Original Article

Effects of alpha-adrenergic agonists on pain modulation in diffuse noxious inhibitory control

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Background: Diffuse noxious inhibitory control (DNIC) is thought to be mediated by neural networks in supraspinal brain structures. The descending antinociceptive system (DAS) is an important component of the DNIC neural network, but the precise structure of the neural network and the related neurotransmitters have not been examined.

Methods: The study was designed to examine whether systemic administration of the adrenergic agonists dexmedetomidine (DEX) and phenylephrine (PE) influences DNIC in the rat. Changes in the C-fiber reflex evoked by electromyographic activity were recorded following noxious tail immersion in hot water.

Results: Inhibition of the C-fiber reflex by the conditioning stimuli was reduced from 77.1 ± 22.6% to 26.6 ± 38.2% with continuous administration of DEX, and restored to 58.3 ± 29.2% by intramuscular injection of atipamezole hydrochloride (APZ), a selective α₂-adrenoceptor antagonist. Inhibition of the C-fiber reflex was reduced from 75.6 ± 25.8% to 22.7 ± 38.9% with continuous administration of PE, and restored to 84.9 ± 9.7% by intramuscular injection of phentolamine mesylate (PT), an α₁-adrenoceptor antagonist.

Conclusion: The results show that clinical doses of DEX and PE inhibit DNIC, thereby affecting and modulating the intrinsic pain inhibition system. These findings suggest that adrenergic neurons are involved in DNIC.

Key words: Diffuse noxious inhibitory control, dexmedetomidine, phenylephrine, pain modulation, descending antinociceptive system

Introduction

Diffuse noxious inhibitory control (DNIC) is a phenomenon in which the activities of the spinal dorsal horn or trigeminal nucleus convergent neurons (wide dynamic range (WDR) neurons) are selectively and powerfully inhibited by heterotopically applied noxious stimuli to a body area distant from their excitatory receptive fields. Such inhibitory phenomena were initially described in rat¹,² and subsequently in other animal species³,⁴ and humans⁵,⁶. DNIC is mediated by neural networks in supraspinal brain structures and the descending antinociceptive system is an important component of the DNIC neural network. Studies using brain dissection indicate that DNIC is associated with the subnucleus reticularis dorsalis¹¹, a structure in the more caudal part of the brainstem¹²,¹³, but the precise mechanism of the neural network and the related neurotransmitters in DNIC have not been fully elucidated.

Several subtypes of adrenoceptors may also be involved in the descending inhibitory control system. Alpha₂-adrenoceptors are found throughout the body,
including the central nervous system and effector organs such as vascular smooth muscle, and are especially prevalent in tissues innervated by the sympathetic nervous system. Presynaptically, \( \alpha_2 \) receptor activation reduces noradrenaline release, and activation of postsynaptic \( \alpha_2 \) receptors hyperpolarizes neural membranes. Activation of these receptors by noradrenaline is thus included in an inhibitory feedback loop, whereby further release of noradrenaline is inhibited. Injection of phentolamine into the nucleus raphe magnus (NRM) induces a significant increase in the release of both noradrenaline and serotonin in the spinal cord\(^\text{16}\), based on which it was concluded that antinociception induced by blockade of the inhibitory noradrenergic input to the NRM is mediated by activation of spinally-projecting serotonergic and noradrenergic (NA) neurons. Neurons located in the NRM appear to be tonically inhibited by NA neurons\(^\text{17}\). Dexametomidine hydrochloride (DEX) is a relatively selective \( \alpha_2 \)-adrenoceptor agonist with sedative properties. DEX has a greater affinity for \( \alpha_2 \)-adrenoceptors than for \( \alpha_1 \)-adrenoceptors (1620:1) in vitro\(^\text{18}\), and is currently used for sedation of patients in postoperative management or in the intensive care unit. Phenylephrine (PE) is an \( \alpha_1 \)-adrenoceptor agonist that is used to increase the blood pressure in treatment of hypotension or as a decongestant in local anesthesia and in the critical care setting\(^\text{19}\). It is unclear if DNIC is modulated by clinical doses of \( \alpha_2 \) -and \( \alpha_1 \)-adrenoceptor agonists, which have effects on the descending inhibitory control system following either systemic intrathecal or supraspinal administration. Therefore, we designed this study to examine whether systemic administration of adrenergic agonists may affect DNIC following noxious tail immersion in hot water by recording changes in the C-fiber reflex evoked by electromyographic activity in the rat.

