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Effects of norepinephrine on the electrical activities of pain-related neurons in the rat nucleus accumbens

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Abstract:
This study examined the effects of norepinephrine (NE) and phentolamine on the electrical activities of pain-excited neurons (PENs) and pain-inhibited neurons (PINs) in the nucleus accumbens (NAc) of Wistar rats. Trains of electric pulses applied to the right sciatic nerve were used to provide noxious stimulation, and the discharges of PENs and PINs were recorded using a glass microelectrode. Our results revealed that in response to noxious stimulation, NE decreases the evoked discharge frequency of PENs and increases the evoked discharge frequency of PINs in the NAc of healthy rats, whereas phentolamine produced opposite responses. These results demonstrate that NE is involved in the modulation of nociceptive information transmission in the NAc.

Key words:
norepinephrine, phentolamine, electrical activity, pain-related neuron, nucleus accumbens

Introduction

Norepinephrine (NE) is an important neurotransmitter in the rat brain. The noradrenergic system, which uses NE as the main neurotransmitter, participates in multiple brain functions, including arousal, attention, mood, learning, memory, and stress responses [16]. The effects of NE on pain are complex. The role of NE in pain is dependent on its specific site of action in the central nervous system (CNS), the distribution of noradrenergic receptor subtypes, the duration of the pathological pain state, and the situation at that time. The cell bodies of noradrenergic neurons are primarily localized in the medulla oblongata and the pons. The descending inhibitory system, which can modulate pain transmission in the spinal cord, is composed of many brain structures that converge on the brainstem. NE and other chemical substances play important roles in the descending inhibitory system [7]. Some studies have shown that NE inhibits C fibers, which transmit the peripheral noxious stimulation to the spinal cord lateral horn [11].

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The nucleus accumbens (NAc) is located at the junction of the limbic system and the basal ganglia. Various projections from different regions of the brain converge on the NAc, which regulates various bodily functions, such as behavior, drug addiction, schizophrenia, learning, sports memory, cardiovascular activities, etc. [12]. The role of the NAc in analgesia has been debated by scholars. The NAc is rich in endogenous opioid peptides and plays an important role in pain transmission and modulation in the CNS. The NAc may be another structure in which opioid peptides and CCK interact to regulate pain [10]. The ascending projections from the periaqueductal gray matter (PAG) to the NAc and the descending projections from the NAc to the PAG are involved in analgesia. Our laboratory has demonstrated that the glutamic acid, dizocilpine maleate and N-methyl-D-aspartate receptors are involved in the modulation of nociceptive information transmission in the NAc. In recent years, a number of studies have confirmed the presence of NE in the NAc and demonstrated that it plays a central role in the regulation of pain [19].

In the present study, we used extracellular electrophysiological recording techniques to investigate the effects of NE and phentolamine in the NAc on the evoked electrical activities of pain excitet neurons (PENs) and pain inhibited neurons (PINs) in the NAc of healthy rats, and we revealed the role of NE and NAc in central analgesia production and modulation.

Materials and Methods

Animals

Male and female Wistar rats (Animal Center of the second Affiliated Hospital, Harbin Medical University, Certificate No. 09-2-1), weighing 200–260 g were used in this study. The experimental procedures were approved by the Institutional Animal Care and Use Committee at both CCMU and FMMU. All animals were maintained and cared for in compliance with the guidelines set forth by the International Association for the Study of Pain [24]. The number of animals used and their suffering were greatly minimized. The rats were divided into the following 3 groups: (1) control group: intra-NAc injection of normal saline, 0.5 μl; (2) NE group: intra-NAc injection of NE, 2 μg/0.5 μl; and (3) phentolamine group: intra-NAc injection of phentolamine, 2 μg/0.5 μl. All injections were completed within 2 min *via* a microliter syringe.

