Biochemical and Biophysical Research Communications 372 (2008) 179–185

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Morphine dependence changes the role of droperidol on pain-related electric activities in caudate nucleus

Ying Zhang [a], Chunxiao Yang [b], Xianzhang Xu [c], Runsheng Jiao [a], Hongbo Jin [a], Yanhong Lv [d], Huike Yang [d], Manying Xu [a,*]

[a] Department of Physiology, Harbin Medical University, Xuefu Road, No. 174, Harbin 150081, China
[b] Department of Neurology of 2nd Affiliated Hospital, Harbin Medical University, Harbin 150081, China
[c] Heilongjiang Province Hospital, Harbin 150030, China
[d] Department of Anatomy, Harbin Medical University, Harbin 150081, China

ARTICLE INFO

Article history:
Received 17 April 2008
Available online 12 May 2008

Keywords:
Morphine dependence
Pain
Droperidol
Caudate nucleus

ABSTRACT

Droperidol causes the blockage of the dopamine receptors in the central nervous system that are involved in pain transmission. However, the mechanism of action of droperidol in pain-related neurons is not clear, and it is still unknown whether opioids are involved in the modulation of this processing. The present study examines the effect of droperidol on the pain-evoked response of pain-excitation neurons (PENs) and pain-inhibition neurons (PINs) in the caudate nucleus (Cd) of rats. The trains of electric impulses applied to the sciatic nerve were used as noxious stimulation. Our results revealed that droperidol decreased the frequency of PEN discharge, and increased the frequency PIN discharge evoked by the noxious stimulation in the Cd of normal rats, while administration of droperidol to morphine-dependent rats produced the opposite response. Those demonstrated that droperidol is involved in the modulation of nociceptive information transmission in Cd, and there were completely opposite responses to painful stimulation between normal and morphine-dependent rats after administration of droperidol.

Droperidol is a compound similar to haloperidol, which was discovered at Janssen Pharmaceuticals in 1961. It functions via the blockade of dopamine receptors in the subcortical, midbrain, and brainstem reticular formation and produces mild alpha-adrenergic blockades and peripheral vascular dilatation. Droperidol is used in clinical treatments as an antiemetic and antipsychotic as a supplement to anesthesia induction [1]. Many efforts have focused on the role of droperidol in patient-controlled analgesia [2,3].

Some reports have suggested that it functions as an analgesic [4]. This property of droperidol is relatively unknown and its mechanism is unclear. The present study focuses on whether droperidol is involved in pain modulation in the caudate nucleus (Cd).

The basal ganglion consists of four main nuclei (striatum, globus pallidus, subthalamic nucleus, and substantia nigra), which provide a major link between the thalamus and the cerebral cortex. The basal ganglion receives information, including pain, from all sensory systems. Over the past four decades, substantial progress has been made in the research of pain and analgesia at the genetic, molecular, cellular, and systemic levels [5]. Although the basal ganglion has been less intensively studied, it also appears to play a role in pain. It has been suggested that particularly the striatum, which comprises the putamen and Cd, plays a significant role in the modulation of pain sensation in both experimental and clinical conditions [6,7]. Stimulating the caudate-putamen nucleus was found to induce analgesia [8].

Opioids are highly effective in the treatment of pain. Massive investments have been made for the research and development of opioid analgesics, resulting in a plethora of compounds with varying affinity and efficacy at all the known opioid receptor subtypes [9,10]. Opioids are also known to be extremely addictive. The mechanism of opioid dependence is still not clear. Morphine dependence leads to adaptive reactions in the central nervous system that occur at the cellular and molecular levels [11]. The limbic structures appear to be responsible for the effects of drug abuse. The mesolimbic dopaminergic system is the primary substrate of opioid action [12]. The altered expression of transcription factors resulted in adaptive changes in the expression of membrane receptors, channels, intracellular signaling proteins, and a plethora of target genes within the mesolimbic system [13,14]. The present study also determined whether opioids altered the response of pain-related neurons to droperidol.

In our study, we focused on the noxious information transmission of the central nervous system in normal and morphine-dependent rats affected by droperidol in the Cd in order to enrich information on relationship between algesia and drug dependence.

* Corresponding author. Fax: +86 451 86697507.
E-mail address: manyingxu@sohu.com (M. Xu).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.05.009

We observed the influence of droperidol on the electrical activities of PENs and PINs in Cd in normal and morphine-dependent rats.