### Materials and Methods

**Animal preparation**

The study was approved by the Ethics Committee of Tokyo Medical and Dental University and the procedures conformed to the Declaration of Helsinki. The experiments were carried out in 20 male Wistar rats weighing 280-400g. The rats were divided into 2 groups: the DEX group (n=10) and the PE group (n=10). The animals were deeply anesthetized with 5% sevoflurane in oxygen for surgery including tracheotomy and cannulation of the jugular vein. After surgery, the animals were anesthetized with 2.5-3.2% sevoflurane with \( \text{N}_2\text{O}/\text{O}_2 \) mixture (about 2 minimum alveolar concentration (MAC)). Throughout the experiments, the animals were artificially ventilated (rate: 50-75 / min, tidal volume: 2.5-3.5ml) by a mechanical respirator (SN-480-7, Shimano, Tokyo, Japan). The partial pressures of \( \text{O}_2 \), end-tidal \( \text{CO}_2 \) and sevoflurane were monitored intermittently with a capnometer (Respina 1H26, NEC San-ai, Tokyo, Japan). The partial pressure of end-tidal \( \text{CO}_2 \) was adjusted to between 2.5 and 3.2%. Body temperature was measured with a rectal probe and maintained at 37.5 ± 0.3 °C using a heat controller (ATS-1100, Nihon Kohden, Tokyo, Japan). Normal saline (NS) was administered continuously at a rate of 0.5 ml/kg/hr until administration of the experimental agents.

Test stimuli and electromyographic recordings (C-fiber reflex)

Electromyographic (EMG) recordings were obtained using a previously described method\(^\text{20}\). Test stimuli were applied to the digits of the left hind paw. Two non-insulated platinum-iridium needle electrodes were inserted subcutaneously into the medial aspect of the fourth toe and lateral aspect of the fifth toe, innervated by the sural nerve for nerve stimulation. Ten sequential square-wave electrical stimuli of 2-ms duration were delivered with a frequency of 0.1 Hz from a constant current stimulator (SEN-7203, Nihon Kohden, Japan). The electromyographic activity elicited by electrical stimulation within the receptive field of the ipsilateral sural nerve was recorded from the biceps femoris muscle. EMG responses were also recorded through another pair of non-insulated platinum-iridium needles inserted through the skin into the biceps femoris muscle. The EMG responses were biphasic with fast and late components and the C-fiber reflex was defined as the late component of the EMG. The stimuli intensities and EMG responses were fed into an oscilloscope for continuous monitoring and also into a computer system (Power Lab, Chart v 4.2/Scope v 3.6.11 for Windows), which digitized and recorded the EMG from 50 ms before until 500 ms after the start of stimulation. The digitized EMG recordings were full-wave rectified and integrated within a time-window from 250 ms to 350 ms after the start of stimulation. The intensities of the C-fiber reflexes were expressed as the integrated value, with the integrals in millivolts \( \times \) milliseconds (mV \( \times \) ms) and the current intensities in milliamperes (mA).
Conditioning thermal stimuli

Thermal conditioning stimuli were applied using a thermoregulated (50 °C) and agitated waterbath (SD mini, Taiotec, Saitama, Japan), into which the tail was immersed up to two-thirds of its length. These conditioning stimuli were applied for the duration of 1 min, with an interval of 15 min between successive tests. The interval of 15 min was chosen to allow examination of the effects of the stimuli and to avoid any phenomenon of sensitization of the skin receptors\(^{21}\), which could introduce bias into the results. A conditioning test was performed during the control period.

Experimental protocol

After the surgery, the concentration of sevoflurane in the nitrous oxide/oxygen mixture (1:1) was lowered to 2.5-3.2%. Thereafter, NS was injected at a rate of 0.5 ml/kg/hr until DEX or PE was administered. To determine the threshold of the C-fiber reflex, stimuli of 2.7-4.7 mA were applied to the sural nerve receptive field and these resulted in stable EMG responses with minimal fluctuations. A three-sequence stimulation applied at intervals of 5 min was used to determine the threshold of the C-fiber, and the threshold current that elicited minimal fluctuation was determined from the mean value of these currents.

