Title: Involvement of Dopaminergic System in the Dentate Gyrus of the Hippocampus in Modulating the Orofacial Pain-Related Behaviors in the Rats

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Abstract

Chemical stimulation of the lateral hypothalamus (LH) induces analgesia by formation of neural circuitries with multiple brain regions. The involvement of hippocampal dopaminergic receptors in the LH stimulation-induced antinociception in certain animal models of pain has been documented, however, considering the fact that the neural circuitries involved in the mediation of orofacial pain are not the same as those that mediate the other types of pain, the present study aims to detect the role of dopamine receptors within the dentate gyrus (DG) in the antinociceptive responses induced by LH stimulation in an animal model of orofacial pain. Male Wistar rats (220-250 g) were implanted with two separate cannulae into the LH and DG on the same side. D1- or D2-like dopamine receptor antagonist, SCH23390 or Sulpiride (0.25, 1, and 4 μg) were microinjected into the DG, five minutes prior to intra-LH injection of carbachol (250 nM). The animals were then injected with formalin 1% (50 μl; sc) into the upper lip lateral to the nose and subjected to the orofacial formalin test. Intra-DG administration of SCH23390 or Sulpiride attenuated the antinociceptive responses induced by intra-LH microinjection of carbachol during orofacial formalin test. The findings of the current study suggest that chemical stimulation of the LH modulates the orofacial pain possibly through activation of the DG dopaminergic neurons. Due to the high incidence and prevalence of orofacial pain in the general population, understanding how such neuronal circuitry modulates nociceptive processing will advance the search for novel therapeutics.

Keywords: Orofacial pain; D1-like dopamine receptor; D2-like dopamine receptor; Dentate gyrus; Lateral hypothalamus; Rat
1. Introduction

Inflammation in the orofacial region is known to frequently cause persistent pain in the orofacial region and is considered as a serious public health issue. Central and peripheral mechanisms are involved in the persistent ectopic orofacial pain associated with orofacial inflammation (Imbe et al., 2001). The diagnosis, etiology, and management of orofacial pain are complex, multidisciplinary, and multifactorial processes (Crandall, 2018). Among all pain models, the formalin test is a widely used and the most reliable model for producing and quantifying nociception in the rat's trigeminal region. Besides, the formalin test is considered more relevant to clinical pain than the other animal models of pain (Raboisson and Dallel, 2004).

The applied chemical stimulus, i.e., formalin injection into the orofacial receptive field produces a typical biphasic nociceptive response: a brief early phase and a prolonged late phase (Shields et al., 2010). It has been believed that the early phase is caused by the direct effect of formalin chemical stimulation on the nociceptors, which caused the activation of the C-fibers and the late phase is due to the inflammatory response in the peripheral tissue (Tjølsen et al., 1992).

Neurons containing orexin are localized exclusively in the lateral hypothalamus (LH), and their projections are widely distributed in multiple brain regions such as the limbic system, locus coeruleus, periaqueductal gray matter, reticular formation, and trigeminal caudate nucleus [for review see (Kang et al., 2021)]. Chemical stimulation of LH attenuated the nociceptive responses in several animal pain models such as acute and chronic, as well as orofacial pain [for review see (Razavi and Hosseinzadeh, 2017)]. Orexin neurons project from the LH to dopaminergic neurons in the ventral tegmental
area (VTA), and VTA dopamine neurons project to the nucleus accumbens (NAc), amygdala, hippocampus, and prefrontal cortex, forming the mesocorticolimbic dopamine system (Korotkova et al., 2003).