Materials and methods

Experimental animals. Male and female Wistar rats (Animal Centre of the Second Affiliated Hospital, Harbin Medical University, Certificate No.09-2-1) weighing between 230 and 260 g were used in this study. Rats were randomly and equally divided into the following four groups (n = 8 per group): (1) normal rats that were injected 10 μL saline into the lateral ventricle, (2) normal rats that were injected 1 μg/8 μL droperidol into the lateral ventricle, (3) morphine-dependent rats that were injected 10 μL saline into the lateral ventricle, and (4) morphine-dependent rats that were injected 1 μg/8 μL droperidol into the lateral ventricle. All injections were completed within 2 min via a microliter syringe. Morphine hydrochloride was injected subcutaneously [15] (Table 1).

Neurosurgery and electrophysiological studies. Routine surgery was performed after rats were rendered unconscious by anesthesia with a mixture of 500 mg/kg ethyl carbamate and 50 mg/kg chloridized curarine injected into their abdominal cavities, at an artificial respiration of 60 times/min. The right sciatic nerves were isolated. The head of the rat was then fixed on the cerebral solid orientation apparatus and oriented according to the B coordinate system of Pellegrino's atlas. A stainless steel tube with an external diameter of 0.8 mm was inserted into the lateral ventricle (A: 0.1; R: 1.5; H: 3.0) for injection. Two skull windows were opened and covered with liquid paraffin. The rats were fixed on a stereotaxic frame (SN-2, Narishige, Japan). Single-unit recordings were performed with a glass microelectrode (0.5–1.0 mm, DC resistance 10–30 MΩ) filled with KCl (3 mol/L). The glass microelectrode was inserted by a micromanipulator (SM-21, Narishige, Japan) into the Cds (A: 2.0–2.8 mm; L: 1.5–3.5 mm; H: 3.5–5.5 mm) [16]. The electrical activity was amplified by a microelectrical amplifier and recorded by the biological experimental system, monitored at the same time with an oscilloscope (VC-10, Nihon Konden, Japan). As the neural discharges were recorded, electrical stimulation of the sciatic nerves was performed through a double stainless steel electrode (delay, 0; interval, 5 ms; duration, 0.3 ms; train, 5) (SEN-3301, Nihon Konden, Japan) as noxious stimulation. Articular movement and hair touching were set as the non-noxious stimulation to identify the pain-reactive neurons. The discharge of each neuron was recorded 3 times every 2 min, and the complete recording duration was 30 min.

Definition of neurons. PENs and PINs are pain-related neurons. PENs are defined as the neurons that respond by increasing the discharge frequency to noxious stimuli [17], while PINs are defined as the neurons that respond by decreasing the discharge frequency to noxious stimuli [18]. The net increased value (NIV, Hz) refers to the difference in the PENs or PINs between the average frequency of evoked discharges after noxious stimulation and the average frequency of the discharges within 2 s before noxious stimulation. Inhibitory duration (ID, ms) refers to the latency time from the noxious stimulation to the appearance of the PIN discharges.

Table 1
Dose (mg/kg) and time (h) of morphine injection

Day	8:00	12:00	16:00
1	5	5	5
2	10	10	10
3	20	20	20
4	40	40	40
5	50	50	50
6	60	60	60

Statistical analysis. Data were scanned to a computer with Powerlab = 8 s (ADInstruments) and analyzed with Chart v5.3 software (Australia). All data were expressed as mean ± SEM, and they were analyzed with SPSS 13.0 software. Statistical differences were evaluated by one-way ANOVA, and statistical significances were determined at the level of $p < 0.05$.

Results

Effect of droperidol on the electrical activities of PENs in the Cds of normal rats

In normal rats in the droperidol group, the NIV of PENs was 11.43 ± 2.07 Hz, and their latency was 203.71 ± 11.87 ms. Shortly after the intracerebral ventricular injection of droperidol, the NIV of PEN began to reduce and latency began to prolong (Fig. 1B). These effects peaked at 10 min after administration; the NIV decreased to 6.78 ± 1.03 Hz, reducing by 40.68% as compared with that before administration; and the latency prolonged to 277.83 ± 18.53 ms. During 6–18 min after administration, the NIV and latency of PEN showed obvious changes as compared with those before administration and with those of the control group (Fig. 1C and D). Twelve minutes after administration, the NIV and latency of PEN returned to the values observed before treatment.

Effect of droperidol on the electrical activities of PINs in the Cd of normal rats

After intracerebral ventricular droperidol injection, the NIV of PIN began to increase and the ID began to decrease (Fig. 2B). Four minutes after the injection, the average NIV of PIN significantly increased from −5.82 ± 1.02 to −4.35 ± 0.78 Hz, and the ID decreased from 1.81 ± 0.24 to 1.46 ± 0.33 s. These reactions peaked at 10 min after injection. During 6–20 min after injection, the NIV and ID values of PIN exhibited obvious changes as compared with those before administration and with those of the control group (Fig. 2C and D).

