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Effects of dopaminergic agents on carrageenan hyperalgesia after intrathecal administration to rats

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Abstract

The present study explored the role of dopaminergic transmission in spinal cord in a model of carrageenan-induced inflammatory pain by examining the effects of selective agonists and antagonists of dopamine receptors. The results were as follows: (1) *trans*-(−)-4*aR*-4,4*a*,5,6,7,8,8*a*,9-octahydro-5-propyl-1*H*-pyrazolo[3,4 − *g*] quinoline hydrochloride (LY171555), a dopamine D_2 receptor agonist, produced anti-hyperalgesia (150 and 300 nmol) or hypoalgesia (300 nmol) in the inflamed hindpaws and non-inflamed hindpaws, respectively; spiperone hydrochloride (8-[4-(4-fluorophenyl)-4-oxobutyl]-1-phenyl-1,3,8-triazaspiro[4,5]decan-4-one hydrochloride), a dopamine D_2 receptor antagonist, decreased the pain threshold of non-inflamed hindpaws (300 nmol). (2) (±)-SKF38393 hydrochloride ((±)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol hydrochloride), a dopamine D_1 receptor agonist, had no effect on either hindpaw, even at a higher dose (300 nmol); *R*(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (*R*(+)-SCH23390 hydrochloride), a dopamine D_1 receptor antagonist, induced anti-hyperalgesia in the inflamed hindpaws (300 nmol). The present results suggest that the dopaminergic system in the spinal cord is involved in the central modulation of inflammatory hyperalgesia, and that the different effects are probably induced by different receptors.

Keywords: Dopaminergic agent; Carrageenan; Dopamine D_1 receptor; Dopamine D_2 receptor; Anti-hyperalgesia; Hypoalgesia; Spinal cord

1. Introduction

Increasing evidence shows that the central dopamine system is involved in the modulation of nociception at the supraspinal and the spinal cord levels, respectively. In the spinal cord level, descending dopaminergic fibers mainly emanate from the dopamine-containing neurons of the diencephalic A_{11} area (Björklund and Skagerberg, 1984; Hökfelt et al., 1979). Focal electrical stimulation in the diencephalic A_{11} dopamine cell area suppresses nociceptive responses of spinal multireceptive neurons, and this effect is prevented by iontophoretically applied dopamine D_2 receptor antagonists (Fleetwood-walker et al., 1988). Moreover, intrathecal (i.t.) administration of apomorphine or of a dopamine D_2 receptor agonist *trans*-(−)-4*aR*-4,4*a*,5,6,7,8,8*a*,9-octahydro-5-propyl-1*H*-pyrazolo[3,4 − *g*] quinoline hydrochloride (LY171555), also produces antinociception, and these effects are blocked by a dopamine D_2 receptor antagonist; while a dopamine D_1 receptor agonist, (±)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol hydrochloride ((±)-SKF38393 hydrochloride) has no effect on nociception (Barasi and Duggal, 1985; Barasi et al., 1987; Gao et al., 1998; Jensen and Yaksh, 1984; Liu et al., 1992). These results demonstrate that spinal dopaminergic transmission, especially the dopamine D_2 receptor, exerts an important role in the process of pain modulation.

The role of dopamine receptors of the spinal cord in inflammatory pain has not been evaluated yet. The carrageenan model of inflammatory pain in rats is a good one because of its many similarities to clinical inflammatory diseases, namely the large edematous response and persistent, strong hyperalgesia (Winter et al., 1962). Therefore, in the present study, carrageenan-induced hyperalgesia was used as an animal model to investigate the effects of selective agonists or antagonists of dopamine D_1 and D_2 receptors in the spinal cord on inflammatory hyperalgesia.

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2. Materials and methods

2.1. Animals

This study was conducted in concordance with the guidelines of the Ethical Standards of the International Association for the Study of Pain (Zimmermann, 1983). Sixty-two male Sprague–Dawley rats (weighing 200–225 g) were used in the experiments. The rats were housed in groups of 3–5 animals per cage, allowed free access to food and water with a natural day/night cycle, and were allowed to adapt to the laboratory for at least 3 days before the experiment.

2.2. Carrageenan-induced inflammation

Peripheral inflammation was induced by intraplantar injection (i.pl.) of carrageenan (2 mg/200 μl of 0.9% normal saline; λ-carrageenan, Sigma) in the left hindpaw of non-anaesthetized rats according to the method described by Winter et al. (1962).

