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NSAID loxoprofen inhibits high threshold or wide dynamic range neuronal responses in the rat at different time-courses

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Abstract:
The onset of the antinociceptive effect with loxoprofen sodium (LOX), a non-steroidal anti-inflammatory drug, was examined electrophysiologically during carrageenan-induced hindpaw inflammation in the rat. Extracellular recordings were made from either wide dynamic range (WDR) or high threshold (HT) neurons in the dorsal horn. Recordings from the same neuron were continued for at least 3 h after the injection of carrageenan. Three hours after the induction of inflammation, either a fresh solution of LOX (1 mg/kg) or distilled water was directly administered into the stomach through PE 50 tubing. LOX significantly reduced inflammation-increased background activity and noxious heat-evoked responses in both HT and WDR neurons, whereas distilled water did not produce any change. A significant difference in the onset of the inhibitory effect of LOX was observed between HT and WDR neurons. The results show that WDR neurons precede HT neurons regarding inhibition of nociceptive processing in the dorsal horn after administration of LOX.

Key words:
loxoprofen sodium, antinociception, dorsal horn neurons

Introduction

Nociceptive impulses are conducted from peripheral tissues to the dorsal horn of the spinal cord. Dorsal horn neurons which respond to noxious stimuli are classified as high threshold (HT) or wide dynamic range (WDR), according to their responses to brush or pinch stimuli [2]. HT neurons respond almost exclusively to noxious mechanical stimuli, and WDR neurons respond to both innocuous and noxious mechanical stimuli. The activity of these neurons is altered following the development of inflammation in the peripheral receptive fields of neurons. For example, the background activity and responses to noxious stimuli markedly increase during inflammation [18]. These phenomena may constitute the neurophysiological basis of spontaneous pain and hyperalgesia.

Loxoprofen sodium (LOX), a non-steroidal anti-inflammatory drug (NSAID), is popular in Japan for relieving inflammatory pain. LOX has also been widely used in several countries in Asia and South America. Like other NSAIDs, LOX inhibits prosta-

glandin synthesis by blocking cyclooxygenase at the site of inflammation [1, 3, 4, 7, 8, 14, 19, 20]. A reduction in quantity of prostaglandin reduces the frequency of nociceptive impulses conducted *via* primary afferent fibers from the inflammation site to the spinal dorsal horn. With regard to the pain mechanism, it is obvious that the decrease in nociceptive impulses conducted *via* primary afferent fibers results in the reduction of nociceptive activity of dorsal horn neurons. However, there are few reports comparing HT and WDR neurons in the nociceptive activity of dorsal horn neurons following the administration of LOX. We report here that there is a significant difference between HT and WDR neurons with regard to the onset of the inhibitory effect of LOX.

Materials and Methods

The experiments in this study were approved by the Institutional Animal Care and Use Committee of Showa University and were in accordance with guidelines of the International Association for the Study of Pain [22].

Animals

Experiments were performed on male Sprague-Dawley rats (n = 46) weighing 250–330 g. The animals were anesthetized with muscle relaxation using sodium pentobarbital (50 mg/kg *ip*) and sodium pancuronium (3 mg/kg *iv*). After tracheotomy, animals were artificially ventilated to maintain an end-tidal CO_2 level between 3.5 and 4.5%. Anesthesia and neuromuscular block were maintained during the experiment by infusing *iv* a mixture of 50 mg of sodium pentobarbital and 5 mg of sodium pancuronium in 44 ml of 0.9% NaCl at a rate of 0.04 ml/min. An adequate level of anesthesia was ensured from the absence of pupillary dilation or increases in heart rate in response to noxious heating of the skin. The core body temperature was maintained at approximately 37.5°C using a thermostatically controlled heating blanket. A laminectomy was performed to expose lumbar enlargement (T_{12}-L_4) of the spinal cord. The head and the vertebral column were fixed rigidly in a stereotaxic frame. The exposed spinal cord was covered with warm mineral oil after the dura was removed. At the end of surgical procedures, PE 50 tubing was inserted into the stomach through the mouth and was held in place by adhering the tubing to the mandibular incisor teeth. In the present study, LOX and distilled water were directly applied into the stomach through PE 50 tubing.

