EFFECTS OF MORPHINE AND INDOMETHACIN ON EVOKED NEURONAL RESPONSES OF VENTROBASAL THALAMIC NEURONES: SITE OF ACTION OF ANALGESIC DRUGS IN NON-ADJUVANT ARTHRITIC RATS

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Single neuronal activity was recorded extracellularly in the ventrobasal (VB) nucleus of the thalamus in non-adjuvant arthritic rats under urethane (1200 mg/kg, i.p.) anesthesia. The effects of morphine and indomethacin on the evoked responses elicited by noxious stimuli such as transcutaneous electrical stimulation (TES) or tibial nerve electrical stimulation (TNES), or non-noxious stimulation such as repetitive brushing were examined. Intravenous administered morphine (0.1, 0.3 and 1 mg/kg) depressed the evoked responses elicited by either TES or TNES without affecting any background activities. In contrast, intravenous administered indomethacin (1, 3 and 10 mg/kg) depressed the evoked responses induced by TES, but failed to depress the evoked responses induced by TNES. At doses of 3 and 10 mg/kg, indomethacin slightly depressed the background activities of the nociceptive neurones. Depressant effects of morphine were restored by intravenous naloxone (0.5 mg/kg) administration, but not observed in case of indomethacin. The evoked responses induced by non-noxious stimulation failed to depress either indomethacin or morphine administration. These results suggest that the site of action of indomethacin in non-adjuvant arthritic rats is mainly in the periphery. In contrast, morphine produced an antinociceptive action due to the central mechanism.

Keywords — non-adjuvant arthritic rat; ventrobasal thalamic neurone; noxious stimulation; nociceptive neurone; non-noxious stimulation; non-nociceptive neurone; morphine; indomethacin; naloxone

INTRODUCTION

The original suggestion of Mitchell and Hellon indicate that the nociceptive-specific neurones with a restricted receptive field existed in the ventrobasal (VB) thalamus of non-adjuvant arthritic rats. Recently, Guibaud et al. and Peschanski et al. have shown that a large number of the VB neurones in non-adjuvant arthritic rats were driven exclusively by peripheral noxious mechanical, thermal and visceral stimuli and some of responses encoded an intensity of applied noxious heat. Moreover, Benoist et al. reported that the evoked responses of the VB thalamic neurones to noxious stimuli were depressed by intravenously administered morphine in non-adjuvant arthritic rats.

In our previous studies, using two types of noxious stimuli such as transcutaneous electrical stimulation (TES; which is mediated by peripheral cutaneous nociceptors, primary afferents and central pain pathways) and tibial nerve electrical stimulation (TNES; which is mediated by primary afferents and central pain pathways), we have observed the selective depressant actions of intravenous administered morphine and indomethacin on the evoked responses of the nociceptive neurones in adjuvant arthritic rats. Moreover, our results suggest that an antinociceptive mechanisms 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 the peripheral site.

In the present study, the effects of morphine and indomethacin on the neuronal responses of the VB thalamic neurones in non-adjuvant arthritic rats were investigated under the same experimental conditions as in adjuvant arthritic rats.

MATERIALS AND METHODS

*Animals* — A total of 113 male Sprague-Dawley rats weighing 200–240 g were used. Food and water were given *ad libitum* during the experimental period.

*Surgical Preparation and Electrophysiological Recording* — The animal was anesthetized with urethane (1200mg/kg, *i.p.*) and was placed in a stereotaxic head-holder (Narishige). The body temperature was maintained between 37 and 38 °C by using a heating pad controlled with a rectal thermistor (KN-474, Narume). A small hole was made in the skin and skull (3 mm × 3 mm) and the dura mater was carefully removed. The area surrounding the wound was sprayed with 8% xylocaine. The electrodes were stereotaxically placed in the VB thalamus, according to the atlas of Albe-Fessard *et al.* (A: 48–56, L: 2–3.5, H: +4–+6). Recordings were made using glass micropipette electrodes (impedance 5–10 MΩ) filled with a mixture of 0.5 M sodium acetate and 2% pontamine sky blue. 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.

*Noxious Stimulation* — (1) TES: Of 58 neurones, the effects of various doses of morphine (0.1, 0.3 and 1 mg/kg) and indomethacin (1, 3 and 10 mg/kg) were tested on the evoked responses elicited by TES in 58 non-adjuvant arthritic rats. A TES was delivered through a pair of stainless-steel needles (outer diameter: 0.5 mm) inserted into the subplantar pad of the contralateral hind paw to the recording sites. One Hz rectangular pulse of 1 ms duration and 2–10 mA intensity was used.