Effect of droperidol on the electrical activities of PENs in the Cd of morphine-dependent rats

In the morphine-dependent rats administered droperidol, the NIV of PEN was 15.86 ± 2.83 Hz, and the latency was 188.32 ± 7.54 ms. Shortly after the intracerebral ventricular administration of droperidol, the NIV of PEN began to increase and the latency began to decrease (Fig. 3B). These effects peaked at 10 min after administration; the NIV increased to 21.53 ± 2.87 Hz, enhancing by 35.75% as compared with that before administration, and the latency reduced to 123.42 ± 5.09 ms. During 6–18 min after administration, the NIV and latency of PEN showed obvious changes as compared with those before administration or with those of the control group (Fig. 3C and D). Twelve minutes after administration, the NIV and latency of PEN returned to the values observed before treatment.

Effect of droperidol on the electrical activities of PINs in the Cd of morphine-dependent rats

After intracerebral ventricular injection of droperidol, the NIV of PIN started decreasing and ID started increasing (Fig. 4B). Four minutes after the intracerebral ventricular administration of droperidol, the average NIV of PIN significantly reduced from −2.14 ± 0.56 to −2.57 ± 0.75 Hz, and the ID prolonged from 418.20 ± 63.67 ms to 404.25 ± 38.01 ms. These reactions peaked at 12 min after injection. During 6–20 min after injection, the