2.3. Withdrawal responses to heat stimuli

Based on a previously described method (Hargreaves et al., 1988; Gao et al., 2000), we determined the withdrawal response latency of each hindpaw using the model 336 combination unit of paw stimulation (IITC, Life Science Instruments, USA). Rats were tested prior to carrageenan and drug administration, and at 15, 30, 45, 60, 75 and 90 min after drug administration. For testing, the rats were placed in a clear plastic cage on a raised glass platform and allowed 15 min to adapt. Each hindpaw received four stimuli, alternating between paws. The interstimulus interval for each paw was at least 1 min, and withdrawal latency for each paw was defined as the mean of the last three trials to eliminate the large variability. The heat source was maintained at a constant intensity, which produced a stable withdrawal latency of approximately 10–12 s during the training period. A 20-s cut-off was imposed on the stimulus duration to prevent tissue damage. Hyperalgesia to heat was defined as a decrease in withdrawal latency.

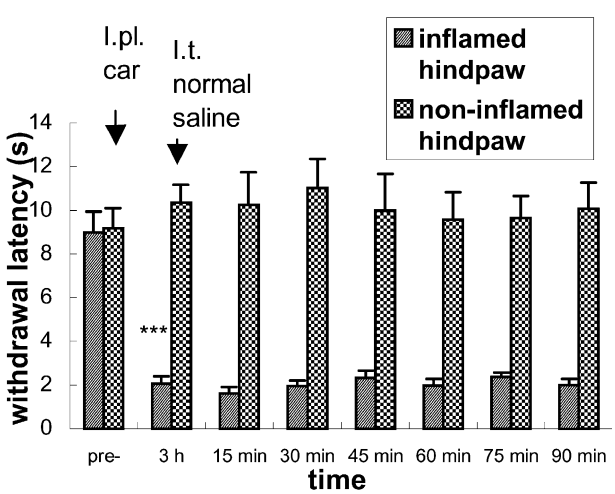

Fig. 1. Hyperalgesia produced at 3 h after i.pl. carrageenan and effects of i.t. normal saline at 3 h on hyperalgesia ($n = 5$). $^{***}P < 0.001$ vs. pre-carrageenan.

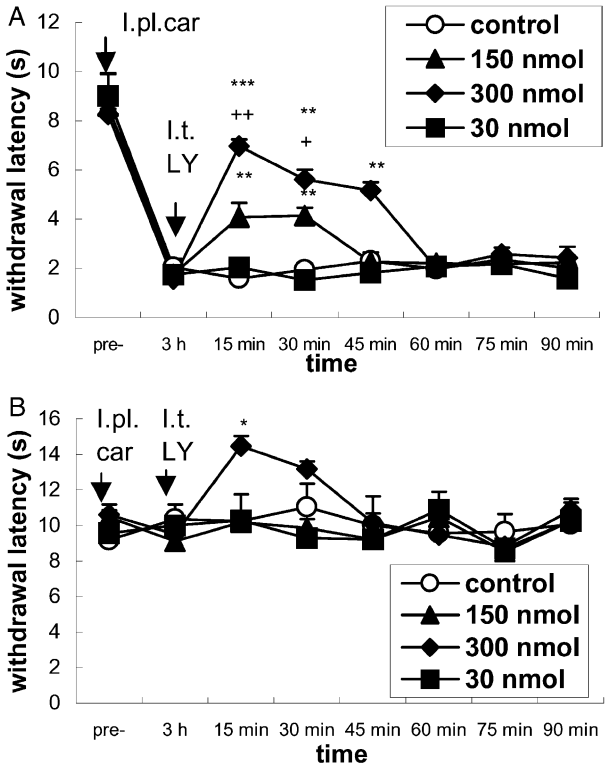

Fig. 2. Effects of i.t. dopamine D_2 receptor agonist, LY171555, on carrageenan-induced hyperalgesia. (A) In inflamed hindpaws, the dose of 150 nmol produced significant anti-hyperalgesia at 15 min ($P < 0.01$) and 30 min ($P < 0.01$) ($n = 6$) after i.t. administration, and 300 nmol further potentiated the anti-hyperalgesia at 15 min ($P < 0.01$) and 30 min ($P < 0.05$) ($n = 7$), but with 30 nmol there was no change in the control group ($n = 6$); (B) in non-inflamed hindpaws, only the dose of 300 nmol produced hypoalgesia at 15 min ($P < 0.05$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control; $+P < 0.05$, $++P < 0.01$ vs. 150 nmol.

