ELSEVIER

European Journal of Pharmacology 406 (2000) 53–58

ejp

www.elsevier.nl/locate/ejphar

Effects of dopaminergic agents on carrageenan hyperalgesia in rats

Xiu Gao, Yu-qiu Zhang, Gen-cheng Wu *

Department of Neurobiology, State Key Laboratory of Medical Neurobiology, Shanghai Medical University, Shanghai 200032, People's Republic of China
Key Laboratory of Neurobiology, Shanghai Institute of Physiology, Chinese Academy of Sciences, Shanghai 200031, People's Republic of China

Received 31 January 2000; received in revised form 14 August 2000; accepted 18 August 2000

Abstract

The present study explored the role of central dopaminergic transmission in a model of carrageenan-induced inflammatory pain by examining the effects of selective agonists and antagonists of dopamine receptors. The results were as follow: (1) LY171555 (*trans*-(−)-4a*R*-4,4a,5,6,7,8,8a,9-Octahydro-5-propyl-1*H*-pyrazolo[3,4-g]quinoline hydrochloride), dopamine D_2 receptor agonist, produced anti-hyperalgesia or hypoalgesia 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), dopamine D_2 receptor antagonist, decreased the pain threshold of the non-inflamed hindpaws. (2) (±)-SKF38393 hydrochloride ((±)-1-Phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol hydrochloride), dopamine D_1 receptor agonist, produced anti-hyperalgesia or hypoalgesia when administered in a high dose (600 nmol), and decreased the pain threshold of non-inflamed hindpaws when administered in a low dose (150 nmol); *R*(+)-SCH23390 hydrochloride (*R*(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride), dopamine D_1 receptor antagonist, induced anti-hyperalgesia or hypoalgesia, respectively. The present study suggests that the dopaminergic system is involved in the central modulation of inflammatory hyperalgesia, and that the different effects are probably induced by the different receptors.

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

1. Introduction

More and more evidences show that the central dopamine system is involved in the modulation of nociception. At the supraspinal level, dopaminergic neurons of the substantia nigra and ventral tegmental area are considered part of the basal ganglia circuitry involved in pain modulation (Chudler and Dong, 1995). Dopamine might tonically inhibit nociception in the substantia nigra-striatum/mesolimbic/mesocortical circuits, because lesion of dopaminergic neurons of the substantia nigra-ventral tegmental area results in hyperalgesic responses (Saadé et al., 1997). In addition, stimulation of the substantia nigra increases latency in the hot-plate test or tail-flick test (Sandberg and Segal, 1978; Segal and Sandberg, 1977; Jurna et al., 1978), and decreases C-fiber responses (Blinn et al., 1980). An increased release of dopamine in nucleus accumbens produces hypoalgesia mediated by dopamine D_1 and D_2 receptors (Altier and Stewart, 1993, 1998). Moreover, a local increase of endogenous dopamine in the rostral agranular insular cortex also produces sustained anti-nociception (Burkey et al., 1999). All these reports suggest that the dopaminergic system in the brain plays an important role in pain modulation.

The carrageenan model of inflammatory pain in rats is a good one because of its many similarities to clinical inflammatory diseases, namely, the large oedematous response and persistent strong hyperalgesia (Winter et al., 1962). It has been observed that L-dopa and some dopaminergic agonists (intracerebroventricular (i.c.v.) administration) inhibit paw oedema and show an anti-inflammation action (Hore et al., 1997). But the role of selective dopamine D_1 or D_2 agents on inflammatory hyperalgesia is still unclear. The aim of the present study was to investigate the effects of agonists or antagonists of central dopamine D_1 and D_2 receptors on carrageenan-induced hyperalgesia.

* Corresponding author. Department of Neurobiology, State Key Laboratory of Medical Neurobiology, Shanghai Medical University, 138 Yi Xue Yuan Road, Shanghai 200032, China. Tel.: +86-21-64041900-2397; fax: +86-21-64174579.

E-mail addresses: gcwu@shmu.edu.cn, hsmnwk@public9.sta.net.cn (G. Wu).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00649-X

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). Seventy-two male Sprague–Dawley rats (weighing 200–225 g) were used in the experiments. The rats were housed in groups of three to five per cage, allowed free access to food and water with a natural day/night cycle, and acclimatized to the laboratory 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 hind paw of non-anaesthetized rats according to the method described by Winter et al. (1962).

