The Role of the Paraventricular Nucleus and Pituitary Gland in Morphine Analgesia

Masaaki HASHIMOTO, Shoichiro OHGAMI and Yukichi YONEMASU

Department of Neurosurgery, Asahikawa Medical College, Asahikawa, Hokkaido

Abstract

The authors investigated the analgesic effects of small morphine doses injected into the paraventricular nucleus (PVN) of normal rats and hypophysectomized (Hx) rats. An injection cannula was stereotactically inserted into the PVN or third ventricle. On the 5–7th postoperative day, morphine (7μg) was injected and the pain threshold (paw lick latency: PLL) was measured using a hot plate analgesia meter (52.0 ± 0.1°C). PVN morphine injection caused significantly longer PLL than the control (physiological saline) in both normal and Hx rats. Ventricular morphine injection did not increase PLL over the control. PVN is a site of morphine action. The analgesia induced by PVN morphine injection was not affected by hypophysectomy, or induced by leaking of morphine into the third ventricle.

Key words: paraventricular nucleus, morphine, analgesia, hypophysectomy, hypothalamo-hypophyseal system

Introduction

The hypothalamus and pituitary gland have important functions in the endocrine system. They are also closely related with pain and have been targets for analgesic operations such as posterior hypothalamotomy or hypophysectomy.

Morphine has a direct action on the dorsal horn of the spinal cord. It also induces analgesia by acting on the supraspinal level and by suppressing the dorsal horn of the spinal cord in the descending direction. At the supraspinal level, the important sites of morphine action are the periaqueductal gray in the brainstem and the nucleus reticularis paragigantocellularis. Superior to the brainstem, the sites of morphine action are the lateral nuclei of the thalamus and the hypothalamus. Contradictory reports suggest that morphine injection into the hypothalamus does and does not have an analgesic effect. Very little is therefore known about the relationship between morphine analgesia and the hypothalamus.

Animal experiments suggest that hypophysectomy does not increase the pain threshold. However, morphine administration in the hypophysectomized (Hx) rat reinforces the analgesic effect, and pituitary gland extract enhances morphine analgesia. Therefore, the relationship between morphine analgesia and the pituitary gland is also unclear.

There is a close relationship between the pituitary gland and the paraventricular nucleus (PVN) in the anterior hypothalamus. To investigate the role of the hypothalamo-hypophyseal system in pain control, we studied morphine analgesia and the feedback mechanism through the hypothalamus by injecting small amounts of morphine into the PVN. We also evaluated the effect of hypophysectomy on morphine analgesia.

Materials and Methods

Twenty-two male Sprague-Dawley rats (260–360 gm) were divided into 4 groups. Group 1: physiological saline (PS) was injected into the PVN of non-Hx rats (n = 5). Group 2: morphine hydrochloride (morphine) was injected into the PVN of non-Hx rats (n = 8). Group 3: morphine was injected into the PVN of Hx rats (n = 5). Group 4: morphine...
was injected into the third ventricle of non-Hx rats (n = 4).

Animals were anesthetized with sodium pentobarbital (45 mg/kg, intraperitoneal injection) and fixed onto a stereotactic frame. A stainless steel guide cannula (0.6 mm outer diameter, 0.3 mm inner diameter) was inserted 1 mm above the right PVN (6.6 mm anterior to the vertical zero plane, 0.3 mm lateral to the midline, 2.2 mm ventral to the horizontal zero plane) chart of Pellegrino et al. The guide cannula was fixed onto the cranial bone with dental acrylic cement. An inner tube of 0.3 mm diameter was inserted to prevent blocking. Group 3 animals underwent microsurgical total removal of the pituitary gland through a parapharyngeal approach before the stereotactic operation. In Group 4 animals, the guide cannula was positioned in the third ventricle (6.2 mm anterior to the vertical zero plane, 0 mm lateral to the midline, 2.6 mm ventral to the horizontal zero plane). An inner tube 0.3 mm in diameter was inserted after confirming cerebrospinal fluid flow.

Morphine or PS solution was injected with the animal at rest and awake on the 5–7th postoperative day. The inner tube was withdrawn, and a stainless steel injection cannula (0.3 mm outer diameter) connected to a 1 µl Hamilton syringe through a polyethylene tube was inserted with the tip contacting the right PVN, located 1 mm from the cannula tip. Morphine solution of 0.7 µl (7 µg) or 0.7 µl PS was injected over about 40 seconds. After injection, the cannula was retained in position for 3 minutes to disperse the solution, and then withdrawn.

The pain threshold was quantitatively determined by the hot plate method (hot plate analgesia meter MK-350, Muromachi Co., Ltd., Tokyo). The paw lick latency (PLL), the time from placing the rat on a hot plate at 52 ± 0.1°C until the animal began to lick the anterior or posterior paw, was determined. The PLL was measured twice before PS or morphine injection and every 30 minutes after injection for 90 minutes. If no paw licking occurred within 60 seconds, the PLL measurement was stopped, and this was considered as complete analgesia. PLL were measured between 14:00 and 17:00. The Wilcoxon test was used to compare the PLL in the control group (Group 1) and the other groups.