2.4. Drugs

The drugs used in this study include the following: (±)-SKF38393 hydrochloride, LY171555, *R*(+)-SCH23390 hydrochloride (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride) and spiperone hydrochloride (8-[4-(4-fluorophenyl)-4-oxobutyl]-1-phenyl-1,3,8-triazaspiro[4,5]-decan-4-one hydrochloride); all of which came from Research Biochemicals (USA). Spiperone hydrochloride was dissolved with the help of ethanol (3 μl) and then diluted in 0.9% normal saline; other drugs were dissolved directly

in 0.9% normal saline. Control animals received an equivalent amount of solvent.

2.5. Implantation of i.t. cannulae

The animals were first anaesthetized with pentobarbitone sodium (40 mg/kg, i.p.), then a PE-10 tube was implanted through the gap between the second and third or third and fourth lumbar spines (L_{2-3} or L_{3-4}) to the subarachnoid space. The cannulated rats were allowed to recover for 3 days and were housed individually. For i.t. administration, the total injection solution 25 μl (15-μl drug solution followed by a normal saline flush of 10 μl) was pushed into the subarachnoid space smoothly within 2 min.

2.6. Statistical analysis

All data were expressed as the means ± S.E.M., and statistical comparison between groups was performed using an analysis of variance (ANOVA) followed by Tukey's test. A level of probability of 0.05 or less was accepted as significant.

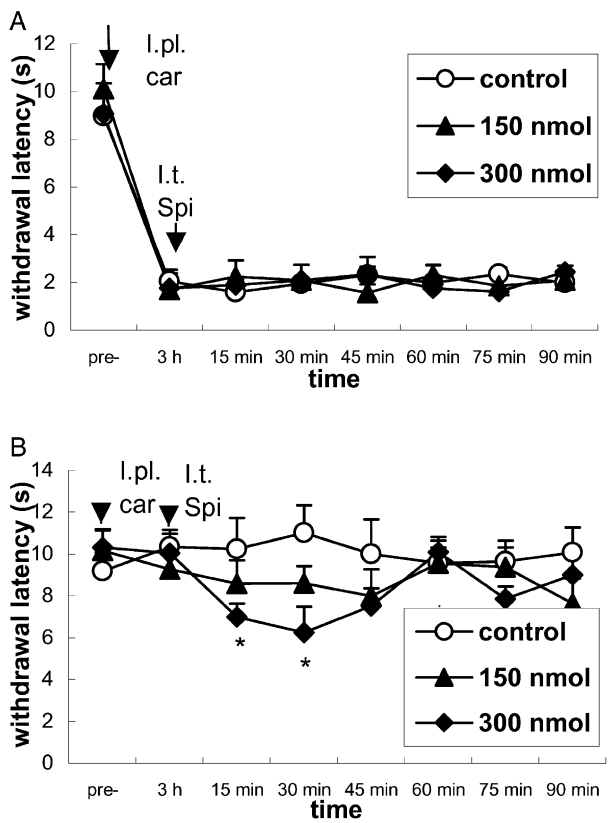

Fig. 3. Effects of i.t. dopamine D_2 receptor antagonist, spiperone hydrochloride, on carrageenan-induced hyperalgesia. (A) In inflamed hindpaws, neither 150 nor 300 nmol influenced the hyperalgesia (respectively, $n = 6$ and $n = 8$); (B) in non-inflamed hindpaws, 300 nmol produced hyperalgesia at 15 min ($P < 0.05$) and 30 min ($P < 0.05$) after i.t. administration. * $P < 0.05$ vs. control.

