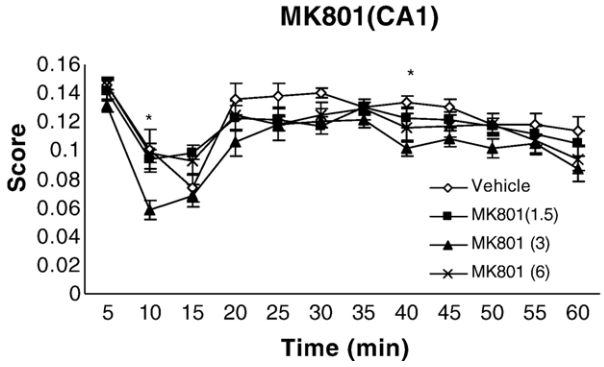

Fig. 2. Formalin test: the effect of MK801 injected into the CA1 region. Data are expressed as the scores of nociceptive behavior induced by formalin injection (50 μl at 5%) in rats treated with MK801 (1.5, 3 and 6 μg/0.5 μl) 5 min before formalin administration. Each animal was observed for 60 min after formalin injection. Each point is the mean±SEM of the accumulative time of nociceptive behavior/5 min. The comparison was done between vehicle and MK801-treated groups ($n=7$). The effective dose of MK801 (3 μg/0.5 μl) had a significant antinociceptive effect only during 10 and 40 timepoints. MK801 had no effect in other time periods or in different doses (*$p<0.05$ *vs.* vehicle).

A score of 1 indicates careful use of the injured paw, with some part of the paw in contact with the floor; the animal limps when walking.

A score of 2 indicates elevation of the paw.

A score of 3 denotes vigorous shaking or licking of the injured paw (distinct from normal grooming behavior).

Scores of each timepoint (5 min intervals) were calculated by the following equation:

$$\frac{0\times T0+1\times T1+2\times T2+3\times T3}{300}=\text{Score}$$

In this equation T0–T3 designates the number of times rats are graded scores of 0–3, respectively. This way, pain behaviors are expressed during every 5 min in the time course data during the initial acute phase (0–5 min) or the second, tonic phase (20–60 min; Coderre et al., 1990; Cohen et al., 1984).

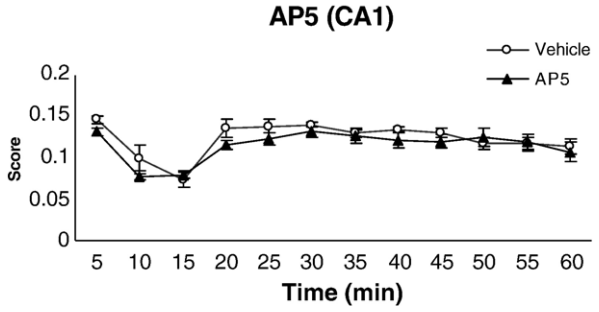

Fig. 4. Formalin test: the effect of AP5 injected into the CA1 region. Data are expressed as the scores of nociceptive behavior induced by formalin injection (50 μl at 5%) in rats treated with AP5 (3.75 μg/0.75 μl) 5 min before formalin administration. Each animal was observed for 60 min after formalin injection. Each point is the mean±SEM of the accumulative time of nociceptive behavior/5 min. The comparison was done between vehicle and AP5-treated groups ($n=7$). AP5 injected into the CA1 region had no effect in the behavior scores of the formalin test (*$p<0.05$).

2.5. Histology

Approximately 1 h after being tested, animals were administered an overdose of ether and their brains were removed and stored in 10% formalin for 48 h. Cannulae placements were verified by performing 100 μm coronal sections. Each subject's data were included in the statistical analysis only if cannula tips were just above the aimed region and the injection tracks were exactly spread in a limited area in the CA1 or dentate gyrus of the hippocampus (Fig. 1).

2.6. Statistical analysis

The effects of drugs were analyzed by one-way and repeated measure ANOVA for the phase data (Figs. 3, 5, 7 and 9) and the time course data (Figs. 2, 4, 6 and 8) respectively. Post hoc analyses were made by LSD test. All results are presented as