The mesolimbic dopamine system modulates sensory and emotional aspects of chronic pain sensation (Yang et al., 2020). Dopamine belongs to the catecholamine family and binds to two types of dopamine receptor families: the D1-like receptor subtypes, including D1 and D5 receptors, and the D2-like receptor subtypes, including D2, D3, and D4 receptors (Missale et al., 1998). It has been reported that orofacial pain syndrome occurs more frequently in patients with dopamine dysregulation such as Parkinson's disease than in the general population (Ford et al., 1996). Besides, a positron emission tomography (PET) study on patients with burning mouth syndrome showed a decrease in the D1/ D2 ratio and dopamine hypofunction in the nigrostriatal dopaminergic pathway (Hagelberg et al., 2003). It has been shown that dopaminergic receptors within the VTA (Matini et al., 2020) and NAc (Haghparast et al., 2020) modulate the antinociceptive responses induced by chemical stimulation of the LH in an animal model of orofacial pain.

On the other hand, the hippocampal formation (HF) receives dopaminergic inputs originating from the VTA and substantia nigra and plays an essential role in pain modulation (Scatton et al., 1980). Dopamine receptors within the dentate gyrus (DG) as the main gateway of the HF participate in the antinociception induced by LH stimulation in animal models of acute and persistent inflammatory pain (Khaleghzadeh-Ahangar et al., 2021; Torkamand et al., 2021). The previous study demonstrated that dopamine receptors in the dorsal hippocampus were involved in suppressing nociception induced by formalin injection into the orofacial region (Shamsizadeh et al., 2013). Considering
the fact that different types of pain based on their origins and different regions of the brain involved different mechanisms in modulating pain, this study aimed to investigate the probable role of dopamine receptors within the DG region in the antinociceptive responses induced by the chemical stimulation of LH using carbachol.

2. Materials and Methods

2.1. Animals and Surgical Procedures

Seventy-eight adult male Wistar rats (220-250 g) were randomly chosen (Pasteur Institute, Tehran, Iran) and were kept in an animal house (3-4 rats per cage, temperature 22 ± 2°C, humidity 50 ± 10 %) with a 12 h light/dark cycle and free access to food and water. All experiments followed the ethical guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication. 8th edition, revised 2011) and were approved by the Research and Ethics Committee of the Faculty of Medicine, AJA University of Medical Sciences (IR.AJAUMS.REC.1400.096), Tehran, Iran. Rats were randomized into control and treatment groups before any assessments were performed.

Animals were anesthetized with a mixture of ketamine 10% (100 mg/kg; ip) and xylazine 2% (10 mg/kg; ip) and were placed in a stereotaxic apparatus (Stoelting, USA). Two stainless steel guide cannulae (23-gauge) were unilaterally lowered and placed 1 mm above the LH and DG regions based on the Paxinos and Watson rat brain atlas (Paxinos and Watson, 2006). Accordingly, the coordinate for the LH was anteroposterior (AP) = 2.65 mm caudal to the bregma, (Lat) = ±1.3 mm lateral to the midline and dorsoventral (DV) = 8.6 mm ventral from the skull surface, and for the DG was as follows: AP = - 4.08
mm, Lat = ±2.2 mm, and DV = 3.6 mm from the skull surface. The guide cannulae were then fixed to the skull surface using dental acrylic cement. Seven days postoperative period was considered for the recovery.

2.2. Drugs and Drug Administration

In this study, carbachol (2-Hydroxyethyl) trimethylammonium chloride carbamate (Tocris Bioscience, Bristol, UK) was diluted in saline (250 nM). The different solutions of D1-like dopamine receptor antagonist, SCH23390, (R)-(+) -7-Chloro-8-hydroxy-3-methyl-1-phenyl-2, 3, 4, 5-tetrahydro-1H-3-benzazepine hydrochloride (Tocris Bioscience, Bristol, UK) were prepared in saline (0.25, 1, and 4 μg). Also, Sulpiride, (S)-5-aminosulfonyl-N-[(1-ethyl-2 pyrrolidinyl) methyl]-2-methoxybenzamide (Tocris Bioscience, Bristol, UK) as a selective D2-like dopamine receptor antagonist was dissolved in dimethyl sulfoxide (DMSO) 12% in different doses (0.25, 1, and 4 μg). All of the used doses were selected according to the pervious study (Shafiei et al., 2018). All of the solutions were made freshly on the experiment day and microinjected into the LH or DG in 0.5 μl volume via a 1-μl-Hamilton syringe using a polyethylene tube (PE-20) connected to a 30-gauge needle.