Current stimuli at 1.5-fold the threshold (test stimuli) were applied 5 min after the threshold was determined. Ten minutes later, the C-fiber reflexes elicited by test stimuli were recorded. Five minutes thereafter, the C-fiber reflex elicited by the same stimulus in the presence of the thermal conditioning stimulus (50 °C water tail immersion) was recorded. Thermal conditioning stimuli were applied 15 sec before and during application of the test stimuli. Fifteen minutes later, NS administration was discontinued and DEX or PE was injected intravenously.

<DEX session>

DEX was injected at a rate of 3 \( \mu g/kg/hr \) for 5 min and then at 0.5 \( \mu g/kg/hr \) until the end of the experiment. EMG responses elicited by the test stimuli were recorded 5 min after the reduction in the rate of the DEX injection and the C-fiber reflex was recorded under the conditioning stimuli after another 5 min. After another 15 min, 0.5 mg/kg of atipamezole (APZ) was injected intramuscularly into the contralateral biceps femoris (right hind paw) and the C-fiber reflex was recorded under the conditioning stimuli 3 min after the injection (Fig. 1 A).

<PE session>

PE was injected at a rate of 5 \( \mu g/kg/min \) for 10
min, followed by NS injection at 0.5 ml/kg/hr. After the termination of PE administration, EMG responses elicited by the test stimuli were recorded and the C-fiber reflex was recorded under the conditioning stimuli after another 5 min. Twenty minutes later, 0.1 mg/kg of phentrimine (PT) was injected intramuscularly into the contralateral biceps femoris (right hind paw) and the C-fiber reflex elicited under the conditioning and test stimuli simultaneously was recorded 3 min after the injection (Fig.1 B).

Data analysis and statistics
The inhibition rate of the C-fiber reflex was calculated as follows:
Inhibition rate = 100 x (1 - [A/B]).
A: Mean C-fiber reflex response in the presence of the conditioning stimuli
B: Mean C-fiber reflex response in the absence of the conditioning stimuli.

One-way ANOVA and a Bonferroni correction were used for comparison of the inhibitory rates with statistical significance accepted at P < 0.05.

Results

C-fiber reflex and DNIC
Electrical stimulation within the receptive field of the sural nerve elicited a two-component response in the ipsilateral biceps femoris muscle. The first component had a short latency (10-100 ms) and a low threshold (1.1-1.2 mA). The second had a longer latency (150-450 ms) and a higher threshold (2.7-4.7 mA) under 2.5-3.2% sevoflurane inhalation. The first component of the reflex response is due to activation of myelinated cutaneous afferent fibers, whereas the second has been demonstrated to be elicited by activation of unmyelinated afferent fibers; therefore, it is referred to as the C-fiber reflex\textsuperscript{23,24}. The present work focused on analysis of this C-fiber reflex elicited by a stimulus intensity of 1.5-fold the threshold intensity.

Typical recordings of EMG responses elicited by such a stimulus are shown in Fig. 2. The C-fiber reflex responses were strongly depressed during the application of noxious thermal conditioning stimuli to the tail, with inhibition of the C-fiber reflex of 77.1 ± 22.6%. During the control period, there were no statistical differences between the experimental groups. The depressive effect elicited by the conditioning stimuli was extended by several minutes during the conditioning period, and an after-effect with inhibition of 14 ± 4% was observed until 3 min after removal of the conditioning stimuli.

Effects of DEX on the C-fiber reflex in the absence of conditioning stimuli
The respective intensities of the C-fiber reflexes (integral values of EMG) were 2847 ± 123 and 2636 ± 250 (mV × ms) before and after administration of DEX, respectively. No change in the reflex response was found after administration of DEX, and this dose of DEX did not induce a significant change in the C-fiber reflex.
in the absence of conditioning thermal stimuli at the low stimulus intensity (1.5-fold the threshold intensity) used in the study (Fig. 2).

