Effects of Morphine and Indomethacin on Evoked Neuronal Responses of Ventrobasal Thalamic Neurones: Site of Action of Analgesic Drugs in Adjuvant Arthritic Rats

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Abstract—A single neuronal activity was recorded extracellularly in the ventrobasal (VB) nucleus of the thalamus in adjuvant arthritic rats under urethane anesthesia (1200 mg/kg, i.p.). The effects of morphine and indomethacin on the evoked responses elicited by noxious stimuli (transcutaneous electrical stimulation and tibial nerve electrical stimulation) and/or non-noxious stimuli were investigated. Intravenous administration of morphine and indomethacin depressed the evoked responses elicited by either transcutaneous electrical stimulation or tibial nerve electrical stimulation without affecting any background activities. By contrast, the responses of all neurones investigated responding to non-noxious stimuli were never depressed by the intravenous administration of morphine and indomethacin. Morphine showed the same depressant effects on the evoked responses activated by both noxious stimuli, but the depressant effects due to indomethacin on the evoked discharges were more sensitively produced by transcutaneous electrical stimulation than tibial nerve electrical stimulation. Depressant effects of morphine were restored by intravenous naloxone administration, but not observed in case of indomethacin. These results suggest that an analgesic mechanism of morphine and indomethacin may reside in the neo-spinothalamic projection system of adjuvant arthritic rats and that the site of action of indomethacin may also reside in a peripheral site. However, the mode of the central action of indomethacin was different from that of morphine.

For evaluating analgesic potency, adjuvant induced polyarthritis in rats has been widely used as a model of chronic inflammatory pain in laboratory animals (1-6).

Using this model, we observed (6) that nociceptive reactions such as vocalization responses were produced by weaker electrical stimuli in freely moving adjuvant arthritic rats than in normal rats. Despite the high sensitivity of adjuvant arthritic rats to pain, the vocalization response was inhibited by a wide variety of analgesic drugs. Especially, the analgesic effects of orally and/or intracerebroventricularly (icv) administered acidic NSAIDs were roughly equivalent to that of subcutaneously and/or icv administered narcotic analgesic drugs (7). It is of interest that some acidic NSAIDs are comparable to some narcotic analgesic drugs in potency in adjuvant arthritic rats. Furthermore, the pain-relieving mechanisms of acidic NSAIDs may reside in both central and peripheral sites.

Recently, using adjuvant arthritic rats, Gautron and Guilbaud (8) showed that the nociceptive-specific neurones with a restricted receptive field existed in the VB thalamus. Moreover, the evoked responses of the VB nociceptive neurones in adjuvant arthritic rats were depressed by the intravenous administration of aspirin (9) and morphine (10). Hereafter, it was thought that the VB thalamic neurones of adjuvant arthritic rats would be applicable to electrophysiological models for studying chronic
inflammatory pain and for various pharmacological approaches in laboratory animals.

The purpose of this investigation was two fold; first, to examine the effects of morphine (narcotic analgesic drugs) and indomethacin (acidic NSAIDs) on the VB thalamic neurones of adjuvant arthritic rats and second, to ascertain whether the mode of action of indomethacin is different from that of morphine.

Materials and Methods

A total of 121 male Sprague-Dawley rats weighing 140–160 g at the time of Mycobacterium butyricum (adjuvant, Difco Laboratories, Detroit, MI) injection were housed in an air-conditioned room at 22±1 °C with a 12 hr light-dark schedule (light on at 7:00). Food and water were given ad libitum during the experimental period.

The rats were injected intradermally at the base of the tail with 0.1 ml of a paraffin oil suspension of the heat-killed adjuvant (0.5 mg). The animals were used for electrophysiological studies between the 15th and 25th day after the adjuvant injection.

Surgical preparation and electrophysiological recording

The animal was anesthetized with urethane (1200 mg/kg, i.p.) and was placed in a stereotaxic head holder (Narishige). The body temperature was maintained between 37°C and 38°C by using a heating pad controlled from a rectal thermistor (KN-474, Natsume).