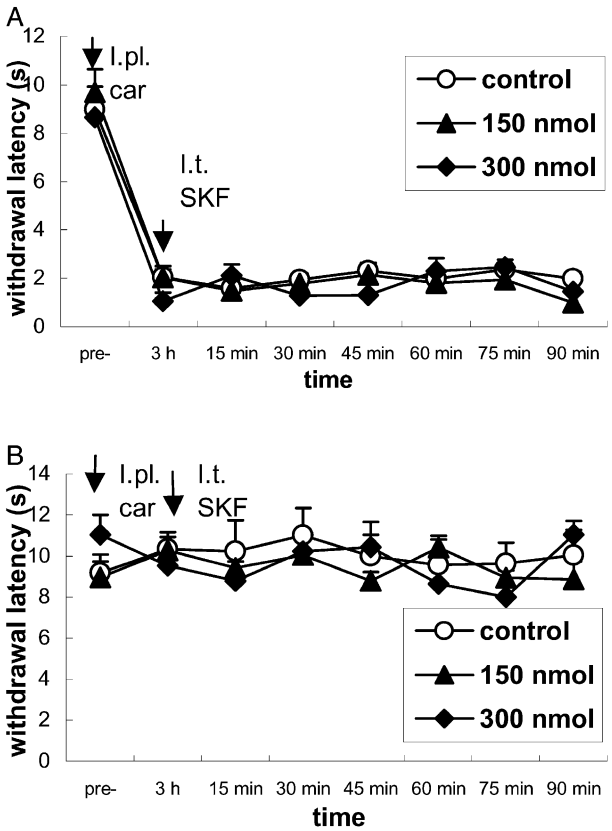

Fig. 4. Effects of i.t. dopamine D_1 receptor agonist, (±)-SKF38393 hydrochloride, on carrageenan-induced hyperalgesia. (A) In inflamed hindpaws; (B) in non-inflamed hindpaws, neither 150 nor 300 nmol had a significant effect compared to control ($n = 5$ and $n = 6$, respectively).

3. Results

3.1. Effects of i.pl. carrageenan and i.t. normal saline

Our study demonstrated that the injection of 2 mg carrageenan produced a marked inflammation of the injected paws and thermal hyperalgesia that peaked at 3 h after injection and showed little change in magnitude through 4.5 h, but the contralateral paws did not change from the pre-test value (Fig. 1). A similar effect was also observed in a previous study (Traub, 1996; Gao et al., 2000). Mean withdrawal latencies of the inflamed hindpaws decreased from 9.0 ± 1.0 s before carrageenan to 2.0 ± 0.3 s at 3 h after carrageenan ($P < 0.001$), and that of the non-inflamed hindpaws did not change significantly (from 9.2 ± 0.9 to 10.3 ± 0.8 s).

As shown in Fig. 1, we administered normal saline at 3 h after carrageenan. During the next 90 min, the withdrawal latencies of the inflamed hindpaws and the non-in-

flamed hindpaws were not significantly different from the pre-normal saline latencies (at 3 h).

3.2. Effects of i.t. administration of the dopamine D_2 receptor-selective agonist LY171555

As shown in Fig. 2, LY171555 (at doses of 30, 150 and 300 nmol) was administered 3 h after carrageenan. In the inflamed hindpaws, the effect of the 30-nmol dose was not different from that of the control, but the doses of 150 and 300 nmol produced significant dose-dependent anti-hyperalgesia for 45min. In the non-inflamed hindpaws, only the 300-nmol dose increased the withdrawal latency to thermal stimulation.

3.3. Effects of i.t. administration of the dopamine D_2 receptor-selective antagonist spiperone hydrochloride

As shown in Fig. 3, spiperone hydrochloride, 150 and 300 nmol, was administered 3 h after carrageenan. In the inflamed hindpaws, none of these doses had an effect on the hyperalgesia. However, in the non-inflamed hindpaws, the dose of 300 nmol induced significant hyperalgesia.

3.4. Effects of i.t. administration of the dopamine D_1 receptor-selective agonist (±)-SKF38393 hydrochloride

As shown in Fig. 4, (±)-SKF38393 hydrochloride, at 150- and 300-nmol doses, was administered 3 h after carrageenan. Neither the inflamed nor the non-inflamed hindpaw showed any effect or all these doses as compared to the control group.

3.5. Effects of i.t. administration of the dopamine D_1 receptor-selective antagonist, R(+)-SCH23390 hydrochloride

As shown in Fig. 5, *R*(+)-SCH23390 hydrochloride, at doses of 150 and 300 nmol, was administered 3 h after carrageenan. In the inflamed hindpaws, the 300-nmol dose produced anti-hyperalgesia. However, in non-inflamed hindpaws, neither 150 nor 300 nmol produced a significant change of withdrawal latency as compared with the control group.