(2) TNES: Of 42 neurones, the effects of various doses of morphine (0.1, 0.3 and 1 mg/kg) and indomethacin (1, 3 and 10 mg/kg) were tested on the evoked responses elicited by TNES in 42 non-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 ms duration and 0.2–2 mA intensity was used.

In an additional 9 non-adjuvant arthritic rats, 9 neurones responding to TES were subjected to examine the effect of naloxone (0.5 mg/kg) on the depressant action of morphine (1 mg/kg, *n* = 5) and indomethacin (10 mg/kg, *n* = 4).

Each of the electrical stimulation was delivered for 30 s every 5–10 min.

*Non-noxious Stimulation* — Of 4 neurones, the effects of morphine (1 mg/kg, *n* = 2) and indomethacin (10 mg/kg, *n* = 2) were tested on the responses elicited by non-noxious stimulation in 4 non-adjuvant arthritic rats. Non-noxious stimulation such as repetitive brushing was applied to 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 s 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 analysed 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 producing a 50% reduction (ED$_{50}$ value) was calculated from the % inhibition.

Drugs — The drugs used were morphine hydrochloride (Shionogi), indomethacin (Sigma), naloxone hydrochloride (Endo), ethyl carbamate (urethane, Wakou), xylocaine spray (Fujisawa) and pentobarbital-Na (nembutal, Dainippon). Morphine (0.1—1 mg/kg) and naloxone (0.5 mg/kg) were dissolved in a saline solution. Indomethacin (1—10 mg/kg) was dissolved in a 0.1 M Tris buffer with pH of 7.9. Morphine, indomethacin and naloxone were slowly injected intravenously (0.2 ml/100 g body weight).

RESULTS
In VB thalamus of non-adjuvant arthritic rats 109 neurones activated by only noxious stimuli were isolated. The rates of the background activities of these neurones ranged between 0—5 Hz (m =1.1 ± 0.1 Hz, n =48). Most neurones isolated were located in the lateral portions of the VB thalamus in the frontal planes between A: 4.8 and A: 5.6 in the atlas of Albe-Fessard et al.$^{18}$

1. Effects of Indomethacin and Morphine on the Response of Nociceptive Neurones

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

(1) TES — The mean latency of the evoked responses calculated from 14 nociceptive neurones was 256.6±8.1 ms (n =14). The mean threshold value of the electrical stimulation calculated from 58 neurones was 4.8±0.6 mA (n =58).

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FIG. 1. 1) Effect of Morphine (1 mg/kg, i.v.) on the Evoked Responses of the VB Neurones Elicited by TES (6 mA) in Non-adjuvant Arthritic Rats

2) Effect of Indomethacin (10 mg/kg, i.v.) on the Evoked Responses of the VB Neurones Elicited by TES (4 mA) in Non-adjuvant Arthritic Rats

Right inset shows the location of the neurone.
As shown in Fig. 1, the evoked responses elicited by TES depressed by morphine (1 mg/kg, Fig. 1-1) and indomethacin (10 mg/kg, Fig. 1-2) by 73.02% (p<0.001, n=11) and 57.03% (p<0.001, n=14), respectively. The depressant effect of morphine became apparent 5 min after administration, and the maximal effect was seen between 5 and 10 min. The depressant effect of

**TABLE I. Antinociceptive Action of Morphine and Indomethacin on Neurones in the VB Thalamus of Non-adjuvant Arthritic Rats**

<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>5</td>
<td>5</td>
<td>224.6±35.0</td>
<td>160.4±43.0</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>TES</td>
<td>10</td>
<td>10</td>
<td>228.9±23.5</td>
<td>92.2±26.1</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>TES</td>
<td>11</td>
<td>10</td>
<td>246.1±18.3</td>
<td>66.4±22.7</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>TNES</td>
<td>6</td>
<td>5</td>
<td>217.8±40.0</td>
<td>189.3±37.8</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>TNES</td>
<td>8</td>
<td>10</td>
<td>250.0±26.1</td>
<td>108.4±36.5</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>TNES</td>
<td>12</td>
<td>10</td>
<td>211.5±25.7</td>
<td>53.7±20.5</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>1.0</td>
<td>TES</td>
<td>8</td>
<td>20</td>
<td>235.3±27.2</td>
<td>184.6±30.8</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>TES</td>
<td>10</td>
<td>30</td>
<td>231.2±25.4</td>
<td>153.2±21.7</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>TES</td>
<td>14</td>
<td>30</td>
<td>214.8±15.1</td>
<td>92.3±17.8</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>TNES</td>
<td>3</td>
<td>20</td>
<td>249.3±34.5</td>
<td>227.3±23.9</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>TNES</td>
<td>3</td>
<td>30</td>
<td>252.7±39.3</td>
<td>200.7±49.5</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>TNES</td>
<td>10</td>
<td>30</td>
<td>220.2±24.8</td>
<td>160.0±16.7</td>
</tr>
</tbody>
</table>