Electrophysiological recordings

Extracellular recordings were made from the dorsal horn with a carbon filament electrode (4–6 MΩ). To try to encounter dorsal horn neurons, spontaneous activities of neurons were carefully observed as the microelectrode was lowered into the dorsal horn. Tactile and pinch stimuli were used as the search stimuli. Tactile (innocuous brush) stimuli were delivered by repeated brushing in a stereotyped manner with a camel hair brush. Pinch stimuli were applied with an arterial clip. The pinch was distinctly painful with a force of 613 g/mm^2 when applied on human skin. Nociceptive dorsal horn neurons were classified as HT or WDR, according to their responses to brush or pinch stimuli. After a single dorsal horn unit was isolated and its receptive field was determined, a unit was subsequently tested for responsiveness to noxious heating of the skin (51°C). A contact feedback-controlled Peltier thermode with an active area of 16 mm^2 was applied to the unit's receptive field, and heat stimuli were delivered for 15 s. Single-unit activity was then fed into a computer data collection system (CED 1401 in acquisition software, Pentium PC), which constructed peristimulus histograms. The recording site of each neuron was verified from a micromanipulator reading of the depth below the spinal cord surface. Le Bars et al. [10, 11] observed that WDR neurons in rats are subject to a powerful inhibition when noxious stimuli are applied to any part of the body and face outside the excitatory receptive field. This form of inhibition is referred to as diffuse noxious inhibitory control (DNIC). HT neurons are not subject to DNIC. In the present study, therefore, the neuron type (WDR or HT) was reconfirmed by responsiveness to pinch stimuli (arterial clip, 613 g/mm^2) applied to the face. Examples of heat-evoked responses of WDR neurons and DNIC are shown in Figure 1.

Induction of hindpaw inflammation

Carrageenan (lambda, Sigma) was used to induce hindpaw inflammation. The carrageenan-induced in-

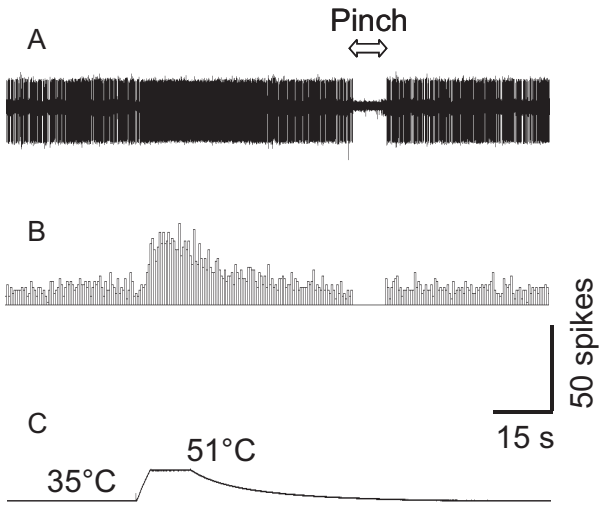

Fig. 1. Heat-evoked response of a WDR neuron and DNIC. For DNIC, pinch stimulation was applied to the face skin. **(A)** Spikes recorded from a WDR neuron. **(B)** Rate histograms constructed from the number of spikes counted every 0.5 s (0.5 s bin width). **(C)** Heat stimulation delivered for 15 s. Pinch stimuli applied to the face skin completely inhibited spontaneous activity of the neuron, which is DNIC. Note that inhibition of spikes resulted in the disappearance of rate histograms

flammation model is characterized by the rapid onset of inflammation causing a restricted distribution of hyperalgesia. Maximum hyperalgesia occurs at 3–4 h after carrageenan injection [5, 6, 9]. To induce hindpaw inflammation, rats received a subcutaneous injection of 6.0 mg of carrageenan in 0.15 ml of saline into the plantar surface of the hindpaws, and nociceptive responses of dorsal horn neurons were measured 3 h after the injection of carrageenan.

Administration of LOX

Loxoprofen sodium (1 mg/kg, pH = 6.43, Daiich-Sankyo) was dissolved in distilled water (DW) immediately before administration. Either 1 ml of LOX solution or 1 ml of DW (sham control) was directly administered into the stomach through PE 50 tubing.

Statistical analysis

Data are represented as the means ± SEM. Statistical analysis was carried out using analysis of variance (ANOVA), and the Least Significant Difference (LSD) test was used for *post-hoc* analysis of differences between individual points. A difference was accepted as significant when $p < 0.05$.

Results

Recordings were made from a total of 46 dorsal horn neurons: 14 HT neurons and 32 WDR neurons in 46 rats. The neurons were in the lumbar enlargement of the spinal cord with their receptive field on the hindpaw or toes. The recording sites were located from 278 to 685 μm below the surface of the spinal cord, suggesting that the cell bodies were located in laminae I–V of the dorsal horn [15].