2.3. Withdrawal responses to heat stimuli

According to a previously described method (Hargreaves et al., 1988), 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 acclimate. 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 in order 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 stimuli duration to prevent tissue damage. Hyperalgesia to heat was defined as a decrease in withdrawal latency.

2.4. Drugs

The drugs used in this study included (±)-SKF38393 hydrochloride ((±)-1-Phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol hydrochloride), LY171555 (*trans*-(−)-4a*R*-4,4a,5,6,7,8,8a,9-Octahydro-5-propyl-1*H*-pyrazolo[3,4-g]quinoline hydrochloride), *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 them came from the Research Biochemicals, USA. Spiperone hydrochloride was dissolved with the help of ethanol and then diluted in 0.9% normal saline; other drugs were directly dissolved in 0.9% normal saline. Control animals received an equivalent amount of solvent.

2.5. Implantation of i.c.v. cannulae

The animals were anaesthetized with pentobarbitone sodium (40 mg/kg, i.p.) and under proper aseptic conditions, an incision was made over the mid-line of the skull and then a small burr hole was made at 2-mm lateral and 1-mm caudal to the bregma mark of the skull. Intracerebroventricular (i.c.v.) cannulation was performed stereotaxically by implanting a stainless steel cannula of 0.8-mm diameter into the right lateral ventricle to a depth of 4 mm below the skull surface (De Balbian Verster et al., 1971). The cannulated rats were allowed to recover for 3 days and were housed individually. For i.c.v. administration, the total injection volume of 25 μl (15-μl drug solution followed by a normal saline flush of 10 μl) was injected into the cerebroventricle smoothly within 1 min.

2.6. Statistical analysis

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

3. Results

3.1. Effects of i.pl. carrageenan and i.c.v. normal saline

Our study demonstrated that injection of 2-mg carrageenan produced a marked inflammation of the injected paws and thermal hyperalgesia that peaked at 3 h after

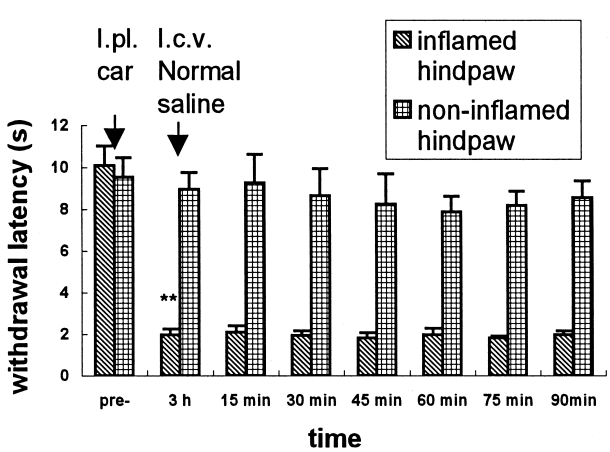

Fig. 1. Hyperalgesia produced at 3 h after i.pl. carrageenan and effects of i.c.v. normal saline at 3 h on the hyperalgesia ($n = 5$). $^{**}P < 0.01$ vs. pre-carrageenan.

injection and showed little change in magnitude through 4.5 h. In the contralateral paws, the latencies were the same as the pretest latencies (Fig. 1). A similar effect was also observed in previous study (Traub, 1996). Mean withdrawal latencies of the inflamed hindpaws decreased from 10.1 ± 0.9 s before carrageenan to 2.0 ± 0.3 s at 3 h after carrageenan ($P < 0.01$), and those of the non-inflamed hindpaws did not change significantly (from 9.5 ± 0.9 to 9.0 ± 0.8 s).

As shown in Fig. 1, we administered normal saline at 3 h after carrageenan. During the following 90 min, the withdrawal latencies of the inflamed hindpaws and the non-inflamed hindpaws were not significantly different from the pre-normal saline latencies (at 3-h point).