Effects of DEX on DNIC

As shown in Fig. 3, inhibition of the C-fiber reflex was reduced from 77.1 ± 22.6% to 26.6 ± 38.2% with continuous administration of DEX. This dose of DEX produced a statistically significant effect in comparison with controls (P<0.05, n=10). The reduction of the inhibition of the C-fiber reflex by DEX was reversed by intramuscular injection of AP2. Inhibition of the C-fiber reflex was 58.3 ± 29.2% following this injection (P<0.05, n=10).

Effects of PE on the C-fiber reflex in the absence of conditioning stimuli

C-fiber reflex responses elicited by the test stimuli increased significantly after administration of PE (P<0.05, n=10) compared with NS administration. A 55.7 ± 35.1% increase of the integral values of the C-fiber reflex activity was observed and lasted for over 30 minutes in both a preliminary study and during the experimental period.

Effects of PE on DNIC

The inhibition of the C-fiber reflex by noxious thermal stimuli was blocked significantly following injection of PE. Inhibition of the C-fiber reflex was reduced from 75.6 ± 25.8% to 22.7 ± 38.9% following PE injection. A statistically significant effect was obtained with this dose of PE compared with controls (P<0.05, n=10). The reduced inhibition of the C-fiber reflex due to PE was reversed by intramuscular injection of PT to 84.9 ± 9.7% (P<0.05, n=10).

Discussion

Effects of noxious conditioning stimuli on the C-fiber reflex

The DNIC system is thought to be involved in pain modulation related to supraspinal brain structures. The current study shows that clinical doses of DEX and PE inhibit DNIC, thereby affecting and modulating the intrinsic pain inhibition system. With anesthetized rats in the present study, electrical stimulation within the region of the sural nerve elicits a C-fiber reflex that is inhibited by noxious conditioning stimuli (as shown Fig. 2). For a stimulus of immersion of the tail in a thermoregulated waterbath, this inhibition is temperature-dependent. This process is reminiscent of DNIC, which modulates the activities of dorsal horn convergent neurons.

In the present study, a thermal stimulus (tail immersion into 50°C hot water) was employed as the conditioning stimulus, as in previous protocols. Therefore, appropriate heterotopic conditioning stimuli for inducing DNIC were used in the study.

Our results indicate occurrence of DNIC in adequately anesthetized rats at about 2 minimum alveolar concentration (MAC) sevoflurane. This phenomena has been reported previously with inhalation of 2
MAC sevoflurane and for other volatile anesthetics, including 0.8 MAC isoflurane and 1.6 MAC halothane.

Effect of DEX on DNIC
No significant difference between the C-fiber reflex elicited by test stimuli was observed following administration of a clinical dose of DEX after NS. However, inhibition of the C-fiber reflex by the conditioning thermal stimuli was blocked by this dose of DEX, which indicates that DEX inhibits DNIC to modulate the pain processing mechanism. One of the physiologically and clinically significant effects of DNIC is to facilitate extraction of nociceptive information by increasing the signal-to-noise ratio between the pool of neurons activated by a noxious stimulus and the remaining population of neurons. Distinction of pain is known to be modulated by supraspinal mechanisms, and blocking of ascending pain information is induced by inhibition of WDR neurons located in lamina III-V in the spinal dorsal horn by heterotopic noxious stimuli. Therefore, DEX may act on the central nervous system to modulate the pain sensation.

The weak analgesic effect of DEX might inhibit the neuronal activity evoked by peripheral noxious inputs in the spinal cord, making the conditioning stimuli insufficient to reduce the C-fiber reflex. However, we believe that the analgesic effect did not influence our results because no changes in the magnitude of the C-fiber reflex were seen between DEX-administered and control periods; and because the analgesic effect of DEX is relatively weak under strong noxious stimulation, such as that provided by the test and conditioning stimuli in this study.

Effect of PE on DNIC
Our results indicate that PE also blocked DNIC, which suggests that systemic administration of a clinical dose of PE may modulate the DNIC mechanism and play an important role in pain processing in the central nervous system. This may occur through effects on noradrenergic neurons in the brainstem, since microinjection of PT (an α₁ antagonist) into the NRM produces hypoalgesia, leading to the conclusion that postsynaptic α₁ noradrenergic receptors may be involved in modulation of nociception in the NRM. The C-fiber reflex elicited by a stimulus of 1.5-fold the threshold intensity following a clinical dose of PE was significantly stronger than under control conditions. Previous reports and this result indicate that PE may modulate NA neurons inhibiting NRM neurons and may reduce powerful inhibition of the spinal cord.