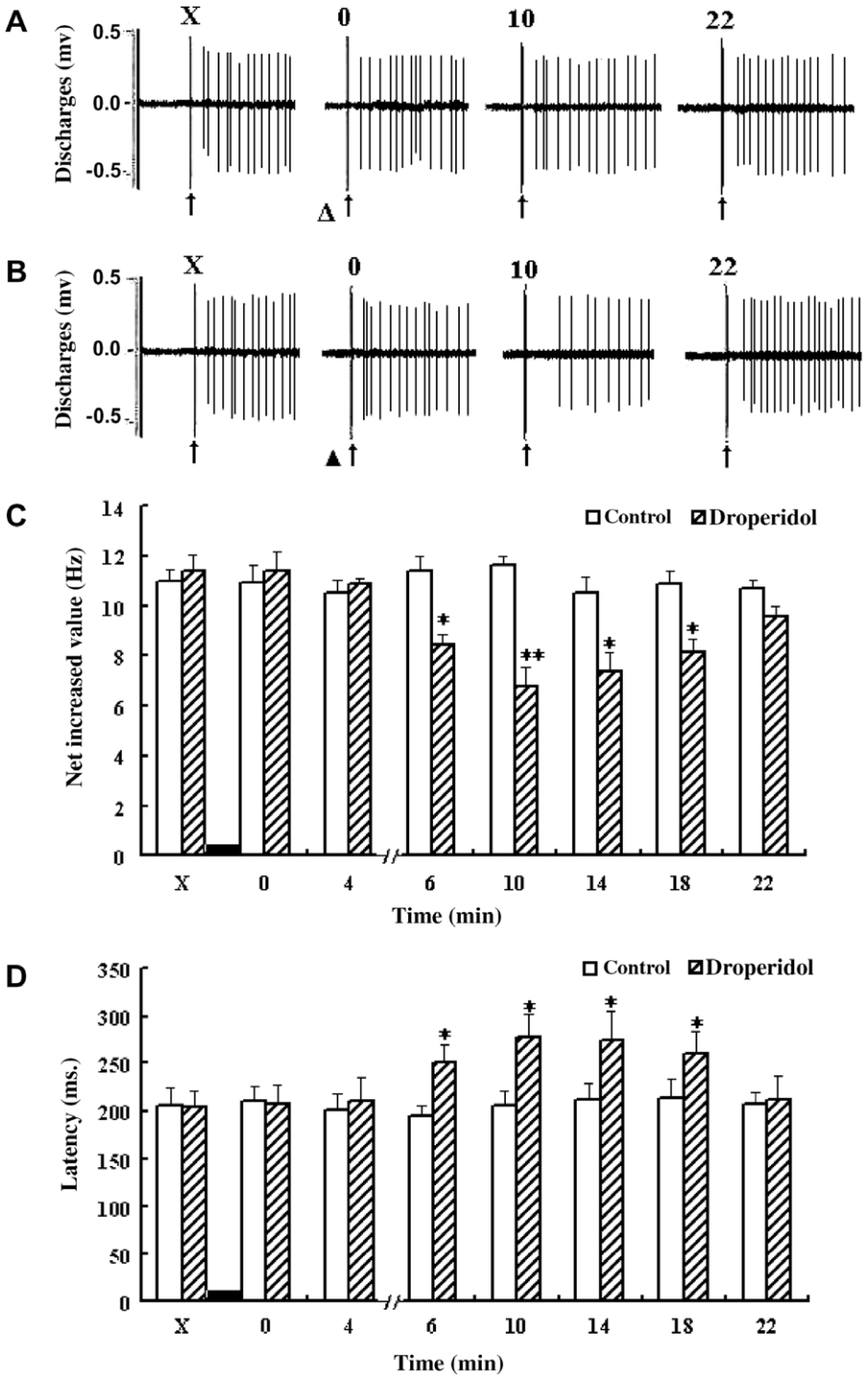

Fig. 1. Effects of intracerebral ventricular injections of saline (A) and droperidol (B) on the evoked discharges of PENs in the Cd of normal rats. ↑, Stimulus artifact; Δ, injection of saline; ▲, injection of droperidol; X, before injection; 0, 10, and 22, time after injection (min). Changes of NIV (C) and latency (D) in Cd PENs of normal rats after intracerebral ventricle injection of droperidol (means ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control). —, injection of substance; X, before injection; 0, 4, . . . ,22, time after injection (min).

NIV and ID of PINs exhibited apparent changes as compared with those before administration and with those of the control group (Fig. 4C and D).

Discussion

We studied the effects of droperidol on the electrical activities of PENs and PINs in Cd in normal and morphine-dependent rats. Our study revealed the following results. First, droperidol, a butyrophenone neuroleptic drug, reduced the NIV and prolonged the latency of PEN. It also increased the NIV and shortened the ID of PIN significantly in normal rats. This result revealed that droperidol inhibited the electrical activities of PEN and enhanced those of PIN. This illustrated that droperidol is involved in the modulation of nociceptive information transmission in Cd. Second, in morphine-dependent rats, droperidol increased the NIV and reduced the latency of PEN. It also decreased the NIV and prolonged the ID of PIN significantly. This result showed that droperidol