Neurosurgery and electrophysiological studies

Routine surgery was performed after rats were anesthetized with 20% urethane injected intraperitoneally (1 g/kg). The right sciatic nerves were isolated. Two skull windows were created and liquid paraffin was used to cover the windows. According to the stereotaxic coordinate system of Pellegrino's atlas [13], the rat was fixed on a stereotaxic frame (SN-2, Narishige, Japan). The rat's head must be in a fixed position such that the interaural line is exactly 5 mm below the level of upper incisor bar. The skull landmark, bregma, was used as the rostrocaudal zero reference point. After 4–10 min, the rat was paralyzed with tubocurarine chloride (1 mg/kg), and artificial ventilation was maintained. The experiment used extracellular recording techniques. Single-unit recordings of neuronal electric activity were performed with a glass microelectrode (tip extreme diameter: 0.5–1.0 μm, DC resistance: 10–30 MΩ) filled with KCl (3 mol/l). The glass microelectrode was inserted using a micromanipulator (SM-21, Narishige, Japan) into the NAc (A: 3.2–4.0 mm; R or L: 1.0–1.8 mm; H: 6.2–7.0 mm) to record the discharges of the neurons in the NAc [13]. Another glass microelectrode was inserted using a micromanipulator (SM-11, Narishige Japan) into the NAc (A: 3.6 mm; R or L: 1.6 mm; H: 6.2 mm) for the administration of drugs. The electrical activity was amplified with a microelectrical amplifier (signal high frequency filter: 3 kHz, low frequency filter: 0.01 s, amplification: 100-fold), recorded with a biological experimental system and concurrently monitored with an oscilloscope (VC-9, 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 ms, interval: 5 ms, intensity: 5 mA, duration: 0.3 ms, train: 5; SEN-3301, Nihon Konden, Japan) to produce noxious stimulation. Articular movement and hair touching were used as the non-noxious stimulation to identify the pain-related neurons. The discharge of each neuron was recorded 3 times every 2 min, and the complete recording duration was 30 min.

Definition of neurons

The neurons recorded in the NAc were divided into 3 types: 1. unallide neurons, which do not react to

noxious stimuli or non-noxious stimuli; 2. convergent neurons, which respond to both noxious stimuli and non-noxious stimuli; and 3. pain-related neurons, which only respond to noxious stimuli. Pain-related neurons can be subdivided into PENs and PINs. PENs are defined as neurons that respond to noxious stimuli by increasing their discharge frequency [22], whereas PINs are defined as neurons that respond to noxious stimuli by decreasing their discharge frequency [18]. This study principally observed and recorded the electrical activities of PENs and PINs. The net increased value (NIV, Hz) refers to the difference in the average frequency of evoked discharges after noxious stimulation and the average frequency of the discharges within 2 s before noxious stimulation between PENs and PINs. Inhibitory duration (ID, ms) refers to the latency between the noxious stimulation and the appearance of the PIN discharges.

Histological identification

At the end of the experiment, 2% pontamine sky blue was diffused through the microelectrode using a negative direct current (30 μA, 15 min) to identify the tip position of the recording microelectrode.

Statistical analysis

Data were uploaded to a computer with Powerlab/8 s (ADInstruments) and analyzed with Chart v5.3 software (Australia). All data were expressed as the mean ± SEM and analyzed with SPSS 13.0 software. Statistical differences were evaluated by one-way ANOVA, and $p < 0.05$ was considered to be statistically significant.

Results

Effects of normal saline on the evoked discharges of pain-related neurons in the rat NAc

In the control group, intra-NAc administration of normal saline produced no significant changes in either the PENs or the PINs (Fig. 1a and Fig. 2a).

Influence of NE on the electrical activities of pain-related neurons in the rat NAc

In the NE group, the average latency of the 22 PENs was 0.19 ± 0.05 s, and the average NIV was 4.36 ± 0.57 Hz. Shortly after the intra-NAc injection of NE, the latency of the PENs began to increase, and the NIVs began to decrease (Fig. 1b). These effects peaked at 6 min after administration; at the peak, the average latency was 0.85 ± 0.14 s, and the average NIV was 0.64 ± 0.01 Hz. During 4–14 min after NE administration, the average latency and NIV of the PENs showed obvious changes compared to the average latency and NIV prior to NE administration or compared to those of the control group ($p < 0.05$, Fig. 3). At 20 min after NE administration, the latency and NIV of the PENs started to return to pretreatment values.