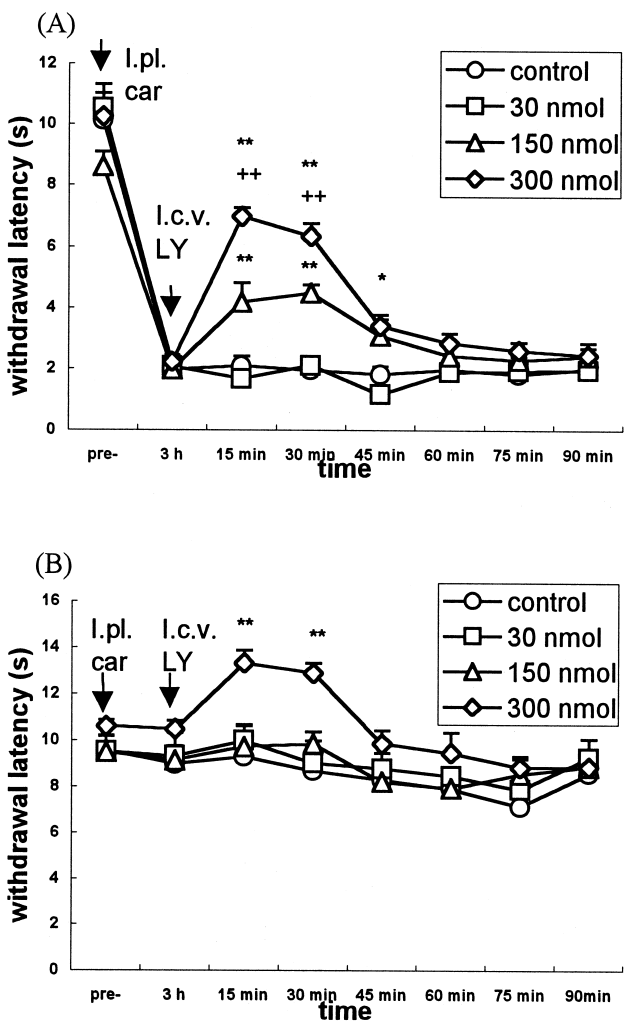

Fig. 2. Effects of i.c.v. dopamine D_2 receptor agonist LY171555 on carrageenan-induced hyperalgesia. (A) In inflamed hindpaws, the dose of 30 nmol did not have an effect different from the control effect ($n = 5$), whereas 150 nmol produced significant anti-hyperalgesia at 15 min ($P < 0.01$) and 30 min ($P < 0.01$) after i.c.v. administration ($n = 6$), and 300 nmol further potentiated the anti-hyperalgesia at 15 min ($P < 0.01$) and 30 min ($P < 0.01$) ($n = 6$); (B) In non-inflamed hindpaws, only the dose of 300 nmol produced hypoalgesia at 15 min ($P < 0.01$) and 30 min ($P < 0.01$). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control $++P < 0.01$ vs. 150 nmol.