2.3. Orofacial Formalin Test

The animals were placed in the lab environment for 30 minutes before initiating the experiments during the acclimation period. Formalin 1% (50 μl; sc) was injected into the upper lip just lateral to the nose using a 29-gauge injection needle. In order to observe the nociceptive response after the formalin injections, rats were placed in a plexiglass box (30 × 30 × 30 cm) with a mirror angled at 45° under the surface of box (Raboisson and
Dallel, 2004). The time that animals spent rubbing the injected area was calculated in 15 blocks of 3 minutes during a total time of 45 minutes. A typical biphasic nociceptive response was observable after the formalin injection (Haghparast et al., 2020), the early phase between 0 and 3 min reflecting the direct chemical stimulation of the nociceptive terminals, and the late phase between 15 and 33 min representing the orofacial inflammatory pain (Supplementary Fig. 1).

2.4. Experimental Design

An effective dose of carbachol (250 nM / 0.5 μl saline) was selected based on the previous study (Supplementary Fig. 2) and microinjected into the LH to examine the effect of the chemical stimulation of LH on orofacial pain modulation (Haghparast et al., 2020). Different doses (0.25, 1, and 4 μg) of SCH23390 or Sulpiride were microinjected into the DG area to investigate the role of intra-DG D1 and D2-like dopamine receptors in carbachol-induced analgesia, five minutes before the microinjection of carbachol (Fig. 1). Five minutes after the drug administration, formalin was injected into the orofacial region, and nociceptive behavior was evaluated. To investigate the effect of sole microinjection of D1- and D2-like dopamine receptors into the DG region, two experimental groups, received only the highest dose of SCH23390 or Sulpiride (4 μg; intra-DG) before intra-LH administration of saline.

2.5. Histological Verification

After the termination of experiments, animals were deeply anesthetized with a high dose of ketamine and xylazine and were transcardially perfused with 0.9% saline and 10% formaldehyde solution. The rats’ brain was then harvested, and 50-μm coronal
sections of the brain were prepared using a rotatory microtome. The cannulae placements in LH and DG regions were checked using the rat brain atlas (Paxinos and Watson, 2006). Only the animals with correct cannula placements (Fig. 2) were included into the final data analysis.

2.6. Statistics

Data analyses were done using GraphPad Prism® 6.0 software. The results are expressed as mean ± SEM (standard error of mean). Kolmogorov-Smirnov goodness-of-fit test was then used to verify the normal distribution and homogeneity of variance. To evaluate the face rubbing time during the early or late phases of orofacial formalin test, one-way analysis of variance (ANOVA) followed by Dunnett's or Newman-Keuls multiple comparison tests was used as needed. Unpaired Student's t-test was used to compare the effect of intra-DG microinjection of the highest dose of SCH23390 or Sulpiride with or without intra-LH microinjection of carbachol on the face rubbing time. Besides, to estimate the effective dose 50% (ED50) of SCH23390 or Sulpiride, the best-fitted line to show the data on scatter plot was drawn, and the ED50 values were estimated during the early and late phases of the orofacial formalin test. The effect size was also calculated by dividing the mean difference between the control and experimental groups during both phase of the orofacial formalin test by the standard deviation of the population from which the different treatment groups were taken. P < 0.05 was considered to be statistically significant.
3. Results