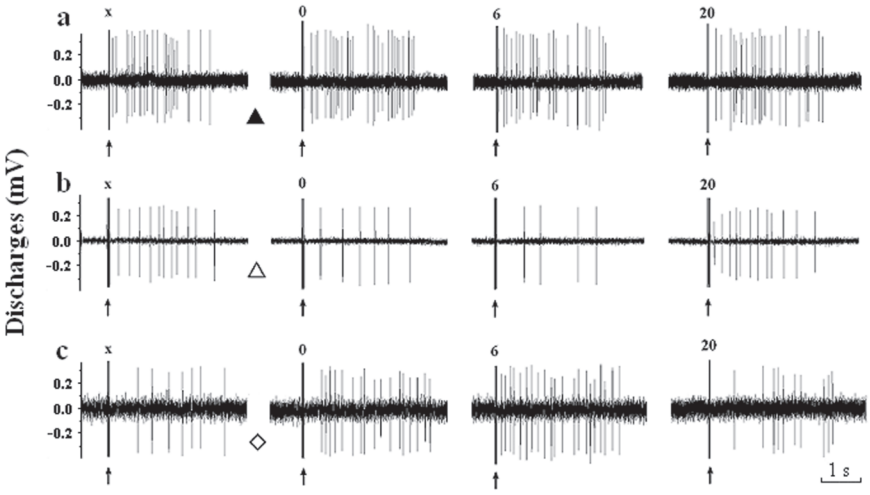

Fig. 1. (**a**) Effects of an intra-NAc injection of saline on the evoked discharges of PENs in the NAc. (**b**) Effects of an intra-NAc injection of NE on the evoked discharges of PENs in the NAc. (**c**) Effects of an intra-NAc injection of phentolamine on the evoked discharges of PENs in the NAc. ↑– Stimulus artifact; ▲– injection of saline; Δ – injection of NE; ◊ – injection of phentolamine; X – before injection; 0, 6, 20 – time after injection (min)

The average ID of the 16 PINs was 1.38 ± 0.69 s, and the NIV was –2.15 ± 0.98 Hz. After the intra-NAc injection of NE, the ID of the PINs began to shorten, and the NIV began to increase (Fig. 2b). These changes peaked at 6 min after NE injection; the average ID decreased to 0.48 ± 0.07 s, and the NIV increased to –0.03 ± 0.01 Hz. During 2–12 min after the injection, the average ID and NIV exhibited clear changes compared to those before the injection or those of the control group ($p < 0.05$, Fig. 4). The average ID and NIV of the PINs started to recover 22 min after the NE injection.

Effects of phentolamine on the electrical activities of pain-related neurons in the NAc of rats

In the phentolamine group, the average latency of the 21 PENs was 0.17 ± 0.02 s, and the NIV was 3.65 ± 0.95 Hz. After the intra-NAc administration of phen-