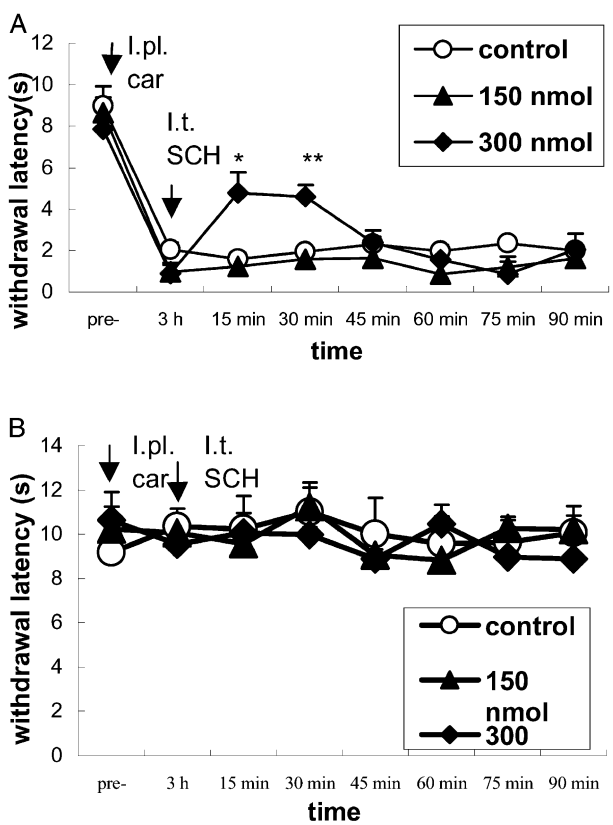

Fig. 5. Effects of i.t. dopamine D_1 receptor antagonist, R(+)-SCH23390 hydrochloride, on carrageenan-induced hyperalgesia. (A) In inflamed hindpaws, the dose of 150 nmol did not influence carrageenan hyperalgesia ($n = 7$), while 300 nmol produced a significant anti-hyperalgesia at 15 min ($P < 0.05$) and 30 min ($P < 0.01$) ($n = 6$); (B) in non-inflamed hindpaws, neither 150 nor 300 nmol had any influence on hyperalgesia. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control.

4. Discussion

Results of previous work in our laboratory suggested that the dopaminergic system in the brain is involved in the central modulation of inflammatory hyperalgesia, and that different effects were probably induced by different receptors (Gao et al., 2000). In the present experiments, we evaluated the role of dopamine receptors in the spinal cord in inflammatory pain using i.t. administration.

The results of our work showed that dopaminergic transmission in the spinal cord was also involved in the central modulation of peripheral inflammatory pain mediated by dopamine D_1 and D_2 receptors. A selective dopamine D_2 receptor agonist produced anti-hyperalgesia or hypoalgesia in the inflamed and non-inflamed hindpaws, respectively, and a selective dopamine D_1 receptor agonist had no significant effect on either hindpaw. These results suggested that the dopamine D_2 receptor exerts an inhibitory action while the dopamine D_1 receptor has no effect on the peripheral nociceptive information in inflammatory pain modulation, which is consistent with previous reports (Barasi and Duggal, 1985; Barasi et al., 1987; Gao et al., 1998; Jensen and Yaksh, 1984; Liu et al., 1992).

To further evaluate the possible role of dopamine receptors in the spinal cord in inflammatory pain, the antagonists of dopamine D_1 and D_2 receptors were tested individually. The results showed that blocking the dopamine D_2 receptor decreased the pain threshold of non-inflamed hindpaw, while the dopamine D_1 receptor antagonist produced anti-hyperalgesia in the inflamed hindpaws. Therefore, the dopamine D_2 receptor may have induced tonic

inhibition, and the dopamine D_1 receptor may have induced tonic excitation in the process of inflammatory pain.

Because of its properties, the dopamine D_2 receptor agonist LY171555 (lesoisomer of LY141865 hydrochloride) has been reported to have a dopamine receptor agonist activity similar to that of LY141865 hydrochloride (Titus et al., 1983), a potent and highly selective dopamine D_2 receptor agonist (Bach et al., 1980; Tsuruta et al., 1981). Furthermore, some reports also demonstrated that the effects of LY171555 in the central nervous system could be blocked by a dopamine D_2 receptor antagonist (Barasi et al., 1987; Barasi and Duggal, 1985). In our experiments, this antagonism of the effects of LY171555 was not detected.

The present results suggest that the dopaminergic system in the spinal cord is involved in the central modulation of inflammatory hyperalgesia, and that different effects may have been induced by different receptors. The dopamine D_2 receptor exerted anti-hyperalgesic or hypoalgesic effects but also tonically inhibited the nociceptive information. The dopamine D_1 receptor exerted tonic excitation and is perhaps related to the production of hyperalgesia.

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