Based on previous studies, formalin injection into the rat’s orofacial region induces a biphasic nociceptive response. Statistical analysis showed that the face rubbing time in formalin-treated group is significantly more than that of saline-treated group [Formalin injection effect: F (1,150) = 762.5, P < 0.0001; Time effect: F (14,150) = 21.13, P < 0.0001; Formalin injection and time interaction effects: F (14,150) = 19.93, P < 0.0001; Supplementary Fig. 1; Adapted from Haghparast et al., 2020 (Haghparast et al., 2020); Springer Nature license agreement number 5244730959475]. Furthermore, one-way ANOVA followed by Dunnett’s multiple comparison test demonstrated that different doses of the intra-LH microinjection of carbachol (62.5, 125 and 250 nM / 0.5 µl saline) significantly attenuated the face rubbing time during both the early [F (3,21) =4.568, P = 0.0151; Supplementary Fig. 2, left panel] and late [F (3,21) = 18.83, P < 0.0001; Supplementary Fig. 2, right panel] phases of orofacial formalin test (Adapted from Haghparast et al., 2020 (Haghparast et al., 2020); Springer Nature license agreement number 5244730959475). Therefore, in the present study, the dose of 250 nM carbachol was chosen for the chemical stimulation of the LH in the next experiments on D1- and D2-like dopamine receptors in the DG region of the hippocampus.

3.1. The blockade of D1-like dopamine receptors within the DG region attenuated the antinociceptive responses induced by LH stimulation

One-way ANOVA followed by Dunnett’s comparison tests indicated that intra-DG microinjection of D1-like dopamine receptor antagonist, SCH23390 (1 and 4 µg/ 0.5 µl saline) before intra-LH carbachol administration (250 nM / 0.5 µl saline), significantly
increased face rubbing behavior during early \([F (3, 30) = 4.646, P = 0.0096; \eta^2 = 0.34; \text{Fig. 3, left panel}]\) and late \([F (3, 30) = 24.84, P<0.0001; \eta^2 = 0.73; \text{Fig. 3, right panel}]\) phases of orofacial pain compared to saline-carbachol group. Besides, one-way ANOVA followed by Newman-Keuls multiple comparison tests indicated that there is a significant difference between SCH23390 at the doses of 4 and 1\(\mu\text{g} / 0.5 \mu\text{l} \text{ saline} (P < 0.001)\) and SCH23390 at the doses of 1\(\mu\text{g} \text{ and } 0.25 \mu\text{g} / 0.5 \mu\text{l} \text{ saline} (P<0.05)\) during the late phase of orofacial formalin test. However, there was no significant difference between the mentioned doses during the early phase of orofacial pain. Unpaired Student's \(t\)-test showed that the highest dose of SCH23390 (4 \(\mu\text{g} / 0.5 \mu\text{l} \text{ saline}) could completely block the antinociceptive responses induced by carbachol, so that there was no significant difference between SCH23390 (4 \(\mu\text{g})\)-carbachol and SCH23390-saline groups in both early \([t_{(13)} = 0.4760, P = 0.642]\) and late \([t_{(13)} = 0.6278, P = 0.5410]\) phases of the orofacial formalin test.

Furthermore, as it has been depicted in Fig. 4, comparison of the different doses of SCH23390 in the percentage of the changes in face rubbing time to carbachol-control group (intra-LH microinjection; 250 nM / 0.5 \(\mu\text{l} \text{ saline}) between the early and late phases of orofacial formalin test demonstrated that the antinociceptive response elicited by carbachol microinjection was reversed by a lower dose of SCH23390 in the late phase (ED50=0.53) than that of the early phase (ED50=1.44) during the orofacial formalin test.

### 3.2. The blockade of D2-like dopamine receptors within the DG region attenuated the antinociceptive responses induced by LH stimulation