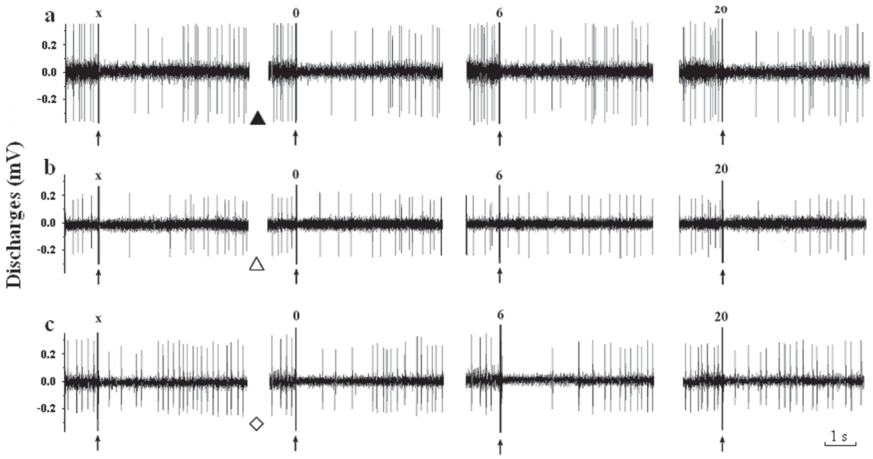

Fig. 2. (**a**) Effects of an intra-NAc injection of saline on the evoked discharges of PINs in the NAc. (**b**) Effects of an intra-NAc injection of NE on the evoked discharges of PINs in the NAc. (**c**) Effects of an intra-NAc injection of phentolamine on the evoked discharges of PINs in the NAc. ↑ – Stimulus artifact; ▲– injection of saline; Δ – injection of NE; ◊ – injection of phentolamine; x – before injection; 0, 6, 20 – time after injection (min)

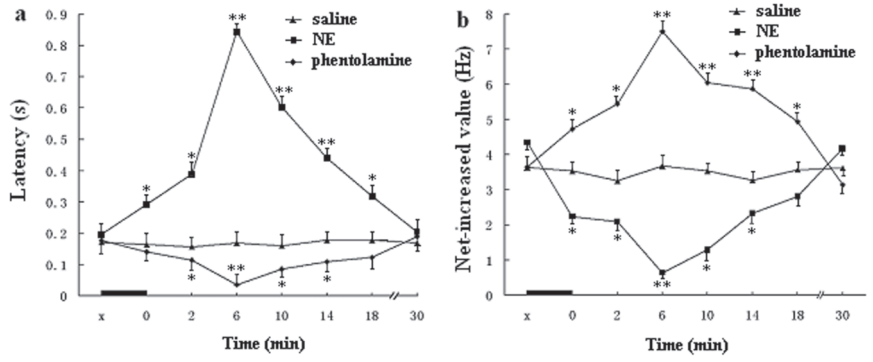

Fig. 3. Influences of intra-NAc injections of different substances on the latency (**a**) and NIV (**b**) of PENs in the NAc. — – injection of substance; x – before injection; 0, 2,……, 30 – time after injection (min); values are expressed as the means ± SEM; * $p < 0.05$, ** $p < 0.01$ compared to the saline group

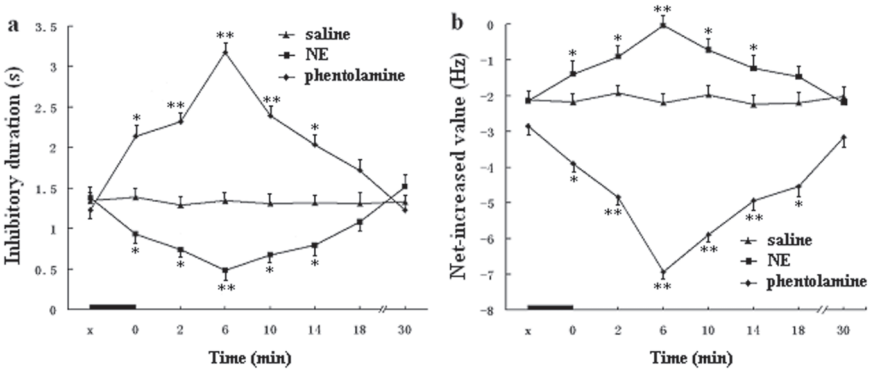

Fig. 4. Influences of intra-NAc injections of different substances on the ID (**a**) and NIV (**b**) of PINs in the NAc. — – injection of substance; x – before injection; 0, 2,……, 30 – time after injection (min); values are expressed as the means ± SEM; * $p < 0.05$, ** $p < 0.01$ compared to the saline group

tolamine, the latency of the PENs began to shorten, and the NIV began to increase (Fig. 1c). These effects peaked at 6 min after administration; the average latency decreased to 0.07 ± 0.01 s, and the NIV increased to 7.51 ± 0.98 Hz ($p < 0.05$, Fig. 3).