Central nervous system effects of DEX and PE
Le Bars et al. have proposed that the mechanism underlying the occurrence of DNIC involves the supraspinal structures, including the subnucleus reticularis dorsalis, a structure in the more caudal part of the brainstem. Conversely, more rostral structures such as the periaqueductal grey, duneiform nucleus, parabrachial area, locus coeruleus / subcoeruleus, rostral ventromedial medulla including the NRM, and the gigantocellular and paragigantocellular nuclei are not involved in DNIC. DEX has sedative properties. Systemic administration of DEX affects the central nervous system. DEX mostly acts on a₂ adrenergic receptors in the locus coeruleus to inhibit noradrenaline release. Descending inhibitory control mediated by the catecholaminergic, serotoninergic and opioid neuronal system is thought to trigger DNIC. Le Bars et al. proposed that DEX was blocked by parenteral administration of DEX and restored by administration of APZ (an a₂ selective antagonist), which indicates that DEX may partially block descending inhibition by stimulating a₂ receptors and causing inhibition of noradrenaline release from catecholaminergic or noradrenergic neurons of the locus coeruleus. This suggests that DNIC is mediated by noradrenaline released from NA neurons.

Systemic administration of a clinical dose of PE modulated the DNIC mechanism and was thought to have no influence on peripheral pain modulation. It was reported that PE had possibility of penetrating the Blood-Brain Barrier (BBB). It has been shown that injection of PT into the NRM induces a significant increase in both norepinephrine and serotonin release in the spinal cord, and this was concluded to indicate that antinociception induced by blocking the inhibitory noradrenergic input to the NRM is mediated by activation of spinally-projecting serotoninergic and noradrenergic neurons. Our results indicate that PE may work as an agonist at the site of NA neurons inhibiting NRM neurons and reduce the activity of the descending inhibitory control system on spinal WDR neurons.

Overall, our results suggest that the neural network of the DNIC loop exists in the middle part of the brainstem, including the locus coeruleus and nucleus raphe magnus, and that different descending inhibitory pathways (the noradrenergic and serotoninergic systems) may be candidates for mediating the DNIC mechanism in supraspinal structures.
Effects of DEX and PE on thermal pain

A specific action of DEX and PE on thermal pain cannot be completely ruled out, because these drugs might affect the thermal threshold. Intraperitoneal administration of DEX increases the thermal threshold in a dose-dependent manner in normal animals and intrathecal administration of DEX produced a thermal antinociceptive effect in rat. Such effects through a peripheral action may have affected the reduction of inhibition of the C-fiber reflex induced by conditioning thermal stimuli. However, there was no significant difference in the C-fiber reflex between DEX and NS administration, suggesting the absence of a thermal antinociceptive effect of DEX. We did not examine the effect of PE on the thermal threshold, but intradermal injection of PE has been shown to produce dose-dependent thermal hyperalgesia. The effects of DEX and PE on thermal pain may differ depending on the administration route and dosage of these drugs and the anesthetics used in the experiment. However, we believe that peripheral effects of DEX and PE on changes of DNIC were minor under the conditions of the study.

Limitations

Finally, some limitations of the study should be noted concerning the administration method of DEX and PE, and the peripheral actions of these drugs. First, intrathecal or supraspinal administration was not used, in contrast to previous studies of the mechanism of DNIC. However, the effects of α-receptor agonists on DNIC has not been tested for systemic, intrathecal or supraspinal administration of DEX and PE. Therefore, systemic injection of DEX and PE was selected at a dose corresponding to the clinical dosage. Second, we did not examine whether the action of DEX and PE on the C-fiber reflex is mediated by motor neurons, motor controls and vegetative effects and whether these drugs might contribute directly to nociceptive processes or a peripheral mechanism of pain modulation. Therefore, effects of peripheral actions of DEX and PE on the C-fiber reflex cannot be excluded, although it has been shown that the nociceptive reflex is not suppressed by an adrenoreceptor agonist.

References

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