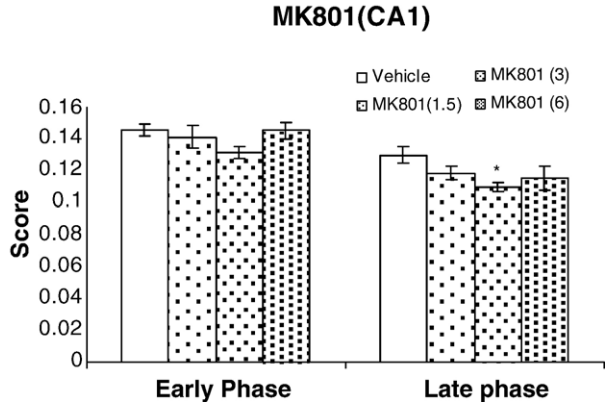

Fig. 3. Average pain scores during the early (0–5 min) and late (25–60 min) phases of the formalin test. Rats received bilateral injections of MK801 into the CA1 region of hippocampus. The effective dose of MK801 (3 μg/0.5 μl) had a significant anti-nociceptive effect in the tonic phase only (*$p<0.05$ *vs.* vehicle).

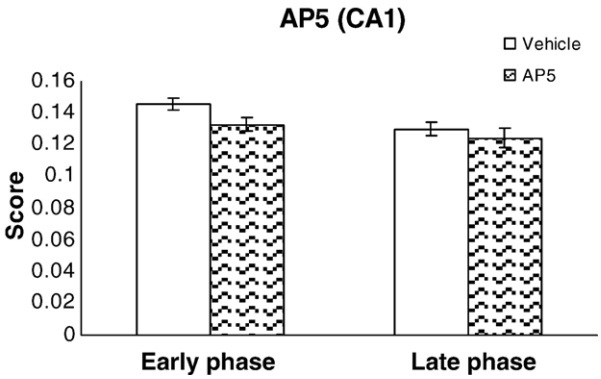

Fig. 5. Average pain scores during the early (0–5 min) and late (25–60 min) phases of the formalin test. Rats received bilateral injections of AP5 into the CA1 region of hippocampus. The AP5 (3.75 μg/0.75 μl) had no effect in the acute and tonic phases (*$p<0.05$ *vs.* vehicle).

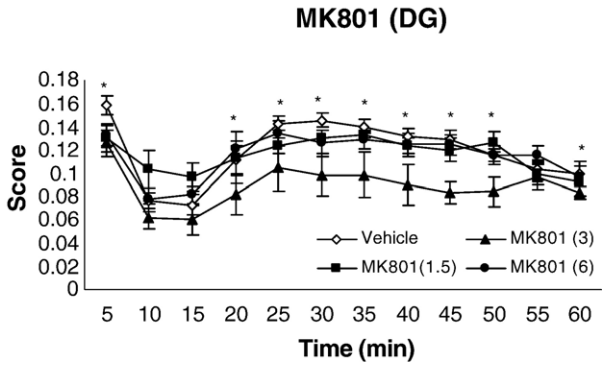

Fig. 6. Formalin test: the effect of MK801 injected into the dentate gyrus. Data are expressed as the scores of nociceptive behavior induced by formalin injection (50 μl at 5%) in rats treated with MK801 (1.5, 3 and 6 μg/0.5 μl) 5 min before formalin administration. Each animal was observed for 60 min after formalin injection. Each point is the mean±SEM of the accumulative time of nociceptive behavior/5 min. The comparison was done between vehicle and MK801-treated groups ($n=7$). The effective dose of MK801 (3 μg/0.5 μl) had a significant antinociceptive effect during 5, 20, 25, 30, 35, 40, 45, 50 and 60 timepoints after formalin injection (*$p<0.05$ *vs.* vehicle).

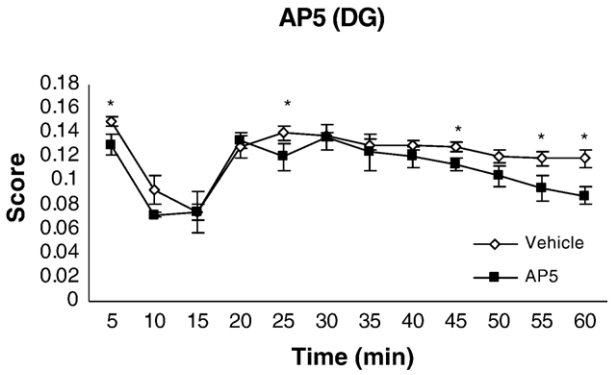

Fig. 8. Formalin test: the effect of AP5 injected into the dentate gyrus. Data are expressed as the scores of nociceptive behavior induced by formalin (50 μl at 5%) in rats treated with AP5 (3.75 μg/ 0.75 μl) 5 min before formalin administration. Each animal was observed for 60 min after formalin injection. Each point is the mean±SEM of the accumulative time of nociceptive behavior/ 5 min. The comparison was done between vehicle and AP5-treated groups ($n=7$). AP5 had a significant antinociceptive effect during 5, 25, 45, 55 and 60 timepoints (*$p<0.05$ *vs* vehicle).

means±S.E.M. In all statistical comparisons, P values<0.05 were considered to be significant.