Changes in background activity and heat-evoked responses during hindpaw inflammation

The subcutaneous injection of carrageenan into the plantar surface of the hindpaw resulted in the development of an edema in the injected hindpaws in all rats tested (n = 38). The edema developed rapidly with noticeable swelling by 3 h; paw thickness increased from 4.58 ± 0.09 mm to 10.87 ± 0.11 mm. Recordings from the same neuron were continued for at least 3 h after the injection of carrageenan. An example of changes in neuronal activity of a WDR neuron following the plantar injection of carrageenan is shown in Figure 2. With the development of edema, both the background activity and heat-evoked responses increased gradually up to 3 h after carrageenan injection. Three hours after the induction of inflammation, the background activity of WDR neurons increased significantly from 5.69 ± 1.73 to 26.50 ± 4.14 spikes/s, and heat-evoked responses increased significantly from 34.13 ± 8.32 to 70.28 ± 9.23 spikes/s. In HT neurons, the background activity increased significantly from 4.38 ± 0.88 to 23.64 ± 3.25 spikes/s, and heat-evoked responses increased significantly from 38.21 ± 7.31 to 81.16 ± 8.17 spikes/s (not shown).

Effects of LOX on dorsal horn neurons of rats non-treated with carrageenan

The effects of LOX (1 mg/kg) on two types of dorsal horn neurons of rats non-treated with carrageenan were examined. The background activity averaged for 1 min before LOX administration was 5.48 ± 1.66

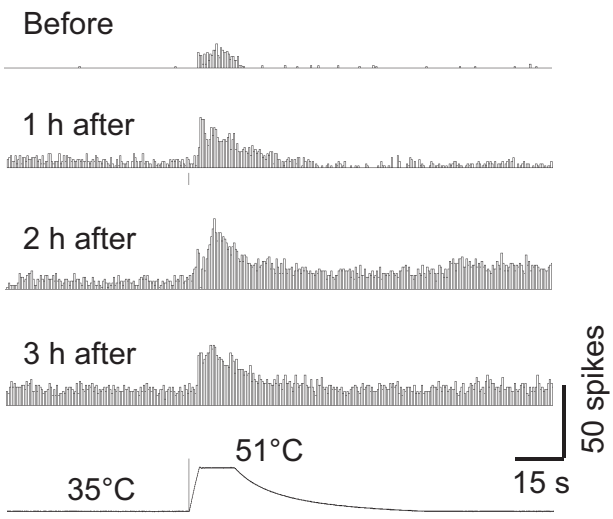

Fig. 2. An example of heat-evoked responses of the same WDR neuron following the induction of hindpaw inflammation. Carrageenan (6 mg in 0.15 ml of saline) was injected into the plantar surface of the hindpaw. Recordings were continued for 3 h after carrageenan injection. Note that both background activity and heat-evoked responses gradually increased after carrageenan injection. This phenomenon was the same in HT neurons (not shown)

spikes/s in WDR neurons (n = 4) and 5.07 ± 1.03 spikes/s in HT neurons (n = 4). Following LOX administration, the background activity did not significantly alter even at 30–33 min after LOX administration, and amounted to 5.26 ± 0.85 spikes/s in WDR neurons and 4.97 ± 1.11 spikes/s in HT neurons, as well as the case of DW administration. In contrast, LOX produced significant reduction of heat-evoked responses in both HT and WDR neurons. The percentage inhibitory effect on HT neurons was similar to that on WDR neurons: 79.7 ± 7.1% and 82.3 ± 6.7%, respectively. An example is shown in Figure 3.

Effects of LOX on dorsal horn neurons in rats with inflammation

The effects of either distilled water or LOX (1 mg/kg) on two types of dorsal horn neurons are shown as representative examples in Figure 4 and are summarized in Figure 5. In Figure 5, background activities were averaged for every 3 min and plotted every 3 min in