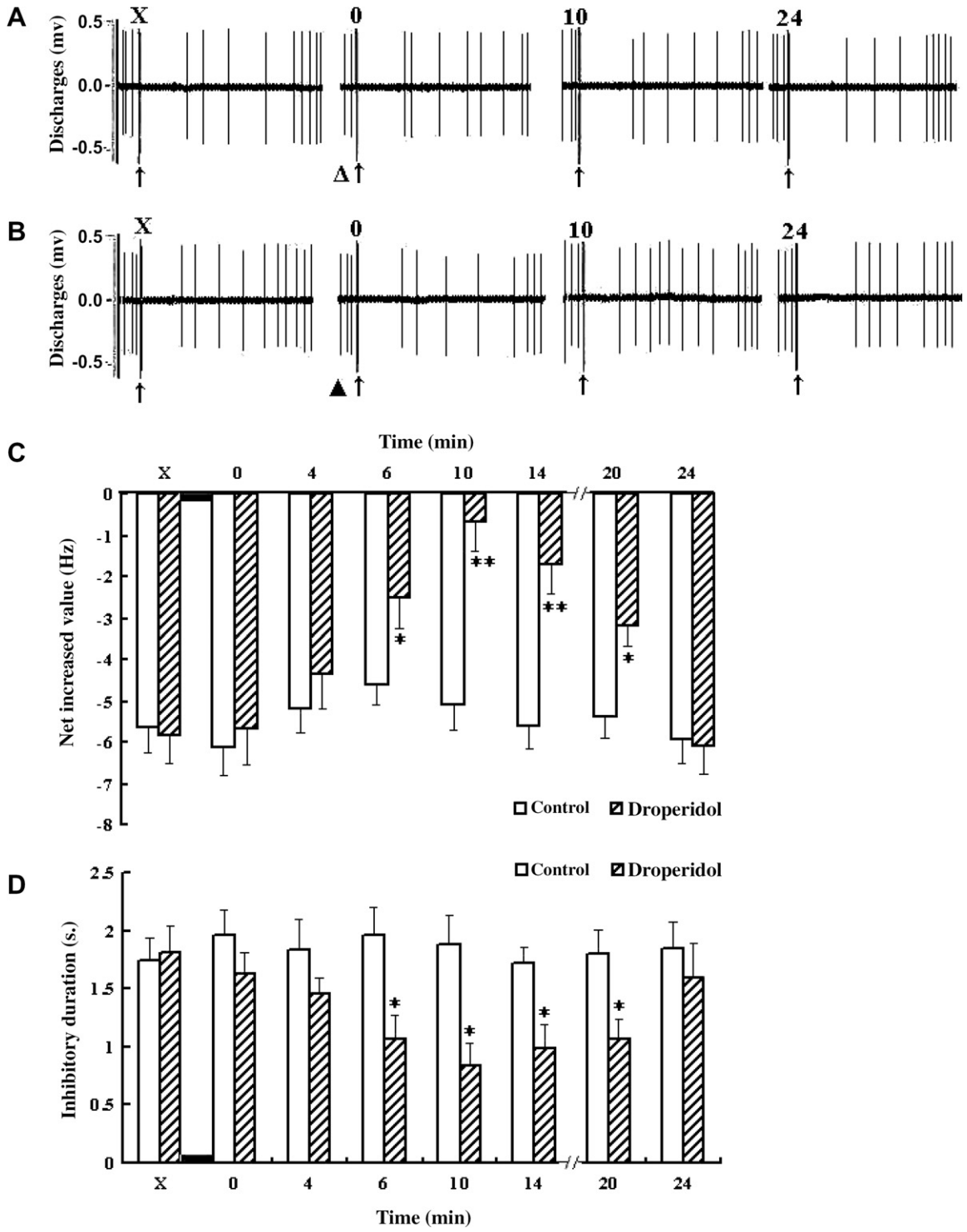

Fig. 2. Effects of the intracerebral ventricular saline injection (A) and droperidol (B) on the evoked discharges of PINs in the Cd of normal rats. ↑, Stimulus artifact; Δ, saline injection; ▲, droperidol injection; X, before injection; 0, 10, and 24, time after injection (min). Changes of NIV (C) and ID (D) in Cd PINs of normal rats after intracerebral ventricular injection of droperidol (means ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control). —, injection of substance; X, before injection; 0, 4,...,24, time after injection (min).

enhanced the electrical activities of PEN and inhibited those of PIN. Those demonstrated that the responses to pain stimulation were considerably different between normal rats and morphine-dependent rats after the intracerebral ventricular injection of droperidol.

Several reports have revealed that droperidol is involved in antinociception and analgesia in many animals, including humans. In clinical studies, spinal or epidural administration of non-opioid agents such as droperidol and neostigmine has been shown to cause analgesia without causing motor dysfunction or neurotoxicity. Epidural droperidol blocks mainly the fast sodium channels, and to a lesser extent, the slow sodium channels and inhibits the formation of action potential [19]. Some results suggest that the addition of droperidol or clonidine to epidural tramadol provides a shorter onset time and a longer duration of analgesia [4]. Lo et al. [2] have reported the morphine-sparing effect of droperidol when used in combination with patient-controlled analgesia, and