A small hole was made in the skin and skull (3 mm x 3 mm), and the dura mater was carefully removed. The area surrounding the wound was sprayed with xylocaine. The electrodes were stereotaxically placed in the VB thalamus, according to the atlas of Albe-Fessard et al. (11) (A 4.8–5.6, L 2–3.5, H +4–+6). Recordings were made using glass micropipette electrodes filled with a mixture of 0.5 M sodium acetate and 2% pontamine sky blue. The electrodes had an initial resistance of 5–10 MΩ. At the end of each experiment, the recording sites were marked with a dye. The animal was sacrificed under deep pentobarbital anesthesia and perfused with 10% formalin. Frozen 50 μm thick sections of the whole brain were cut using a freezing microtome (MA-101, Komatsu) and stained with hematoxylin and eosin. The recording sites were verified using a microscope (Fig. 1).

Techniques of peripheral stimulation

1. Noxious stimulation

1) Transcutaneous electrical stimulation: In 45 neurones, effects of various doses of morphine (0.1, 0.3 and 1 mg/kg) and indomethacin (0.1, 0.3 and 1 mg/kg) were tested on the evoked responses elicited by transcutaneous electrical stimulation of 45 adjuvant arthritic rats. A transcutaneous electrical stimulation was delivered through a pair of stainless-steel needles (1/4) inserted through the subplantar pad of the contralateral hind paw to the recording sites. One Hz rectangular pulse of 1 msec duration and 0.5–5 mA intensity was used.

2) Tibial nerve electrical stimulation: In 57 neurones, effects of various doses of morphine (0.1, 0.3 and 1 mg/kg) and indomethacin (0.3, 1 and 3 mg/kg) were tested on the evoked responses elicited by tibial nerve electrical stimulation of 57 adjuvant arthritic rats. The contralateral tibial nerve of the hind paw was severed and allowed to soak in warm paraffin (38°C). The nerve was placed on a pair of platinum hook electrodes (3–4 mm apart) and centrally stimulated. One Hz rectangular pulse of 0.5 msec duration and 0.1–0.8 mA intensity was applied to the nerve.

In an additional 11 adjuvant arthritic rats,
11 neurones responding to tibial nerve electrical stimulation were subjected to examine the effect of naloxone (0.5 mg/kg) on the depressant action of morphine (1 mg/kg, n=6) and indomethacin (3 mg/kg, n=5).

Each of the electrical stimulations were delivered for 30 sec every 5–10 min.

2. Non-noxious stimulation

In 5 neurones, the effects of morphine (1 mg/kg, n=3) and indomethacin (3 mg/kg, n=2) were tested on the responses elicited by non-noxious stimuli in 5 adjuvant arthritic rats.

Non-noxious stimuli such as brushing or tapping were applied to the inflamed area of the contralateral hind paw by a hairbrush.

Data analysis

Single unit activities were monitored on an oscilloscope (VC-10, Nihon Kohden) and converted to a uniform voltage pulse by a window discriminator (DSE-325P, Dia Medical). The pulses were integrated at 1 sec epochs and displayed on an ink-writing oscilloscope (WT-645G, Nihon Kohden).

The degree of the evoked responses to noxious stimuli was expressed as the total number of spikes generated in response to each application of noxious stimuli. The evoked activities were analyzed by measuring the total number of spikes produced during noxious stimulation from which the number of spikes emitted during an equivalent period before the noxious stimulation were subtracted. Electrophysiological recordings were continued for at least 60 min after the drug administration.

The % inhibition was determined by comparing the pre- and post-drug values. The dose that produced a 50% reduction (ED50 values) was calculated from the % inhibition.

Drugs

The drugs used were morphine hydrochloride (Shionogi), naloxone hydrochloride (Endo), indomethacin (Sigma), ethyl carbamate (urethane, Wakou), xylocaine spray (Fujisawa) and pentobarbital sodium (nembutal, Dainippon). Morphine (0.1–1 mg/kg), naloxone (0.5 mg/kg) and urethane (1200 mg/kg) were dissolved in saline solution. Indomethacin (0.1–3 mg/kg) was dissolved in a 0.1 M Tris buffer with a pH of 7.9. Morphine, indomethacin, naloxone were slowly injected intravenously. The injection volume of all solutions was 0.2 ml/100 g of body weight.