The average ID of the 18 PINs was 1.23 ± 0.18 s, and the NIV was –2.85 ± 0.99 Hz. After the injection of phentolamine, the ID began to prolong, and the NIV began to reduce (Fig. 2c). At 6 min after phentolamine administration, the average ID of the PINs increased to 3.17 ± 0.48 s, and the NIV decreased to –6.94 ± 0.03 Hz. At 24 min after phentolamine administration, the average ID and NIV of the PINs returned to the values observed before treatment ($p < 0.05$, Fig. 4).

Discussion

We studied the effects of NE and phentolamine on the electrical activities of PENs and PINs in the NAc of normal rats. The results of our study revealed that NE inhibits the electrical activities of PENs and enhances those of PINs. Additionally, phentolamine enhanced the electrical activities of PENs and inhibited those of PINs, demonstrating the antagonism between the effects of phentolamine and those of NE in the NAc. These results illustrate that NE is involved in the modulation of nociceptive information transmission in the NAc. Furthermore, PENs and PINs may be considered to be indices of pain research [15, 21]. PENs and PINs have opposite responses to identical substances, which may account for the effects of NE and phentolamine on pain modulation.

The analgesic effect of catecholamines is not constant (especially NE). Previous experiments in our laboratory have demonstrated that intracerebroventricular NE injections (10 μg/10 μl) produced analgesic effects [8]. In this study, we demonstrated that NE injection into the NAc also has an analgesic effect. NE is widely distributed in the brain. The descending inhibitory system, which can modulate spinal cord pain transmission, is composed of many brain structures that converge on the brainstem. NE, 5-hydroxytryptamine (5-HT), and other chemicals play important roles in the descending inhibitory system [7]. NE inhibits peripheral C fibers that transport the noxious stimulation to the spinal cord lateral horn [11]. NE reuptake inhibitors have been shown to reduce the early pain sensations caused by noxious stimulation [6]. Monoamines, including NE, dopamine (DA), 5-HT, etc., regulate the excitability of dorsal horn neurons and nociceptive pain through different neurotransmitter receptor subtypes [2]. NE has little effect on pain sensations under basal conditions, but long-lasting pain may promote NE-mediated inhibition of pain due to negative feedback [14]. 5-HT and NE are involved in pain modulation *via* descending inhibitory pathways in the brain and spinal cord [20]. Previous experiments have demonstrated that an intrathecal injection of NE produces a stronger analgesic effect than 5-HT, which suggests that there is a close relationship between the noradrenergic system and the pain modulation system [9].

The pain-suppressing system, which involves the activation of mesolimbic DA neurons, is naturally triggered by exposure to stress, pain or both [1]. Our laboratory has confirmed that the NAc contains PENs and PINs that have specific responses to nociceptive stimulation [23]. The NAc receives projections from glutamatergic, serotonergic, and noradrenergic neurons. The NAc contains a large number of endogenous opioid peptides and plays an important role in the control of pain transmission and modulation. The NAc may be another structure that modulates pain through the interaction between opioid peptides and cholecystokinin (CCK) [4]. A body of evidence suggests that dopamine (DA) is co-released with NE from noradrenergic terminals of the prefrontal cortex [4, 5]. Previous studies on descending inhibition of pain have focused on the dopamine-rich NAc, a striatal subdivision that contains a NE-poor rostral portion and a NE-rich caudomedial subdivision [19]. The major neurotransmitter of the NAc is DA, and DA receptors are abundant in the NAc, especially the D_3 receptor [3, 17]. Studies have confirmed that there are equal levels of NE and DA neurotransmitters in the NAc [4]. Phentolamine is an NE receptor antagonist. In our study, phentolamine had the opposite effect of NE on the electrical activities of pain-related neurons in the NAc.

In conclusion, our results indicate that NE modulates nociception by inhibiting PEN activity and potentiating PIN activity, whereas the α-adrenoceptor antagonist phentolamine produced opposite effects. Further investigations are required to determine which mechanism predominates in the nociception mediated by the NAc.

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