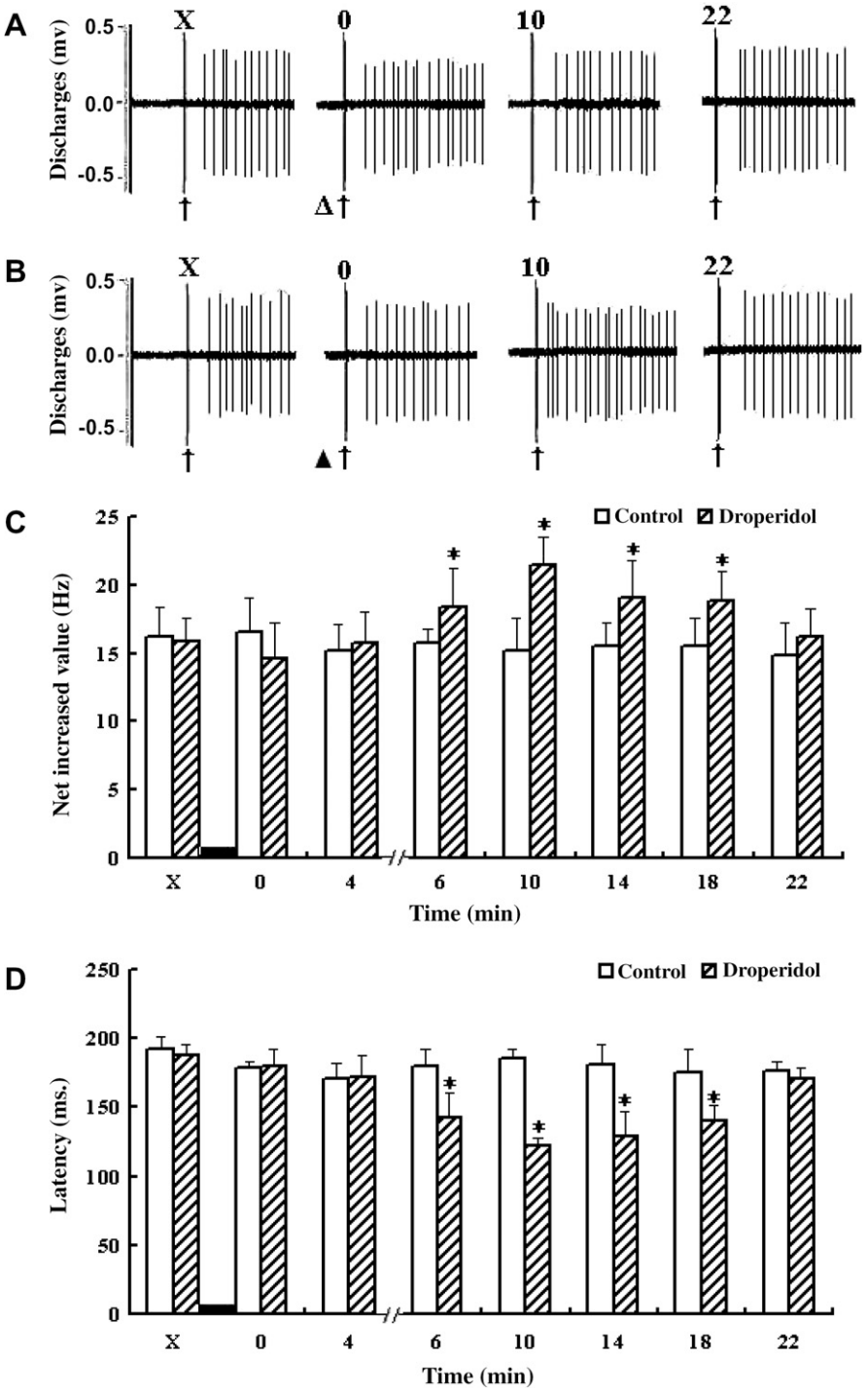

Fig. 3. Effects of intracerebral ventricular injection of saline (A) and droperidol (B) on the evoked discharges of PENs in the Cd of morphine-dependent rats. ↑, Stimulus artifact; Δ, injection of saline; ▲, injection of droperidol; X, before injection; 0, 10, and 22, time after injection (min). Changes of NIV (C) and latency (D) in Cd PENs of morphine-dependent rats after intracerebral ventricular injection of droperidol (means ± SEM, $^{*}P < 0.05$ vs. control). —, injection of substance; X, before injection; 0, 4, . . . , 22, time after injection (min).

it has already been observed in patients undergoing rotator-cuff repair surgery [20].

Animal studies suggest that the basal ganglia play a major role in the processing of somatosensory information, including noxious stimuli [6]. Evidences indicate that the striatum and striatal dopamine receptors are involved pain regulation in humans. Pain is a common symptom in patients with nigrostriatal dopaminergic hypofunction. Our results demonstrated that droperidol is involved in the modulation of nociceptive information transmission in Cd. This regulation may be mediated by dopamine receptors. Striatal administration of dopamine D2 receptor agonists (such as quinpirole) and dopamine D2 receptor antagonists (such as eticlopride) suppresses and enhances pain-related responses in animal experiments, respectively [21]. Interestingly, systemic administration of dopamine D2 receptor antagonists may also produce antinociception, probably due to opioidergic mechanisms [22].

Considerably different responses to pain stimulation were observed between normal and morphine-dependent rats. This might