Results

In the VB thalamus of adjuvant arthritic rats, 175 neurones activated by only noxious stimuli were isolated. One-third of them (57/175) showed spontaneous 'paroxysmal' discharges which could not be distinguished from the evoked responses. These neurones were omitted from this pharmacological investigation. All VB neurones displayed a long-lasting afterdischarge. The mean duration of these afterdischarges calculated from 41 neurones with responses to electrical stimulation of 30 sec duration was 92.1 ±12.8 sec (n=41). No clear relationship was established between these discharge rates and the degree of arthritis. The rates of the background activities of these neurones ranged between 0 and 5 Hz (m=1.2±0.1 Hz, n=41). Most neurones isolated were located in the lateral portions of the VB thalamus where the frontal planes were between A 4.8 and A 5.6 in the atlas of Albe-Fessard et al. (11).

Effect of morphine and indomethacin on the responses of nociceptive neurones

During the application of electrical stimulation, the evoked discharges of the nociceptive neurones increased progressively and reached their maximal values after a certain delay.

1) Transcutaneous electrical stimulation:

The mean latency of the evoked responses calculated from 18 nociceptive neurones was 154.6±17.9 msec (n=18). The mean threshold values of the electrical stimulation calculated from 45 neurones were 2.3±0.1 mA (n=45).

At the dose of 1 mg/kg, i.v., morphine (Fig. 2) markedly depressed the evoked responses in all 8 neurones (88.79%, P<0.001, n=8). The same dose of indomethacin (Fig. 3) also depressed the evoked responses in 8 of the 9 neurones (70.75%, P<0.01, n=9). The depressant effect of morphine became apparent from 5 min after the administration, and the maximal effect was seen between 5 and 15 min. The depressant effect of indomethacin was
Fig. 2. 1: Effect of morphine (1 mg/kg) on the responses of the VB neurones elicited by transcutaneous electrical stimulation (2 mA) in adjuvant arthritic rats. a: control, b: 15 min after, c: 90 min after the administration of morphine. 2: Recordings from neurones in the VB thalamus showing a maximal firing rate at each time (a, b, c). "2" correspond to those "1". Arrows point to stimulus artifacts. Right inset shows the location of the neurone.

Fig. 3. 1: Effect of indomethacin (1 mg/kg) on the responses of the VB neurones elicited by transcutaneous electrical stimulation (2 mA) in adjuvant arthritic rats. a: control, b: 30 min after, c: 120 min after the administration of indomethacin. 2: Recordings from neurones in the VB thalamus showing a maximal firing rate at each time (a, b, c). "2" correspond to those "1". Arrows point to stimulus artifacts. Right inset shows the location of the neurone.
observed 5 min after the administration. In spite of the variability in the time course of the depressant effect, its maximal effect was observed between 20 and 30 min after administration.

The effects of lower doses of morphine (0.1 and 0.3 mg/kg) and indomethacin (0.1 and 0.3 mg/kg) on the responses of the VB nociceptive neurones were examined. As shown in Table 1, morphine depressed the evoked responses of VB neurones in a dose-dependent manner. The intravenous administration of indomethacin at 0.1 mg/kg failed to depress the evoked responses of the VB nociceptive neurones in all the cases examined (22.90%, NS, n=6). With 0.3 mg/kg, the depressant effect of indomethacin was observed in 4 of the 8 neurones (53.59%, P<0.01, n=8).

The ED50 values of morphine and indomethacin on the evoked responses to transcutaneous electrical stimulation were 0.22 mg/kg and 0.32 mg/kg, respectively (Table 2).

2) Tibial nerve electrical stimulation: The mean latency of the evoked responses calculated from 15 nociceptive neurones was 136.0±13.0 msec (n=15). The mean threshold values of the electrical stimulation were 0.58±0.05 mA (n=57).