3. Results

3.1. Experiment 1

Formalin injection into the left paw resulted in the typical biphasic response (Fig. 2). The NMDA antagonist MK801 injected bilaterally into the CA1 hippocampal region 5 min before the formalin test had a significant effect only at a dose of 3 μg/0.5 μl, compared to the saline control group. This effect was apparent during the late phase only (25–60 min, $F_{4,30}=3.5$; $p<0.019$; Fig. 3), and during the 10 and 40 timepoints (Fig. 2).

3.2. Experiment 2

Formalin injection into the left paw of rat resulted in the typical biphasic response (Fig. 4). The NMDA antagonist AP5 (3.75 μg/0.75 μl) injected bilaterally into the CA1 hippocampal region 5 min before the formalin test had no effect during the early (0–5 min) and late (25–60 min) phases of the formalin test, as compared to the saline control group (Fig. 5).

3.3. Experiment 3

Formalin injection into the left paw resulted in a typical biphasic response (Fig. 6). Compared to the saline group, bilateral injection of 3 μg/0.5 μl MK801 into the dentate gyrus 5 min before the formalin test resulted in a significant decrease in the overall pain behavior scores of rats. This effect of MK801was apparent in both the acute (0–5 min, $F_{4,26}=2.9$; $p<0.04$) and tonic (25–60 min, $F_{4,26}=3.5$; $p<0.02$) intervals of the formalin test (Fig. 7) and during the 5, 20, 25, 30, 35, 40, 45, 50, 60 timepoints in the time course data (Fig. 6). In other concentrations (1.5 and 6 μg/0.5 μl) MK801 had no effect on pain behavior.

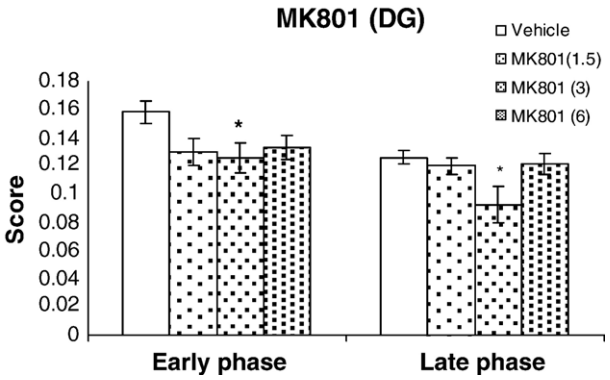

Fig. 7. Average pain scores during the early (0–5 min) and late (25–60 min) phases of the formalin test. Rats received bilateral injections of MK801 into the dentate gyrus The effective dose of MK801 (3 μg/0.5 μl) had a significant antinociceptive effect in the acute and tonic phases (*$p<0.05$ *vs*. vehicle).

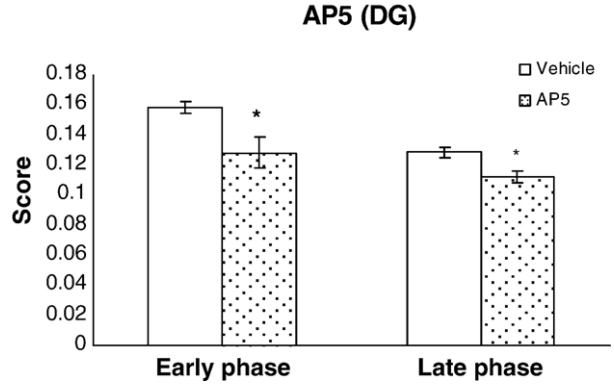

Fig. 9. Average pain scores during early (0–5 min) and late (25–60 min) phases of the formalin test. Rats received bilateral injections of AP5 into the dentate gyrus. AP5 had a significant antinociceptive effect in the acute and tonic phases (*$p<0.05$ *vs*. vehicle).

3.4. Experiment 4

Formalin injection into the left paw of the rat resulted in the typical biphasic response (Fig. 8). Compared to the saline group, bilateral injection of the NMDA antagonist AP5 (3.75 μg/0.75 μl) into the dentate gyrus 5 min before the formalin test resulted in a significant decrease in the overall pain behavior scores of rats. This effect was apparent in both the acute (0–5 min, $F_{2,16}=4.6$; $p<0.026$) and tonic (25–60 min, $F_{2,16}=4.3$; $p<0.03$) intervals of the formalin test (Fig. 9) and during the 5, 25, 45, 55, 60 timepoints (Fig. 8).