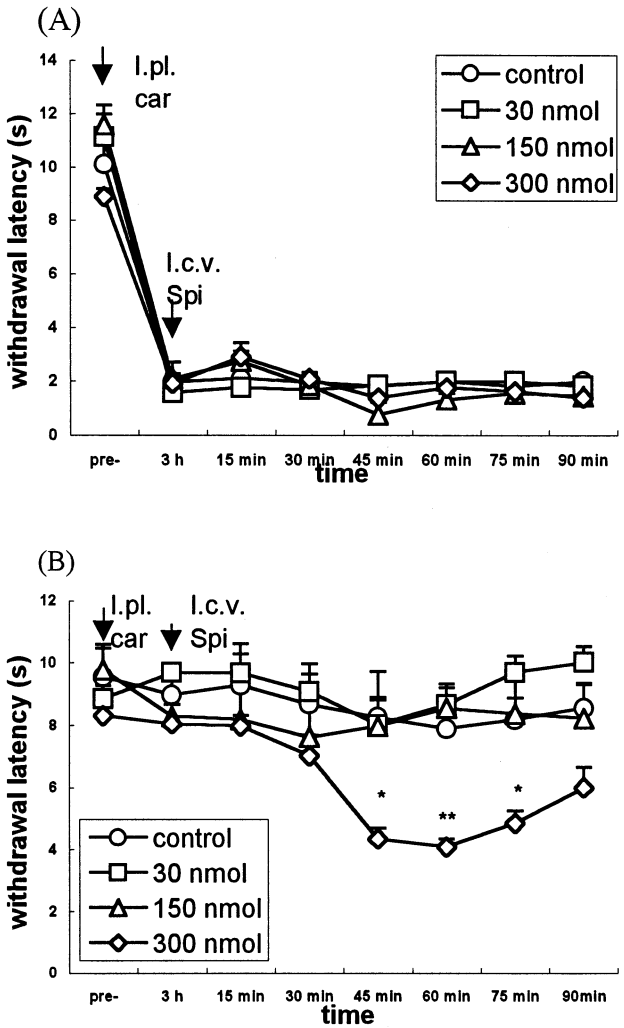

Fig. 3. Effects of i.c.v. dopamine D_2 receptor antagonist spiperone hydrochloride on carrageenan-induced hyperalgesia. (A) In inflamed hindpaws, neither 30, 150 nor 300 nmol influenced the hyperalgesia (respectively, $n = 5$, $n = 6$ and $n = 5$); (B) In non-inflamed hindpaws, the highest dose of spiperone hydrochloride produced hyperalgesia at 45 min ($P < 0.05$), 60 min ($P < 0.01$) and 75 min ($P < 0.05$) after i.c.v. administration. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control.

3.2. Effects of i.c.v. Administration of the dopamine D_2 receptor-selective agonist LY171555

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

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

As shown in Fig. 3, spiperone hydrochloride at 30, 150 and 300 nmol was administered 3 h after carrageenan. In

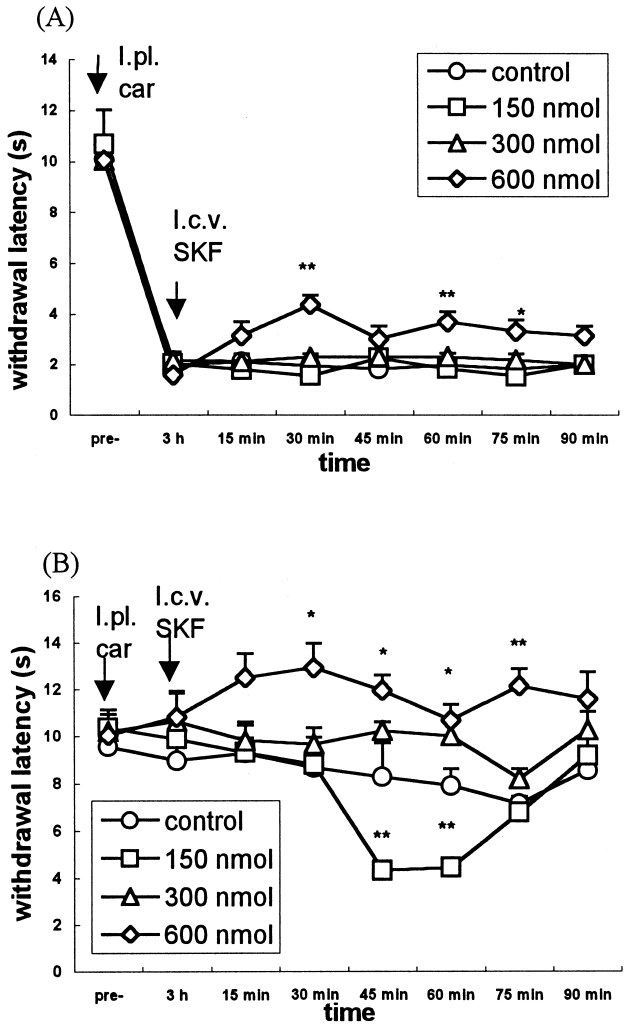

Fig. 4. Effects of i.c.v. dopamine D_1 receptor agonist (±)-SKF38393 hydrochloride on carrageenan-induced hyperalgesia. (A) In inflamed hindpaws, neither 150 nor 300 nmol had a significant effect compared with the control ($n = 5$ and $n = 6$, respectively), while the dose of 600 nmol had an anti-hyperalgesic effect at 30 min ($P < 0.01$), 60 min ($P < 0.01$) and 75 min ($P < 0.05$) after i.c.v. administration ($n = 8$); (B) In non-inflamed hindpaws, 600 nmol also produced hypoalgesia at 30, 45, 60 and 75 min, but 150 nmol decreased the withdrawal latency, leading to hyperalgesia, at 45 min ($P < 0.01$) and 60 min ($P < 0.01$). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control.

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.c.v. administration of the dopamine D_1 receptor-selective agonist (±)-SKF38393 hydrochloride

As shown in Fig. 4, (±)-SKF38393 hydrochloride at 150, 300 and 600 nmol was administered 3 h after carrageenan. In the inflamed hindpaws, the doses of 150 and 300 nmol did not significantly influence carrageenan-induced hyperalgesia, but 600 nmol had an anti-hyperalgesic effect. In the non-inflamed hindpaws, i.c.v. administration of 150 nmol (±)-SKF38393 hydrochloride produced significant hyperalgesia, while 600 nmol prolonged the withdrawal latency.