As shown in Fig. 4 and Table 1, the evoked responses elicited by tibial nerve electrical

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg, i.v.)</th>
<th>Mode</th>
<th>N</th>
<th>Peak time (min)</th>
<th>Total number of spikes</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pre-drug</td>
<td>Post-drug</td>
</tr>
<tr>
<td>Morphine</td>
<td>0.1</td>
<td>TES</td>
<td>7</td>
<td>5</td>
<td>643.1±141.9</td>
<td>433.6±139.3**</td>
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<tr>
<td></td>
<td>0.3</td>
<td>TES</td>
<td>7</td>
<td>15</td>
<td>553.8±112.2</td>
<td>276.1±96.6*</td>
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<td></td>
<td>1.0</td>
<td>TES</td>
<td>8</td>
<td>15</td>
<td>620.1±108.1</td>
<td>69.5±43.0***</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>TNES</td>
<td>9</td>
<td>5</td>
<td>619.4±94.0</td>
<td>407.8±85.4*</td>
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<td>15</td>
<td>575.3±99.8</td>
<td>244.6±80.3*</td>
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<td></td>
<td>1.0</td>
<td>TNES</td>
<td>9</td>
<td>15</td>
<td>582.8±90.2</td>
<td>101.4±42.9**</td>
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<tr>
<td>Indomethacin</td>
<td>0.1</td>
<td>TES</td>
<td>6</td>
<td>20</td>
<td>547.1±103.4</td>
<td>421.8±101.2</td>
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<tr>
<td></td>
<td>0.3</td>
<td>TES</td>
<td>8</td>
<td>30</td>
<td>614.3±105.9</td>
<td>285.1±60.0**</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>TES</td>
<td>9</td>
<td>30</td>
<td>584.0±101.7</td>
<td>170.8±43.0**</td>
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<tr>
<td></td>
<td>0.3</td>
<td>TNES</td>
<td>8</td>
<td>20</td>
<td>582.8±100.4</td>
<td>369.9±92.0</td>
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<tr>
<td></td>
<td>1.0</td>
<td>TNES</td>
<td>10</td>
<td>30</td>
<td>415.5±87.3</td>
<td>180.7±54.0**</td>
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<tr>
<td></td>
<td>3.0</td>
<td>TNES</td>
<td>14</td>
<td>30</td>
<td>538.2±62.5</td>
<td>129.1±40.5***</td>
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</tbody>
</table>

102 neurones were recorded in 102 adjuvant arthritic rats. TES: transcutaneous electrical stimulation (m=2.3±0.1 mA, n=45). TNES: tibial nerve electrical stimulation (m=0.58±0.05 mA, n=57). N: number of neurones. Significantly different from pre-drug values. **P<0.05. ***P<0.01. ****P<0.001. t-test, paired samples.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Mode</th>
<th>ED50 (95% C.L.) mg/kg, i.v.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>TES</td>
<td>0.22 (0.08–0.63)</td>
</tr>
<tr>
<td></td>
<td>TNES</td>
<td>0.21 (0.08–0.53)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>TES</td>
<td>0.32 (0.11–0.93)</td>
</tr>
<tr>
<td></td>
<td>TNES</td>
<td>0.66 (0.23–1.91)</td>
</tr>
</tbody>
</table>

See Table 1 explanation. The ED50 values and 95% confidence limits (C.L.) were calculated from the % inhibition.
stimulation were markedly depressed by morphine (1 mg/kg, Fig. 4-1) and indomethacin (3 mg/kg, Fig. 4-2). The time course of the depressant effects of morphine and indomethacin delivered by tibial nerve electrical stimulation was similar to that of transcutaneous electrical stimulation. The depressant effects of morphine (0.1 and 0.3 mg/kg) and indomethacin (0.3 and 1 mg/kg) were observed in a dose-dependent manner, as shown in Table 1.