4. Discussion

These results indicate that the competitive and noncompetitive NMDA antagonists AP5 and MK801 administered into the dentate gyrus significantly reduce pain behavior in both the acute and tonic phases of the formalin test. In contrast, injection of these compounds into the CA1 region of the hippocampus was significantly less effective: AP5 had no significant effect on nociceptive behaviors both in the acute and tonic phases, whereas only one of the three tested doses of MK801 (3 μg) changed nociceptive behavior of rats in the tonic, but not the acute phase of the formalin test.

Previous studies, including a study performed in our lab, have shown that the hippocampal formation is involved in nociception (Delgado, 1955; Khanna and Sinclaire, 1992; McKenna and Melzack, 1992; Seltzer et al., 1991; Soleimannejad et al., 2006). Granular cells in the dentate gyrus are glutamatergic and GABAergic and receive synapses from neurons of the entorhinal cortex through the perforant pathway (Amaral and Witter, 1995; Vizi and Kiss, 1998). In turn, these granular cells send their axons into the CA3 area of the hippocampus via the mossy fiber pathway (Amaral and Witter, 1995). Granular cells axon terminals in the CA3 area synapse onto both glutamatergic pyramidal neurons and GABAergic interneurons (Amaral and Witter, 1995; Vizi and Kiss, 1998). In strata radiatum and oriens of the hippocampal CA1 area, most glutamate-releasing nerve terminals derive from axon collaterals of CA3 pyramidal cells. The two main targets of this input are the spines of pyramidal cells and the dendritic shafts of the different kinds of interneurons. Fast synaptic transmission in these synapses is mostly mediated by AMPA- and NMDA-type glutamate receptors (Baude et al., 1995; Nusser et al., 1998a; Racca et al., 2000).

In dentate gyrus NMDA antagonists may act on glutamatergic granular cells and inhibit the activity of these cells. This support previous finding that disruption of neural activity in the dentate gyrus reduces formalin pain (McKenna and Melzack, 1992).

Our results show that the NMDA antagonist MK801, injected into the CA1area of the hippocampus, reduces pain during the late phase after formalin injection. Since the CA1 GABAergic interneurons have NMDA receptors (see above; Baude et al., 1995; Nusser et al., 1998a; Racca et al., 2000) it is possible that MK801 exerts its antinociceptive effect by inhibiting these interneurons in the hippocampal CA1 area. This suggestion support previous findings that subcutaneous formalin increased firing rate of a majority of the field CA1 putative GABAergic interneurons and induced a long lasting depression of synaptic excitability of CA1 pyramidal cell (Khanna, 1997; Khanna and Zheng, 1999).

The present data show that AP5, injected into the CA1 area of hippocampus, had no significant effect on nociceptive behaviors both in the acute and tonic phases. Our findings are in agreement with McKenna's finding that AP5 injections at sites in the CA region did not reduce overall pain behavior (McKenna and Melzack, 2001). In contrast to the AP5 data, we found that MK801 reduces nociception in the second phase of the formalin test when injected into the CA1 area, supporting the anti-nociceptive role of NMDA antagonist when applied into other brain sites (McKenna and Melzack, 2001). It is possible, therefore, that MK801, being a noncompetitive antagonist of NMDA receptors, exert significant anti-nociceptive effect in a dose of 3 μg but not 1.5 or 6 μg, in contrast to the AP5 effect. This difference between MK801 and AP5 is supported by previous study, showing that they differed in their ability to protect against hippocampal hypoxic neuronal damage (Schurr et al., 1995). This difference could be explained by differential activity at the receptor site (Hardman et al., 1996).

The present study confirms that the CA1 region of hippocampus and dentate gyrus is involved in neural processing related to persistent pain. AP5 or MK801 injected into the dentate gyrus may attenuate directly or indirectly acute and tonic nociceptive signals to forebrain, or other brain structures, receiving efferent outflow from the hippocampal formation. It seems that NMDA receptors in the CA1 region may have a role in persistent nociceptive behavior. The precise mechanism(s) through which MK801 causes reduced nociceptive behavior is not clearly understood at present.

In conclusion, our data suggest that NMDA-sensitive mechanisms in the dentate gyrus modulate both acute and tonic noxious sensory processing. In contrast, the hippocampal CA1 region might modulate tonic pain behavior only.

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