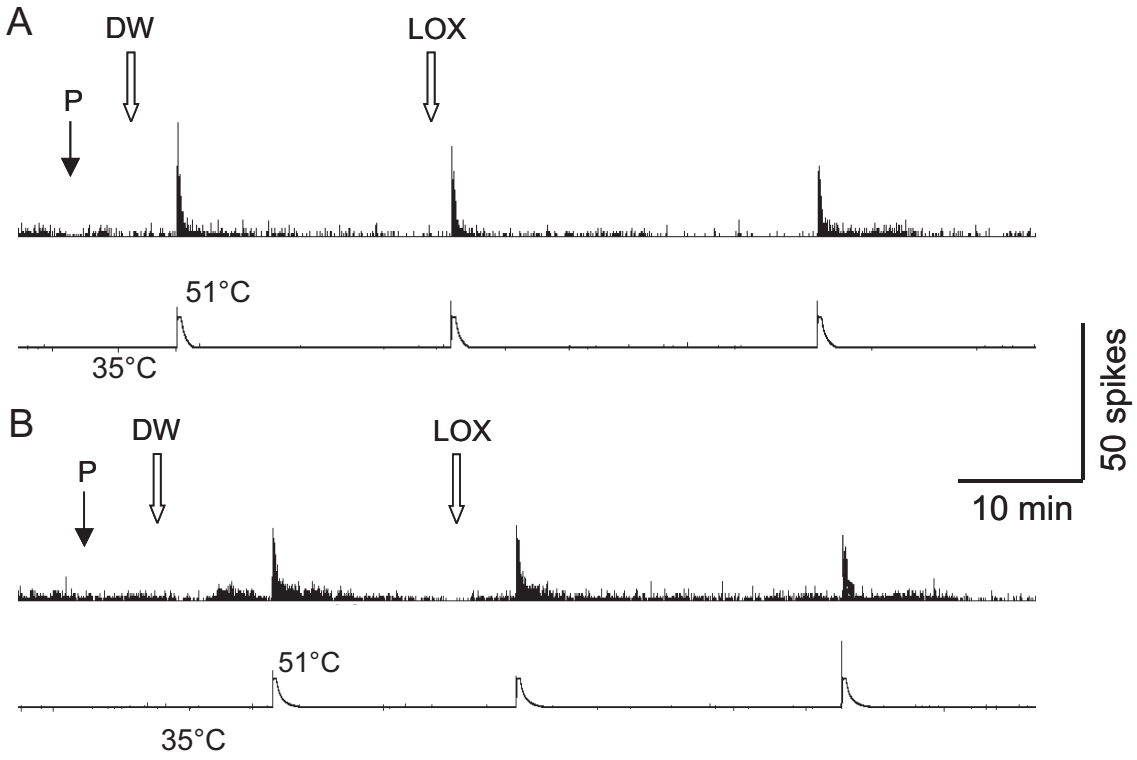

Fig. 3. An example of effects of either DW or LOX (1 mg/kg) on background activity and heat-evoked responses in rats non-treated with carrageenan. Rate histograms (0.5 s bin width) were constructed for 1 h following DW administration. **(A)** Rate histograms from a WDR neuron in which pinch-induced inhibition was observed. **(B)** Rate histograms from an HT neuron in which pinch-induced inhibition was not observed. DW – distilled water, LOX – loxoprofen sodium, P – pinch stimuli applied to the face skin