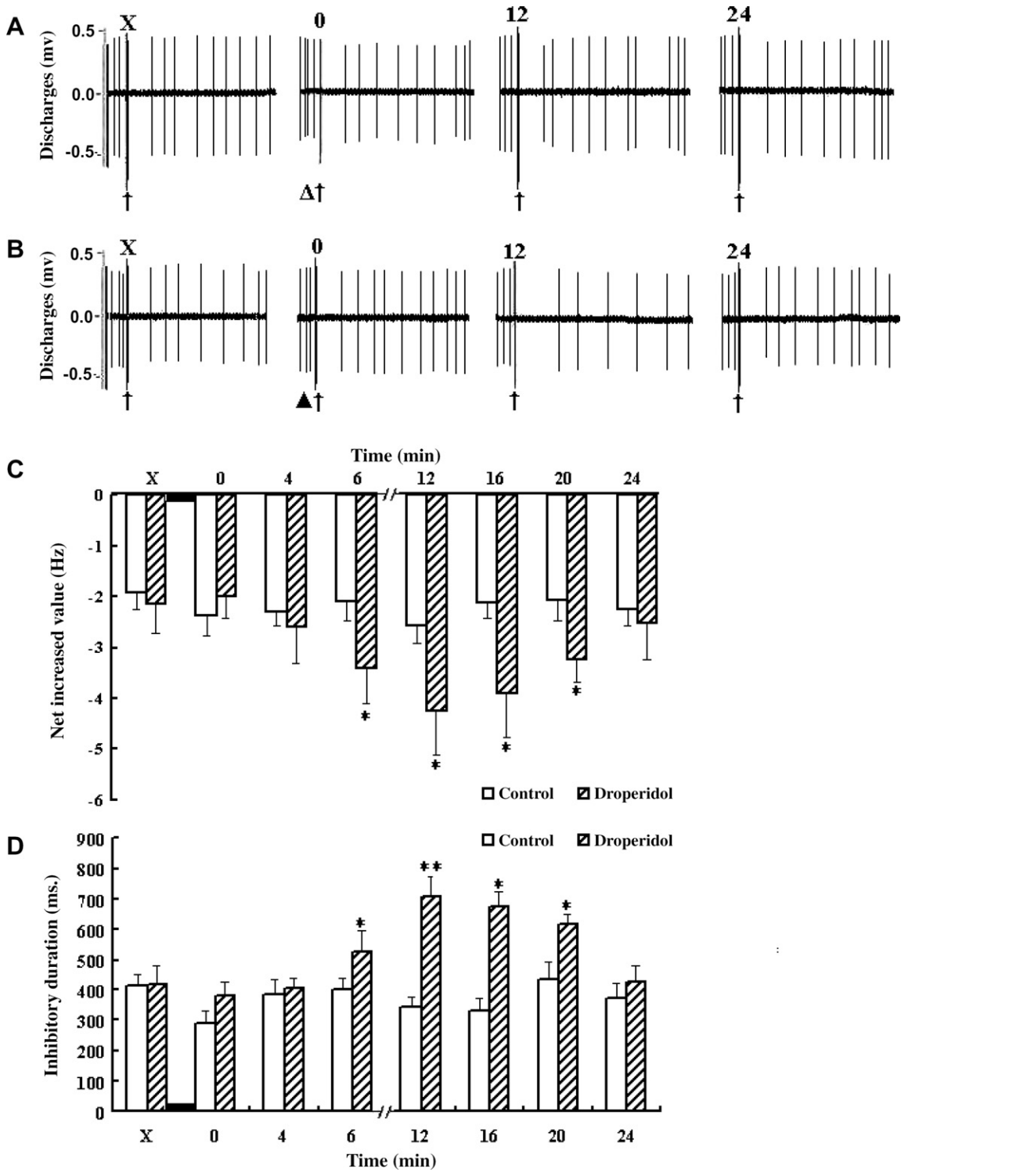

Fig. 4. Effects of intracerebral ventricular injections of saline (A) and droperidol (B) on the evoked discharges of PINs in the Cd of morphine-dependent rats. ↑, Stimulus artifact; Δ, injection of saline; ▲, injection of droperidol; X, before injection; 0, 10, and 24, time after injection (min). Changes of NIV (C) and ID (D) in Cd PINs of morphine-dependent rats after intracerebral ventricular injection of droperidol (means ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control). —, injection of substance; X, before injection; 0, 4,...,24, time after injection (min).

be because the activity of the dopamine pathway changes adaptably to opioids [23]. The pCREB levels were super-induced when SKF 82958 (agonist of dopamine receptor) and dopamine were added to cultures that were treated chronically with morphine and subsequently administered naloxone to precipitate withdrawal. These data suggest that dopamine receptor-mediated pathways are augmented by chronic morphine [24]. In morphine-dependent rats, the expression of DA receptor subtypes change. It is reported that, there was no alteration in D1R mRNA and a 25% decrease in D2R mRNA in the caudate putamen, 2 h after the final morphine injection. Moreover, in the same RNA extracts, D3R mRNA displayed significant increases of 85% in the caudate-putamen and 165% in the ventral midbrain, including the substantia nigra and ventral tegmental areas [25]. In conclusion, these results indicate that dro-

peridol is involved in pain modulation in Cd. In addition, the difference in the responses of pain-related neurons in Cd to droperidol by morphine could be related to the adaptable changes of dopaminergic nerves. Further studies on these issues will deepen our knowledge of pain modulation and dependence.

Acknowledgment

This work was supported by National Natural Science Foundation of China (30240058)