100 nociceptive neurones were recorded in 100 non-adjuvant arthritic rats. TES: transcutaneous electrical stimulation (m=48±0.6 mA, n=58). TNES: tibial nerve electrical stimulation (m=1.02±0.11 mA, n=42). n: number of neurones.
a) Significantly different from pre-drug values, p < 0.05, t-test, paired samples.
b) Significantly different from pre-drug values, p < 0.01, t-test, paired samples.
c) Significantly different from pre-drug values, p < 0.001, t-test, paired samples.

**TABLE II. The 50% Inhibitory Doses of Morphine and Indomethacin on the Evoked Responses of Nociceptive neurones in Non-adjuvant Arthritic Rats**

<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>0.24 (0.08 — 0.74)</td>
</tr>
<tr>
<td></td>
<td>TNES</td>
<td>0.34 (0.16 — 0.73)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>TES</td>
<td>6.20 (2.20 — 21.50)</td>
</tr>
<tr>
<td></td>
<td>TNES</td>
<td>&gt; 10.00</td>
</tr>
</tbody>
</table>

See Table I explanation. The ED50 values and 95% confidence limits (C.L.) were calculated from the % inhibition.
indomethacin was observed 5 min after 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 depressant effects of morphine (0.1 and 0.3 mg/kg) and indomethacin (1 and 3 mg/kg) were observed in a dose-dependent manner, as shown in Table I. The ED₅₀ values of morphine and indomethacin on the evoked responses to TES were 0.24 and 6.2 mg/kg, respectively (Table II).

(2) TNES — The mean latency of the evoked responses calculated from 12 nociceptive neurones was 246.3 ± 9.7 ms (n = 12). The mean threshold value of the electrical stimulation was 1.02 ± 0.11 mA (n = 42).

At the dose of 1 mg/kg, the evoked responses were markedly depressed (74.75%, p < 0.001, n = 12) by intravenous-administered morphine (Fig. 2-1). The effects of lower doses of morphine (0.1 and 0.3 mg/kg) were tested. As shown in Table I, morphine depressed the evoked responses in a dose-dependent manner. The time course of the depressant effect of morphine delivered by TNES was similar to that of TES. The ED₅₀ value of morphine on the evoked responses to TNES was 0.34 mg/kg (Table II).

Intravenous indomethacin (1, 3 and 10 mg/kg) failed to depress the evoked responses of the VB nociceptive neurones induced by TNES (Fig. 2-2, Table I).

At indomethacin doses of 3 and 10 mg/kg, the background activities of 2 out of the 13 neurones and that of 8 out of the 24 neurones were depressed, although the degree of depression was slight and was not observed following morphine and other dose of indomethacin administrations.

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**FIG. 2.** 1) Effect of Morphine (1 mg/kg, i.v.) on the Evoked Responses of the VB Neurones Elicited by TNES (0.8 mA) in Non-adjutant Arthritic Rats

2) Effect of Indomethacin (10 mg/kg, i.v.) on the Evoked Responses of the VB Neurones Elicited by TNES (1.2 mA) in Non-adjutant Arthritic Rats

Right inset shows the location of the neurone.
Vehicle injection never induced any significant modification of the responses. The depressant effects of morphine and indomethacin on the nociceptive neurones are summarized in Table I.

2. 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 depres-

![Graph showing effects of morphine and indomethacin on neurones]

FIG. 3. 1) Effects of Morphine (1 mg/kg, i.v.) and Naloxone (0.5 mg/kg, i.v.) on the Responses of the VB Neurones Elicited by TES (5 mA)

Following naloxone administration, the depressant effect was reversed.

2) Effects of Indomethacin (10 mg/kg, i.v.) and Naloxone (0.5 mg/kg, i.v.) on the Responses of the VB Neurones Elicited by TES (4 mA)

Following naloxone administration, the depressant effect was not reversed. Right inset shows the location of the neurone.

<p>| TABLE III. Effect of Naloxone on the Depressant Action of Morphine and Indomethacin. |
|---------------------------------|---------------------------------|-----------------|</p>
<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>5</td>
<td>231.6±28.6</td>
<td>64.8±18.8</td>
<td>208.3±21.9</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>4</td>
<td>252.5±26.6</td>
<td>98.7±16.4</td>
<td>97.6±17.4</td>
</tr>
</tbody>
</table>

*In 9 non-adjuvant arthritic rats, 9 neurones responding to TES were tested. Naloxone (0.5 mg/kg, i.v.) was administered 10 min to 20 min after morphine or indomethacin administration.*

n: number of neurones.

a) Significantly different from pre-drug values, $p < 0.001$, t-test, paired samples.

b) Significantly different from post-drug values, $p < 0.001$, t-test, paired samples.
sant effects of morphine (1 mg/kg, n = 5) and indomethacin (10 mg/kg, n = 4). Naloxone was administered 10 to 20 min after the morphine or indomethacin administration.