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

As shown in Fig. 5, *R*(+)-SCH23390 hydrochloride at 150, 300 and 600 nmol was administered 3 h after carrageenan. In neither the inflamed nor the non-inflamed hindpaws did 150 and 300 nmol exert an effect different from that of the control, while 600 nmol had an anti-hyperalgesic or hypoalgesic effect.

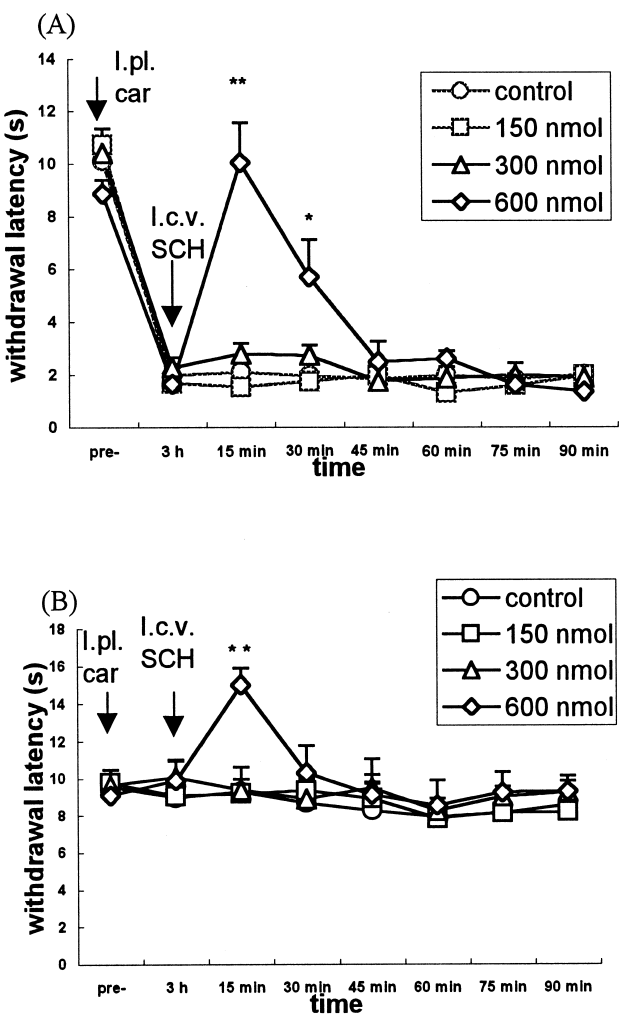

Fig. 5. Effects of i.c.v. dopamine D_1 receptor antagonist *R*(+)-SCH23390 hydrochloride on carrageenan-induced hyperalgesia. (A) In inflamed hindpaws, neither 150 nor 300 nmol had any influence on carrageenan-induced hyperalgesia (respectively, $n = 5$), while 600 nmol produced a significant anti-hyperalgesic effect compared with the control at 15 min ($P < 0.01$) and 30 min ($P < 0.05$) ($n = 5$); (B) In non-inflamed hindpaws, the effect was similar to that in inflamed hindpaws at 15 min ($P < 0.01$) after i.c.v. *R*(+)-SCH23390 hydrochloride. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control.

4. Discussion

In the rat, a dopaminergic system mediating pain modulation has been reported in many studies, but results have not always been identical. For example, some investigations demonstrated that selective and non-selective dopaminergic agonists produced anti-nociceptive effects (Gorlitz and Frei, 1972; Tricklebank et al., 1984; Lin et al., 1989; Morgan and Franklin, 1991). Conversely, many data indicated that these agents caused hyperalgesia (Tulunay et al., 1975, 1976; Ben-Sreti et al., 1983). The discrepancies might lie in the different routes of administration, different doses of dopaminergic agents, or different methods to measure the pain threshold, and different pain model, etc. In the present study, we observed the actions of dopamine D_1 and D_2 receptor agonists and antagonists on carrageenan-induced inflammatory hyperalgesia by using i.c.v. drug administration and radiant heat stimuli.