References

[1] K. McKeage, D. Simpson, A.J. Wagstaff, Intravenous droperidol: a review of its use in the management of postoperative nausea and vomiting, Drugs 66 (2006) 2123–2147.
[2] Y. Lo, Y.Y. Chia, K. Liu, N.H. Ko, Morphine sparing with droperidol in patient-controlled analgesia, J. Clin. Anesth. 17 (2005) 271–275.
[3] S. Inoue, H. Mitsuhata, T. Kawakami, K. Shimohata, Y. Hirabayashi, N. Seo, Addition of 0.1% bupivacaine to buprenorphine and droperidol in patient-controlled epidural analgesia improved postoperative pain scores on coughing after gynecological surgery, J. Clin. Anesth. 17 (2005) 167–171.
[4] M.D.E. Gürses, M.D.H. Sungurtekin, M.D.E. Tomatir, M.D.C. Balci, M.D.M. Gönüllü, The addition of droperidol or clonidine to epidural tramadol shortens onset time and increases duration of postoperative analgesia,Can. J. Anesth. 50 (2003) 147–152.
[5] Y.Q. Zhang, Z.Q. Zhao, R.R. Ji, Emotional distress and related memory of pain: a neurobiological review, Neurosci. Bull. 21 (2005) 10–17.
[6] E.H. Chudler, W.K. Dong, The role of the basal ganglia in nociception and pain, Pain 60 (1995) 3–38.
[7] E.H. Chudler, Response properties of neurons in the caudate-putamen and globus pallidus to noxious and non-noxious thermal stimulation in anesthetized rats, Brain Res. 812 (1998) 283–288.
[8] G.J. Wu, Z.Q. Chen, H. Shi, Roles of globus pallidus in acupuncture analgesia and exciting caudate-putamen nucleus-induced analgesia, China J. Neurosci. 18 (2002) 621–625.
[9] John T. Willams, Macdonald J. Christie, Olivier Manzoni, Cellular and synaptic adaptations mediating, Physiol. Rev. 81 (2001) 299–330.
[10] U.M. Stamer, F. Stüber, The pharmacogenetics of analgesia, Expert Opin. Pharmacother. 8 (2007) 2235–2245.
[11] Ryszard Przewlocki, Opioid abuse and brain gene expression,Eur. J. Pharmacol 500 (2004) 331–349.
[12] Davidson, J.A. Stamford, Neurochemical evidence of functional A10 dopamine terminals innervating the ventromedial axis of the neostriatum in vitro voltammetric data in rat brain slices, Brain Res. 615 (1993) 229–239.
[13] E.J. Nestler, From neurobiology to treatment: progress against addiction, Nat. Neurosci. 5 (2002) 1076–1079.
[14] S. Ammon, P. Mayer, U. Riechert, H. Tischmeyer, V. Hollt, Microarray analysis of genes expressed in the frontal cortex of rats chronically treated with morphine and after naloxone precipitated withdrawal, Brain Res. Mol. Brain Res. 12 (2003) 113–125.
[15] C.Y. Zhao, L.X. Yan, N. Lu, J.Y. Zhang, M.Y. Xu, Making the model quickly for morphinomania in rats, J. Harbin Med. Univ. 35 (2001) 257–258.
[16] L.J. Pellegrino, A.S. Pellegrino, A.J. Cushman, A Stereotaxic Atlas of the Rat Brain, second ed., Plenum Press, New York, 1979, pp. 81–85.
[17] X.T. Zhang, The integration of thalamus in the process of acupunctureanalgesia, Sci. China 1 (1973) 28–52.
[18] M.Z. Sun, L.S. Chen, H.L. Gu, J. Cheng, L.S. Yue, Effect of acupuncture on unit discharge in nucleus parafascicularis of rat thalamus, Sheng Li Xue Bao 32 (1980) 207–213.
[19] A. Olschewski, M.E. Brau, G. Hempelmann, W. Vogel, B.V. Safronov, Differential block of fast and slow inactivating tetrodotoxin-sensitive sodium channels by droperidol in spinal dorsal horn neurons, Anesthesiology 92 (2000) 1667–1676.
[20] S. Yamamoto, H. Yamaguchi, M. Sakaguchi, S. Yamashita, T. Satsumae, Preoperative droperidol improved postoperative pain relief in patients undergoing rotator-cuff repair during general anesthesia using intravenous morphine, J. Clin. Anesth. 15 (2003) 525–529.
[21] J.E. Magnusson, K. Fisher, The involvement of dopamine in nociception: the role of D1 and D2 receptors in the dorsolateral striatum, Brain Res. 855 (2000) 260–266.
[22] T. Weizman, C.G. Pick, M.M. Backer, T. Rigai, M. Bloch, S. Schreiber, The antinociceptive effect of amisulpiride in mice is mediated through opioid mechanisms, Eur. J. Pharmacol. 478 (2003) 155–159.
[23] Lynette C. Dawsa, Paul D. Callaghana, José A. Morónb, Kris M. Kahliga, Toni S. Shippenbergb, Jonathan A. Javitchc, Aurelio Gallia, Cocaineincreases dopamine uptake and cell surface expression of dopamine transporters, Biochem. Biophys. Res. Commun. 290 (2002) 1545–1550.
[24] S.C. Pandey, E.H. Chartoff, W.A. Carlezon Jr., J. Zou, H. Zhang, A.S. Kreibich, J.A. Blendy, F.T. Crews, CREB gene transcription factors: role in molecular mechanisms of alcohol and drug addiction, Alcohol Clin. Exp. Res. 29 (2005) 176–184.
[25] R. Spangler, N.L. Goddard, N.M. Avena, B.G. Hoebel, S.F. Leibowitz, Elevated D3 dopamine receptor mRNA in dopaminergic and dopaminoceptive regions of the rat brain in response to morphine, Brain Res. Mol. Brain Res. 111 (1–2) (2003) 74–83.