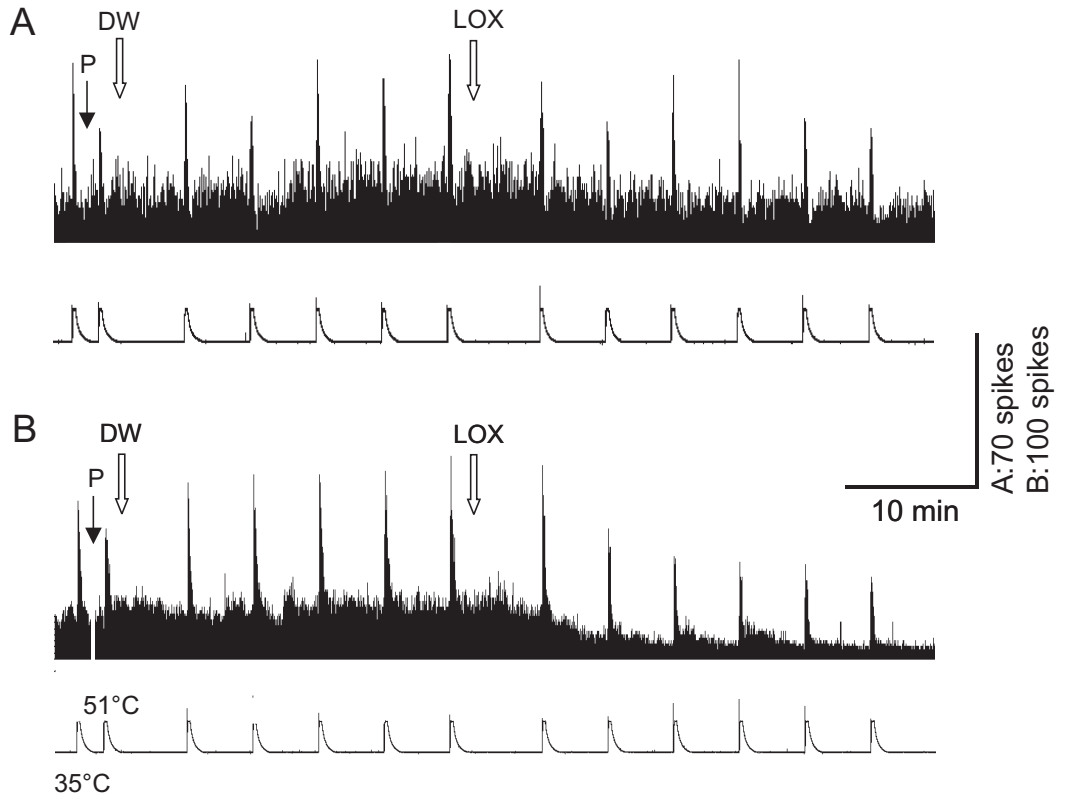

Fig. 4. An example of effects of either DW or LOX (1 mg/kg) on background activity and heat-evoked responses. Rate histograms (0.5 s bin width) were constructed for 1 h following DW administration. **(A)** Rate histograms from an HT neuron in which pinch-induced inhibition was not observed. **(B)** Rate histograms from a WDR neuron in which pinch-induced inhibition was observed. DW – distilled water, LOX – loxoprofen sodium, P – pinch stimuli applied to the face skin

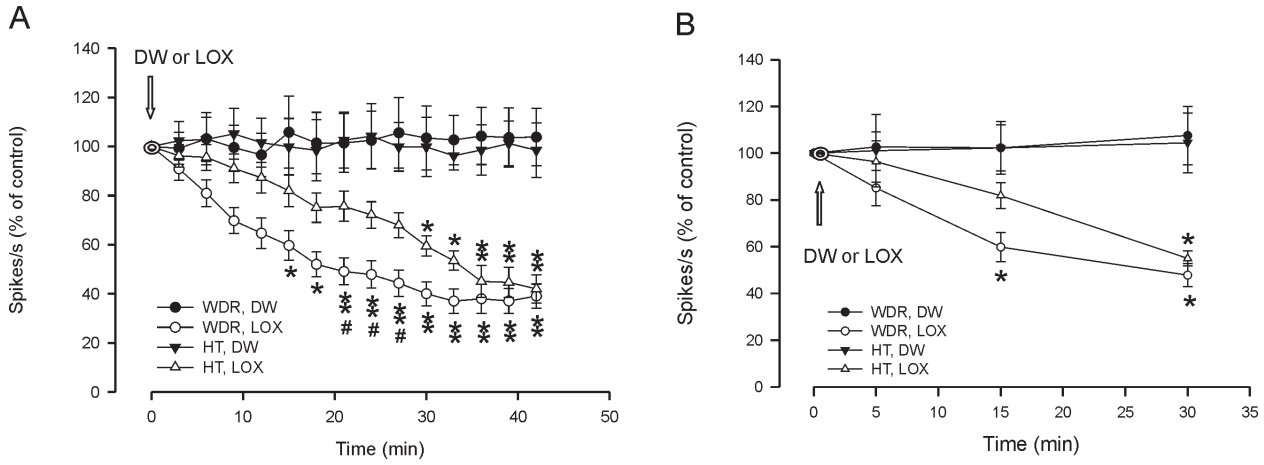

Fig. 5. Graphs summarize antinociceptive effects with LOX (1 mg/kg) on background activities **(A)** and heat-evoked responses **(B)** in HT (n = 10) and WDR (n = 28) neurons. Background activities and heat-evoked responses following administration of either DW or LOX are expressed as a percentage of the control. In **(A)** and **(B)**, 100% (control) were discharges before administration of either DW or LOX. Background activities were averaged for every 3 min and plotted every 3 min in the graph. For example, the mean background activity for 0–3 min was plotted at 3 min of the time scale (abscissa) in the graph. Note that the onset of the antinociceptive effect of LOX was significantly different between HT and WDR neurons. DW – distilled water, LOX – loxoprofen sodium. * $p < 0.05$, ** $p < 0.01$, significantly different from the control. # $p < 0.05$, significantly different between the values of HT and WDR neurons