The present work showed that dopaminergic transmission in the brain was 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 hindpaws and non-inflamed hindpaws, respectively, and blocking this receptor decreased the pain threshold of non-inflamed hindpaws. These results suggested the tonic inhibition by dopamine D_2 receptors of peripheral nociceptive information and were similar to the previous reports (Michael-Titus et al., 1990; Morgan and Franklin, 1991; Rooney and Sewell, 1989). Because of its properties, the dopamine D_2 receptor agonist LY171555 (leso-isomer of LY141865 hydrochloride) has been reported to have the dopamine receptor agonist activity 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 role of the dopamine D_1 receptor was somewhat puzzling. On one hand, the dopamine D_1 receptor perhaps induced tonic excitation of inflammatory pain pathways, because blocking the dopamine D_1 receptor produced anti-hyperalgesia or hypoalgesia. But on the other hand, a low dose of a D_1 receptor agonist lowered the pain threshold, leading to hyperalgesia, while a higher dose had the opposite effect, the mechanism of which cannot be explained clearly now. Probably, it was related to the partial activation of dopamine D_2 receptors when a high dose of (±)-SKF38393 hydrochloride was used. (Phillips et al., 1992).

As mentioned in the Introduction, at the supraspinal level, dopaminergic neurons and dopamine receptors of the substantia nigra-striatum/mesolimbic/mesocortical circuits have a key role in pain modulation (Chudler and Dong, 1995; Saadé et al., 1997). The present study confirmed the effect of dopamine receptors, but the results were different from those of other investigations (Altier and Stewart, 1993, 1998; Burkey et al., 1999). The discrepancy might partly result from the different effects of dopamine receptors in different brain areas. The results of our study showed the integrated effects of dopamine D_1 or D_2 receptors in the entire brain.

In summary, the present study suggested that the dopaminergic system in the brain is involved in the central modulation of inflammatory hyperalgesia, and that probably different effects were induced by the different receptors. The central dopamine D_2 receptor exerted anti-hyperalgesic or hypoalgesic effects but also tonically inhibited the nociceptive information. The dopamine D_1 receptor actions induced by different doses of D_1 receptor agonist are still unclear, but are perhaps related to the development of hyperalgesia.

Acknowledgements

This work were supported by the National Natural Science Foundation of China (No. 39970925) and The Research Fund for the Doctoral Program of Higher Education (No. 9835).

References

Altier, N., Stewart, J., 1993. Intra-VTA infusions of the substance P analogue, DiMe-C7, and intra-accumbens infusions of amphetamine induce analgesia in the formalin test for tonic pain. Brain Res. 628, 279–285.

Altier, N., Stewart, J., 1998. Dopamine receptor antagonists in the nucleus accumbens attenuate analgesia induced by ventral tegmental area substance P or morphine and by nucleus accumbens amphetamine. J. Pharmacol. Exp. Ther. 285, 208–215.

Bach, N.J., Kornfeld, E.C., Jones, N.D., Chaney, M.O., Dorman, D.E., Paschal, J.W., Clemens, J.A., Smalstig, E.B., 1980. Bicyclic and tricyclic ergoline partial structures. Rigid 3-(2-aminoethyl)pyrroles and 3- and 4-(2-aminoethyl) pyrazoles as dopamine agonists. J. Med. Chem. 23, 481–491.

Barasi, S., Duggal, K.N., 1985. The effects of local and systemic application of dopaminergic agents on tail flick latency in the rat. Eur. J. Pharmacol. 117, 287–294.

Barasi, S., Ben-Sreti, M.M., Clatworthy, A.L., 1987. Dopamine receptor-mediated spinal antinociception in the normal and haloperidol pretreated rat: effects of sulpiride and SCH23390. Br. J. Pharmacol. 90, 15–22.

Ben-Sreti, M.M., Gonzalez, J.P., Sewell, R.D.E., 1983. Differential effects of SK and F 38393 and LY 141865 on nociception and morphine. Life Sci. 33, 665–668.

Blinn, G., Heinz, G., Jurna, I., 1980. Effects of substantia nigra stimulation on suralis-evoked spinal reflex activity: comparison with the effects of morphine and stimulation in the periaqueductal gray matter. Neuropharmacology 19, 75–85.

Burkey, A.R., Carstens, E., Jasmin, L., 1999. Dopamine reuptake inhibition in the rostral agranular insular cortex produces antinociception. J. Neurosci. 19, 4169–4179.

Chudler, E.H., Dong, W.K., 1995. The role of the basal ganglia in nociception and pain. Pain 60, 3–38.