As it has been shown in Fig. 5, ordinary one-way ANOVA followed by Dunnett's comparison tests indicated that only administration the highest dose of D2-like dopamine
receptor antagonist, Sulpiride (intra-DG; 4 μg/ 0.5 μl DMSO) before intra-LH carbachol microinjection (250 nM / 0.5 μl saline), could significantly increase the face rubbing response during early [F (3, 31) = 4.385, P = 0.0119; η2 = 0.32; Fig. 5, left panel] and late [F (3, 31) = 20.52, P < 0.0001; η2 = 0.69; Fig. 5, right panel] phases of orofacial pain compared to saline-carbachol group. In addition, one-way ANOVA followed by Newman-Keuls multiple comparison tests showed that there is a significant difference between Sulpiride at the doses of 4 and 1μg/ 0.5 μl DMSO (P<0.001) and Sulpiride at the doses of 1μg and 0.25 μg / 0.5 μl DMSO (P < 0.05) during the late phase of orofacial formalin test but not in the early phase. Unpaired Student’s t-test also showed that the highest dose of Sulpiride (4 μg / 0.5 μl DMSO) completely blocked the antinociceptive responses induced by intra-LH microinjection of carbachol, so that there was no significant difference between Sulpiride (4 μg)-carbachol and Sulpiride-saline groups during early [t (13) = 0.1418, P = 0.8894] and late [t (13) = 0.2079, P = 0.8385] phases of the orofacial formalin test.

On the other hand, as shown in Fig. 6, the effect of different doses of Sulpiride in the percentage of the changes in face rubbing time in comparison with the carbachol-control group (intra-LH microinjection; 250 nM / 0.5 μl saline) is measured during the early and late phases of the orofacial formalin test. Accordingly, the antinociception induced by carbachol was reversed by a lower dose of Sulpiride in the late phase (ED50=0.93) compared to the early phase (ED50=1.47) of the orofacial formalin test.
4. Discussion

The present study illustrated that D1- and D2-like dopamine receptors located in the DG region of the hippocampus are essential for antinociceptive responses produced by chemical stimulation of LH during both early and late phases of orofacial pain. The significant findings of the present study were as follows: (i) Blockade of D1-like dopamine receptor via SCH23390 microinjection into this area attenuated the antinociception induced by intra-LH carbachol microinjection during early and late phases of the orofacial formalin test; (ii) Intra-DG microinjection of the D2-like dopamine receptor antagonist, Sulpiride, significantly reversed the analgesia produced by the LH stimulation during both phases of orofacial pain; (iii) The modulatory role of dopamine receptors within the DG region in the LH stimulation-induced antinociceptive responses during the late phase of orofacial pain was noticeably more prominent than that in the early phase.

According to the obtained results, the DG region of the hippocampus is involved in antinociception produced by the chemical stimulation of LH. Neuroanatomical tracing revealed that LH produces a vast network of connections via sending its neural projections to a large number of brain areas and produces circuits orchestrating pain behaviors. For instance, optogenetic activation of axonal projections of LH neurons to the ventrolateral periaqueductal gray area attenuates nociception via preferentially targeting glutamatergic over GABAergic neurons in persistent inflammatory or neuropathic pain models. However, these neurons produce pathway-specific behavioral effects. So, LH projections to the lateral habenula regulate aversion but not nociception (Siemian et al., 2021). In this respect, it has been shown that the LH-DG neural circuitry participates in pain modulation.
via the LHs' projections to the DG region in multiple animal models of acute and chronic pain (Brojeni et al., 2019; Khaleghzadeh-Ahangar et al., 2021; Torkamand et al., 2021). According to the results of immunohistochemistry studies, the c-fos expression as a sensitive and reliable marker of neuronal activity increased in the hippocampal DG and CA3 regions at two hours following subcutaneous injection of formalin in rats (Ceccarelli et al., 1999). Besides, a significant increase in the c-fos level in the hippocampus and a decrease in phosphorylated cyclic AMP-response element-binding protein was observed following condition place preference induced by intra-LH administration of carbachol (Haghparast et al., 2011). So, it seems that chemical stimulation of LH may activate LH-DG neural circuitry, which can be involved in orofacial pain modulation.