the graph. For example, the mean background activity for 0–3 min was plotted at 3 min of the time scale (abscissa) in the graph. Administration of distilled water did not produce any change in the background activity of either HT or WDR neurons. Following administration of LOX, inflammation-increased background activity of HT neurons (n = 10) gradually decreased, and a significant difference was observed at 27–30 min after LOX administration when compared with the value before LOX administration [$F(10, 99) = 0.97$, $p = 0.04$]. In contrast, LOX quickly reduced inflammation-increased background activity of WDR neurons (n = 28). There was a significant difference at 12–15 min following LOX administration [$F(10, 286) = 2.29$, $p = 0.03$]. At 18–27 min after LOX administration, the decreased background activity of WDR neurons was significantly different from that of HT neurons, although there was no significant difference between the HT and the WDR neurons in background activity after 27 min (Fig. 5A). LOX significantly reduced heat-evoked responses in HT neurons at 30 min after LOX administration [$F(3, 22) = 1.76$, $p = 0.04$], while heat-evoked responses in WDR neurons were significantly decreased already at 15 min after LOX administration [$F(3, 36) = 2.91$, $p = 0.04$] (Fig. 5B).

Discussion

In the present study, we showed for the first time that the onset of inhibitory effects of LOX was different between HT and WDR neurons in the dorsal horn. The onset of the inhibitory effect of LOX on WDR neurons was observed earlier than that on HT neurons. Because no significant difference in the maximal inhibition was observed between HT and WDR neurons, this different time-course of inhibition of HT or WDR neuronal responses was not due to different maximal inhibition. Maixner et al. [12] have reported in monkeys that WDR neurons, but not HT neurons, are involved in the encoding process by which monkeys perceive the intensity of noxious heat stimuli near the detection threshold. This suggests that WDR neurons, but not HT neurons, participate in the processing of painful sensation. In this context, the present result seems to suggest that following administration of LOX, the suppression of painful sensation precedes the improvement of other events accompanying inflammation including emotional and autonomic changes.

Unfortunately, at the present time, we cannot explain the mechanisms underlying the difference between HT and WDR neurons in the onset of inhibitory effect of LOX. The following two possible mechanisms, however, may be considered. First, LOX inhibits prostaglandin synthesis by blocking cyclooxygenase at the site of inflammation [1, 3, 4, 7, 8, 14, 19, 20]. A reduction in quantity of prostaglandins results in the reduction of frequency of nociceptive impulses conducted *via* primary afferent fibers from the site of inflammation to the spinal dorsal horn. However, it is unlikely that the action of LOX on the intensity coding of primary afferent fibers differs between primary afferent fibers that terminate at HT neurons and those that terminate at WDR neurons. It is reasonable to conclude that the difference is related to the specific patterns of synaptic connections within the dorsal horn and to the properties of HT and WDR neurons themselves. Willis and Coggeshall [21] have hypothesized that nociceptive afferent fibers excited by inflammation release excitatory amino acids and peptides, including substance P and calcitonin gene-related peptide, into the dorsal horn, and that these substances cause a sensitization of dorsal horn neurons. In this context, it is likely that the difference in the onset of the inhibitory effect with LOX is due to the difference in the degree of sensitization between HT and WDR neurons. Regarding another mechanism, one behavioral study has suggested that activation of descending serotonergic pathways is involved as a complementary mechanism of antinociception for NSAIDs [13]. This finding generates the idea that administration of LOX activates serotonergic pathways descending from supraspinal structures. The difference in the onset of the inhibitory effect of LOX between HT and WDR neurons might be related to a difference in inhibitory action of descending serotonergic pathways.

Interestingly, heat-evoked responses in rats without inflammation significantly decreased both at HT and WDR neurons when LOX was administered, whereas background activities did not significantly change. A similar result has been shown in a behavioral study using mice. Several NSAIDs, administered either *via ip* or *it* route, were capable of inducing antinociception in two acute pain assays, the writhing test and the tail-flick test [13]. These results may be explained by findings reported by Pitcher and Henry [16, 17] in

which NSAIDs were shown to be more effective in nociceptive types of pain characterized by time or prolonged inputs to primary afferents.

The present study reports that LOX profoundly inhibits nociceptive activity of dorsal horn neurons during inflammation, and that the onset of the inhibitory effect of LOX is significantly different between HT and WDR dorsal horn neurons. Although the mechanisms underlying the difference between HT and WDR neurons in the onset of the inhibitory effect of LOX are unknown at the present time, the present finding may contribute to a better understanding of a possible role of HT and WDR neurons in nociceptive processing under an abnormal pain state, such as inflammation.

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