De Balbian Verster, F., Robinson, C.A., Hengeveld, C.A., Bush, E.S., 1971. Free-hand cerebroventricular injection technique for unanaesthetized rats. Life Sci. 10, 1395–1402.

Gorlitz, B.D., Frei, H.H., 1972. Central monoamines and antinociceptive drug action. Eur. J. Pharmacol. 20, 171–180.

Hargreaves, K., Dubner, R., Brown, F., Flores, C., Joris, J., 1988. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32, 77–88.

Hore, S.K., Dumka, V.K., Kumar, D., Tripathi, H.C., Tandan, S.K., 1997. Central noradrenergic and dopaminergic modulation of Brewer's yeast-induced inflammation and nociception in rats. Indian J. Med. Res. 105, 93–97.

Jurna, I., Heinz, G., Blinn, G., Nell, T., 1978. The effects of substantia nigra stimulation and morphine on α-motoneurones and the tail-flick response. Eur. J. Pharmacol. 51, 239–250.

Lin, Y., Morrow, T.J., Kiritsky-roy, J.A., Terry, L.C., Casey, K.L., 1989. Cocaine: evidence for supraspinal, dopamine-mediated, non-opiate analgesia. Brain Res. 479, 306–312.

Michael-Titus, A., Bousselmame, R., Costentin, J., 1990. Stimulation of dopamine D_2 receptors induces an analgesia involving an opioidergic but not enkephalinergic link. Eur. J. Pharmacol. 187, 201–207.

Morgan, M.J., Franklin, K.B.J., 1991. Dopamine receptor subtypes and formalin test analgesia. Pharmacol., Biochem. Behav. 40, 317–322.

Phillips, S., Gelgor, L., Mitchell, D., 1992. Antinociceptive action of dopamine agonists in the nucleus raphe magnus of rats is mediated by D_2 receptors. Arch. Int. Pharmacodyn. 319, 66–75.

Rooney, K.F., Sewell, R.D.E., 1989. Evaluation of selective actions of dopamine D_1 and D_2 receptor agonists and antagonists on opioid antinociception. Eur. J. Pharmacol. 168, 329–336.

Saadé, N.E., Atweh, S.F., Bahuth, N.B., Jabbur, S.J., 1997. Augmentation of nociceptive reflexes and chronic deafferentation pain by chemical lesions of either dopaminergic terminals or midbrain dopaminergic neurons. Brain Res. 751, 1–12.

Sandberg, D.E., Segal, M., 1978. Pharmacological analysis of analgesia and self-stimulation elicited by electrical stimulation of catecholamine nuclei in the rat brain. Brain Res. 152, 529–542.

Segal, M., Sandberg, D.E., 1977. Analgesia produced by electrical stimulation of catecholamine nuclei in the rat brain. Brain Res. 123, 369–372.

Titus, R.D., Kornfeld, E.C., Jones, N.D., Clemens, J.A., Smalstig, E.B., Fuller, R.W., Hahn, R.A., Hynes, M.D., Mason, N.R., Wong, D.T., Foreman, M.M., 1983. Resolution and absolute configuration of an ergoline-related dopamine agonist, *trans*-4,4a,5,6,7,8,8a,9-Octahydro-5-propyl-1*H*(or 2*H*)-pyrazolo[3,4-g]quinoline. J. Med. Chem. 26, 1112–1116.

Traub, R.J., 1996. The spinal contribution of substance P to the generation and maintenance of inflammatory hyperalgesia in the rat. Pain 67, 151–161.

Tricklebank, M.D., Hutson, P.H., Curzon, G., 1984. Involvement of dopamine in the antinociceptive response to footshock. Psychopharmacology 82, 185–188.

Tsuruta, K., Frey, E.A., Grewe, C.W., Cote, T.E., Eskay, R.L., Kebabian, J.W., 1981. Evidence that LY-141865 specifically stimulates the D-2 dopamine receptor. Nature 292, 463–465.

Tulunay, F.C., Sparber, S.B., Takemori, A.E., 1975. The effect of dopaminergic stimulation and blockade on the nociceptive responses in the mice. Eur. J. Pharmacol. 33, 65–70.

Tulunay, F.C., Yano, I., Takemori, A.E., 1976. The effect of biogenic amine modifiers on morphine analgesia and its antagonism by naloxone. Eur. J. Pharmacol. 35, 285–292.

Winter, W.D., Risley, E.A., Nuss, G.W., 1962. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. 111, 544–547.

Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.