The ED50 values of morphine and indomethacin on the evoked discharges responding to tibial nerve electrical stimulation were 0.21 mg/kg and 0.66 mg/kg, respectively (Table 2).

At a dose of 3 mg/kg indomethacin, background activities of three of the 14 neurones were depressed, although the depression was slight and was not observed by morphine and other doses of indomethacin administrations.

Vehicle injection never induced any significant modification of the responses. The depressant effects of the morphine and indomethacin on nociceptive neurones are summarized in Table 1.

**Effect of naloxone on the depressant action of morphine and indomethacin**

The effect of intravenous administration of naloxone at 0.5 mg/kg was tested for the depressant effects of morphine (1 mg/kg, n=6) and indomethacin (3 mg/kg, n=5). Naloxone was administered 15 min to 30 min after the morphine or indomethacin administration.

The evoked discharges of 6 neurones depressed by morphine were restored by naloxone administration, although complete restoration to the control value was not observed. The initial responses were rapidly recovered to almost the control value (Fig. 5-1, Table 3).

In the 5 neurones, naloxone failed to reduce the depressant effect of indomethacin in every case (Fig. 5-2, Table 3).

**Effect of morphine and indomethacin on the responses of non-nociceptive neurones**

Evoked responses of non-nociceptive neurones activated by repetitive brushing never depressed following morphine (1 mg/kg, n=3) and indomethacin (3 mg/kg, n=2) administrations.
Fig. 5. 1: Effects of morphine (1 mg/kg) and naloxone (0.5 mg/kg) on the responses of the VB neurone elicited by tibial nerve electrical stimulation (0.6 mA). Following naloxone (0.5 mg/kg) administration, the depressant effect was reversed. 2: Effects of indomethacin (3 mg/kg) and naloxone (0.5 mg/kg) on the responses of the VB neurone elicited by tibial nerve electrical stimulation (0.5 mA). Following naloxone (0.5 mg/kg) administration, the depressant effect was not reversed.

Table 3. Effect of naloxone on the depressant action of morphine and indomethacin

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg, i.v.)</th>
<th>N</th>
<th>Pre-drug</th>
<th>Post-drug</th>
<th>Post-naloxone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>1</td>
<td>6</td>
<td>636.6±135.1</td>
<td>85.5±55.5**</td>
<td>543.5±71.2##</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>3</td>
<td>5</td>
<td>631.8±135.4</td>
<td>106.2±32.6**</td>
<td>123.8±57.3</td>
</tr>
</tbody>
</table>

In 11 adjuvant arthritic rats, 11 neurones responding to tibial nerve electrical stimulation were tested. Naloxone (0.5 mg/kg, i.v.) was administered 15 min to 30 min after morphine and indomethacin administrations. N: number of neurones. Significantly different from pre-drug values. **P<0.01, t-test, paired samples. ##P<0.01, t-test, paired samples.

Discussion

In the VB thalamic neurones of the adjuvant arthritic rats, numerous neurones excited by two types of noxious stimulation (transcutaneous electrical stimulation, tibial nerve electrical stimulation) were isolated, and they displayed a long-lasting afterdischarge. In this respect, unanesthetized adjuvant arthritic rats showed nociceptive reactions such as vocalization responses when transcutaneous electrical stimulation was delivered (S. Okuyama and H. Aihara, unpublished observation). The mean latency of the evoked responses elicited by transcutaneous electrical stimulation (m=154.6±17.9 msec, n=18) was also similar to that by tibial nerve electrical stimulation (m=136.0±13.0 msec, n=15). However, the animals did not show the vocalization responses when brushing or tapping was applied to the inflamed area of the hind paws. Therefore, we conclude that transcutaneous electrical stimulation and tibial nerve electrical stimulation were noxious, while brushing and tapping were non-noxious.

Gautron and Guilbaud (8) reported that in the VB complex of adjuvant arthritic rats,
there existed numerous neurones excited by noxious stimuli (joint movement and/or mild pressure) which were characterized with a long-lasting afterdischarge. They dealt with these long-lasting afterdischarges as resulting from one of peripheral inflammatory processes of sensitization phenomena which were produced by the presence of pain-producing substances either in the joint cavity or in the skin overlying the articulation. In the present study, however, a long-lasting afterdischarge was also observed by tibial nerve electrical stimulation (see Methods). Therefore, the long-lasting afterdischarges of these neurones may result in the central neuronal circuitry.