The present study results demonstrated that blockade of dopamine receptors within the DG region could completely block the antinociceptive responses induced by chemical stimulation of LH following formalin injection into the orofacial region. In this regard, the previous study revealed that the dopaminergic receptors located in the VTA contributes in antinociception induced by chemical stimulation of LH during orofacial pain in rats (Matini et al., 2020). In addition, the role of dopaminergic receptors in the NAc in antinociceptive response-induced by chemical stimulation of LH has been demonstrated in formalin-induced orofacial pain (Shafiei et al., 2018). The dentate gyrus plays an essential role in pain information processing. In this respect, it has been documented that microinjection of N-methyl- D-aspartate receptor antagonists, cholinergic agonists, and anesthetics attenuated nociceptive response [for review see (Liu and Chen, 2009)]. We previously reported that the chemical stimulation of LH modulates orofacial pain through activation of orexin receptors in the CA1 area of the hippocampus (Haghparast et al., 2018). On the other hand, the role of dopamine receptors within the
DG region in LH-induced antinociception has been shown in acute and inflammatory pain models (Khaleghzadeh-Ahangar et al., 2021; Torkamand et al., 2021). Orexin-containing neurons originated from the LH project to the brainstem noradrenergic, serotonergic, as well as dopaminergic cells (Peyron et al., 1998; Horvath et al., 1999; Brown et al., 2001). So, it seems that the functions of LH are directly attributed to the activation of orexin receptors or indirectly through neural projections to the other cells, such as dopaminergic neurons. The modulatory role of dopamine neurotransmitter in orofacial pain has been shown. Dopamine depletion of the nigrostriatal pathway caused a hyperalgesic response to orofacial pain (Maegawa et al., 2015). Besides, patients with burning mouth syndrome showed decreased endogenous dopamine levels in the putamen and dopamine hypofunction in the nigrostriatal dopaminergic pathway (Hagelberg et al., 2003). On the other hand, there is an interaction between the opioidergic and dopaminergic systems in the dorsal hippocampus leading to modulation of orofacial pain (Haghparast et al., 2014). Meyer et al. reported that injection of apomorphine as a dopamine receptor agonist into the ventrolateral periaqueductal gray induced robust antinociception during the hot-plate test in rats via inhibition of the GABAergic system, as a common mechanism between dopamine and opioids (Meyer et al., 2009).

According to the present study results, the role of D1- and D2-like dopamine receptors of DG was noticeably more prominent in the late phase of orofacial formalin pain compared to the early phase. Formalin injection into the orofacial receptive field provokes a biphasic orofacial pain and evoked activity in thinly myelinated Aδ and non-myelinated C fibers as well as trigeminal and spinal nociceptive neurons (Raboisson and Dalle, 2004). Increased firing impulses, following external noxious stimulation of the orofacial region, conducted through Aδ- or C-fibers in the central terminals and
depolarize them which subsequently causes the release of certain neurotransmitters such as glutamate, GABA, noradrenaline, serotonin as well as dopamine [for review see (Sessle, 1987)]. The early phase of orofacial formalin nociception is attributed to activating non-myelinated afferent nerve fibers, C fibers. At the same time the late phase results from inflammation of orofacial tissues and the functional changes in the dorsal horn leading to central and peripheral sensitization of trigeminal nociceptive neurons (Takeda et al., 2011). Therefore, differences in the modulatory role of the dopamine system in the early and late phases of orofacial pain may stem from different mechanisms by which dopamine receptors are involved in antinociception-induced by LH stimulation.

Summing up, the present study suggests that chemical stimulation of LH using carbachol microinjection activates the DG dopaminergic neurons, and so, participates as a part of neural circuitry that modulates tonic orofacial pain. Additionally, understanding the critical role of this neural circuitry in orofacial pain modulation offers an appropriate approach to the development of more efficient therapies for alleviating orofacial pain.

Statements and Declarations

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Competing Interests
The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Abbas Haghparast: Conceptualization, designing the study, Data analysis and interpretation of data, and approval the final manuscript. Amir Haghparast: Data acquisition, interpretation of data, writing the manuscript. Mitra Yousefpour: Designing the study, interpretation of data, and approval the final manuscript. Mina Rashvand: Writing the manuscript and approval the final manuscript. Laya Ghahari: Designing the study and approval the final manuscript. Bita Rohani: Data analysis, and approval the final manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are not publicly available due to compare with previous studies in this lab but are available from the corresponding author on reasonable request.