The evoked discharges of 5 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. 3-1, Table III).

In the 4 neurones, naloxone failed to reduce the depressant effect of indomethacin in every case (Fig. 3-2, Table III).

3. Effects of Morphine and Indomethacin on the Responses of Non-nociceptive Neurones

Evoked responses of non-nociceptive neurones activated by repetitive brushing were never depressed following morphine (1 mg/kg, n = 2) and indomethacin (10 mg/kg, n = 2) administration.

DISCUSSION

This study indicates the effects of intravenous administered morphine and indomethacin on the evoked responses of the VB thalamic neurones elicited by noxious and/or non-noxious stimuli in non-adjuvant arthritic rats. In the present study, we used two types of noxious stimuli such as TES and TNES. In this respect, unanesthetized non-adjuvant arthritic rats showed nociceptive reactions such as vocalization responses when TES was delivered (our preliminary experiment, unpublished observations). The mean latency of the evoked responses elicited by TES (m = 256.6 ± 8.1 ms, n = 14) was also similar to that by TNES (m = 246.3 ± 9.7 ms, n = 12). However, the animals did not show the vocalization responses when brushing was applied to the hind paws. Therefore, we conclude that TES and TNES were noxious, while brushing was non-noxious.

Intravenous morphine showed the same depressant effect on the evoked responses elicited by either TES or TNES. The depressant effect of morphine was reversed by the opiate antagonist naloxone. In contrast, intravenous indomethacin depressed the evoked responses elicited by TES, but did not show any effect on the evoked responses elicited by TNES. The depressant effect of indomethacin failed to be attenuated by naloxone. The evoked responses of non-nociceptive neurones failed to be depressed by the administration of morphine or indomethacin. These findings suggest that the main site of antinociceptive action of indomethacin in non-adjuvant arthritic rats may be in the periphery. In contrast, the site of action of morphine may reside in the neospinothalamic projection system.

In our previous study, performed under the same experimental conditions, indomethacin depressed the evoked responses elicited by TNES (ED₅₀: 0.66 mg/kg, i.v.) in adjuvant arthritic rats. Moreover, the depressant effect of drugs on the evoked responses elicited by direct stimulation of tibial nerve indicated that this effect of the drugs was produced by the central antinociceptive actions. Thus, it is suggested that the mode of antinociceptive action of indomethacin was different from non-adjuvant arthritic rats and adjuvant arthritic rats. In other words, the central antinociceptive action of indomethacin is clearly detectable in the presence of chronic inflammation. The depressant effects of indomethacin on the evoked responses elicited by TES were observed in both types of rats. However, a larger dose of indomethacin was required in non-adjuvant arthritic rats than in adjuvant arthritic rats (ED₅₀: 0.32 mg/kg, i.v. in adjuvant arthritic rats vs. ED₅₀: 6.2 mg/kg, i.v. in non-adjuvant arthritic rats). In this respect, it has been established that the strong antinociceptive action of acidic NSAIDs is shown in the presence of inflammation. The results from the present experiment support this concept. In addition, indomethacin inhibits the prostaglandins (PGs) biosynthesis, and PGs mediate or modulate the inflammatory pain both in a peripheral and a central sites. Moreover, our previous studies suggested that adjuvant induced chronic inflammatory pain might be more significantly related to PGs biosynthesis compared with non-adjuvant arthritic rats.

Intravenous morphine showed the same
depressant effects on the evoked responses of the VB neurones in non-adjuvant arthritic rats elicited by either TES (ED$_{50}$: 0.24 mg/kg, i.v.) or TNES (ED$_{50}$: 0.34 mg/kg, i.v.) without affecting any background activities. However, the evoked responses of the non-nociceptive neurones to repetitive brushing were never depressed following morphine administration. These effects of morphine were in agreement with the recent report of Benoist et al. The depressant effect of morphine on the evoked responses elicited by both TES and TNES in non-adjuvant arthritic rats were not significantly different from that obtained in adjuvant arthritic rats. Therefore, it is considered that the antinociceptive action of morphine is the same central mechanism in both types of rats. However, we have no evidence regarding the central antinociceptive mechanism of morphine in our present experiments.

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