A relatively large number of nociceptive neurones displayed a spontaneous 'paroxysmal' discharge. This phenomenon may be related to well-known clinical observations that in rheumatoid disease in humans, flashes of pain can appear spontaneously.

In the present study, using two types of noxious stimuli such as transcutaneous electrical stimulation (which is mediated by peripheral cutaneous nociceptors, primary afferent and central pain pathways) and tibial nerve electrical stimulation (which is mediated by primary afferent and central pain pathways), we have observed the effects of various doses of morphine and indomethacin on the evoked responses of VB thalamic nociceptive neurones in adjuvant arthritic rats. The depressant effects of morphine and indomethacin on the evoked responses elicited by both noxious stimuli were observed in a dose-dependent manner without affecting any background activities. However, the responses of non-nociceptive neurones failed to be depressed by morphine and indomethacin. Our present results confirmed and extended the previous data presented by Guilbaud et al. (9) and Kayser et al. (10), who described that the evoked responses of VB nociceptive neurones in adjuvant arthritic rats were depressed by intravenous administered aspirin and morphine.

The present results showing the depressant effect of drugs on the evoked responses elicited by direct stimulation of tibial nerve indicate that this effect of drugs is produced by the central antinociceptive actions. Morphine showed the same depressant effect on the evoked responses elicited by either transcutaneous electrical stimulation or tibial nerve electrical stimulation. By contrast, the depressant effect of indomethacin was more greatly produced by the transcutaneous electrical stimulation than the tibial nerve electrical stimulation. These findings emphasize that sites of action of indomethacin reside in both peripheral and central ones. This was further supported by our previous findings (7) that the icv and/or systemically administered indomethacin produced a potent analgesic action in adjuvant arthritic rats.

Guzman et al. (12–14), using the cross-perfused spleen technique, have demonstrated that acidic NSAIDs act peripherally at the chemonociceptors and that narcotic analgesic drugs affect the central pain pathways. Although the precise mechanism of the action of acidic NSAIDs is still unknown, it is generally accepted that their analgesic properties are related to the blockade of prostaglandins (PGs) biosynthesis induced by inflammation (15–17). According to Ferreira et al. (16), the concept of inflammatory pain, hyperalgesia induced by inflammation, has a peripheral and a central component. Acidic NSAIDs may exert an analgesic effect by preventing the hyperalgesia induced by a peripheral and/or a central release of PGs. Some acidic NSAIDs, indomethacin, naproxen, ibuprofen and flurbiprofen, that are clinically effective analgesic drugs are known to inhibit PGs biosynthesis in the CNS (18, 19). In addition, it was reported that PGs were released from the CNS following peripheral nerve stimulation (20, 21), and they had an excitatory effect on the dorsal horn interneurones (22).

The depressant effect of indomethacin fail to be attenuated by the opiate antagonist naltroxone. Furthermore, our previous study suggested (6, 7) that adjuvant induced chronic inflammatory pain might be related to the PGs biosynthesis. Therefore, it would be reasonable to assume that antinociceptive effect of indomethacin in adjuvant arthritic rats is mainly due to the inhibition of PGs biosynthesis at a central level as well as a peripheral level.

Our results indicating that morphine de-
pressed the noxious responses of the VB thalamic neurones and its depressant action was reversed by naloxone are in good accordance with the results that morphine depressed noxious evoked responses of neurones in the dorsal horn (23–25), spinal trigeminal nucleus (26), reticular formation (27–29), and reduced the afferent volley in the ventrolateral tract (30).

The results of the present study demonstrate that the neuronal responses of the VB thalamic neurones in adjuvant arthritic rats are an appropriate model for studying the sites of action of analgesic drugs responsible for the clinical efficacy.

References

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