Ethics Approval

This study was performed in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication. 8th edition, revised 2011) and were approved by the Research and Ethics Committee of the Faculty of Medicine, AJA University of Medical Sciences (IR.AJAUMS.REC.1400.096), Tehran, Iran.
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Fig. 1. Graphical scheme of experimental protocol showing the effect of intra-DG microinjection of D1- or D2-like dopamine receptor antagonists on the antinociceptive responses induced by intra-LH microinjection of carbachol during orofacial formalin test. 
LH, Lateral hypothalamus; DG, Dentate gyrus.
Fig. 2. Schematic illustrations of the rat’s brain section showing the approximate location of microinjections in (A) the lateral hypothalamus and (B) the dentate gyrus of hippocampus [□ = Vehicle (saline), ○=Vehicle (saline or DMSO), ● = Treatment (Carbachol or SCH23390), ■ = Treatment (Sulpiride) and ▲ = Misplacement]. All of the microinjections were performed unilaterally. cc, corpus callosum; ic, internal capsule; CPu, Caudate putamen (striatum); VMH, Ventromedial hypothalamic nucleus; D3V, Dorsal 3rd ventricle; DA, Dorsal hypothalamic area; f, fornix; LH, Lateral hypothalamus; PeFLH, Perifornical part of lateral hypothalamus; LV, Lateral ventricle; MCLH, Magnocellular nucleus of the lateral hypothalamus; mt, Mammillothalamic tract; DG, Dentate gyrus; GrDG, Granular dentate gyrus; PoDG, Polymorph layer of the dentate gyrus; MTu, Medial tuberal nucleus; 3V, 3rd ventricle; MoDG, Molecular dentate gyrus; CA1, Cornu ammonis area 1; CA2, Cornu ammonis area 2; CA3, Cornu ammonis area 3; PeF, Perifornical nucleus; SLu, Stratum lucidum of the hippocampus; Po, Posterior thalamic nucleus. Scale bar is 1 mm.
Fig. 3. Effect of intra-DG administration of D1-like dopamine receptor antagonist (SCH23390) on the antinociceptive responses produced by chemical stimulation of LH using carbachol. Administration of SCH23390 (1 and 4 μg/0.5 μl saline) into the DG region of the hippocampus significantly attenuated antinociception induced by chemical stimulation of LH using carbachol (250 nM / 0.5 μl saline) during both early and late phases of the orofacial formalin test. Each point represents the mean ± SEM for 7–8 rats in each group. *P < 0.05, **P < 0.01, and ***P < 0.001 compared to saline-carbachol group or the other groups with the different dose of SCH23390. ns, non-significant.
Fig. 4. A log dose–response curve of the effect of intra-DG administration of SCH23390 on the percentage of the changes in face rubbing time compared to the carbachol-control group during the early and late phases of formalin nociception. The effective dose (ED50) of SCH23390 in the late phase (ED50=0.53) was less than that in the early phase (ED50=1.44).
**Fig. 5.** The effect of D2-like dopamine receptor antagonist (Sulpiride) administration into the DG region on the antinociceptive responses produced by intra-LH microinjection of carbachol. Intra-DG administration of Sulpiride significantly attenuated the antinociception induced by carbachol microinjection into the LH area (250 nM / 0.5 µl saline) during early and late phases of the orofacial formalin test. Each point represents the mean ± SEM for 7–9 rats in each group. *P < 0.05, **P < 0.01, and ***P < 0.001 compared to DMSO-carbachol group or the other group with the different dose of Sulpiride. ns, non-significant.
Fig. 6. A log dose–response curve of the modulatory effect of Sulpiride microinjection into the DG region on the percentage of the changes in face rubbing time compared to the carbachol-control group during the early and late phases of orofacial formalin test. The effective dose (ED50) of Sulpiride in the late phase (ED50=0.93) was less than that in the early phase (ED50=1.47).