Table 1 summarizes the experimental results. There was no difference between the PLL before and after PS injection in Group 1. In Group 2, the PLL peaked at 30 minutes after morphine injection into the PVN and it was prolonged at both 60 and 90 minutes after the injection. The PLL was significantly greater at 30 and 60 minutes compared with Group 1 (p < 0.01). The PLL in Group 3 was prolonged up to 90 minutes after morphine injection peaking at 30 minutes. All values were significantly longer compared with the PLL of the control group (p < 0.01). Comparison of Groups 2 and 3 showed there was no statistically significant difference in analgesic effect. In Group 4, the PLL did not differ from the PLL in the control group. No analgesic effect was obtained by the dose of morphine (7 µg) administered in this study.

Figure 2 shows the location of the cannula tip. The injection cannula was at the PVN in Groups 1–3. In Group 4, the cannula had punctuated into the third ventricle. All pituitary glands were completely extracted in Group 3.

Discussion

Our results show the pain threshold was apparently increased in the non-Hx group compared with the control group, but it was also increased in Hx rats.

**Results**

Table 1 summarizes the experimental results. There was no difference between the PLL before and after PS injection in Group 1. In Group 2, the PLL peaked at 30 minutes after morphine injection into the PVN and it was prolonged at both 60 and 90 minutes after the injection. The PLL was significantly greater at 30 and 60 minutes compared with Group 1 (p < 0.01). The PLL in Group 3 was prolonged up to 90 minutes after morphine injection peaking at 30 minutes. All values were significantly longer compared with the PLL of the control group (p < 0.01). Comparison of Groups 2 and 3 showed there was no statistically significant difference in analgesic effect. In Group 4, the PLL did not differ from the PLL in the control group. No analgesic effect was obtained by the dose of morphine (7 µg) administered in this study.

Figure 2 shows the location of the cannula tip. The injection cannula was at the PVN in Groups 1–3. In Group 4, the cannula had punctuated into the third ventricle. All pituitary glands were completely extracted in Group 3.

**Discussion**

Our results show the pain threshold was apparently increased in the non-Hx group compared with the control group, but it was also increased in Hx rats.
Table 1  Paw lick latency before and after injection of physiological saline or morphine

<table>
<thead>
<tr>
<th>Group</th>
<th>Before injection</th>
<th>30 minutes</th>
<th>Time after injection</th>
<th>60 minutes</th>
<th>90 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.51 ± 2.26</td>
<td>6.29 ± 3.13</td>
<td>7.14 ± 2.28</td>
<td>6.74 ± 2.35</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>&gt;60.0*</td>
<td>59.63 ± 3.38*</td>
<td>32.9 ± 26.64</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>&gt;60.0*</td>
<td>59.2 ± 1.79*</td>
<td>46.8 ± 19.33*</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>—</td>
<td>9.61 ± 2.95</td>
<td>8.13 ± 2.13</td>
<td>7.50 ± 2.30</td>
<td></td>
</tr>
</tbody>
</table>

Group 1: Physiological saline in non-hypophysectomized rats, Group 2: morphine in non-hypophysectomized rats, Group 3: morphine in hypophysectomized rats, Group 4: morphine in the third ventricle of non-hypophysectomized rats. Values are means ± SE (seconds). *p < 0.01 compared with Group 1 (controls).

Foster et al.\textsuperscript{9)} reported that 25 µg morphine injected to the anterior hypothalamus induced analgesia, although the PVN may or may not have been the target. In contrast, analgesia was not obtained by 10 µg morphine injected to the anterior hypothalamus.\textsuperscript{15)} Our experiments suggest that the anterior hypothalamus, especially the PVN, is a very important site of morphine action because the analgesic effect was obtained with a small amount of morphine (7 µg).

Jacquet and Lajtha\textsuperscript{15)} reported no analgesia was observed by the flinch-jump test after microinjection of 10 µg morphine into the anterior hypothalamus of rats, although the injection site was not specified. However, when they injected 10 µg morphine into the third ventricle, the pain threshold increased. Higher morphine doses in the third ventricle do induce analgesia.\textsuperscript{30,32,44)} Therefore, to exclude analgesia caused by morphine leaking into the third ventricle, 7 µg morphine was injected into the third ventricle. No analgesic effect was induced by this relatively low dose in this study (Table 1). Our experiments show that the PVN is very probably the site of morphine action because analgesia is induced by low doses of morphine directly injected to the PVN but not when given to other sites in the anterior hypothalamus or the third ventricle. Recent reports\textsuperscript{7,36)} suggest that morphine injected into the third ventricle of the cat or rat does not activate but rather blocks the descending pain suppression system. Further study on the analgesic effect of morphine in the third ventricle in animals is needed.

What then is analgesic pathway induced by morphine injection into the PVN? Pittman et al.\textsuperscript{34)} reported that electrical stimulation of the PVN neuron caused the level of posterior pituitary hormones measured by perfusion around the spinal cord to rise. These hormones are derived from the terminals of PVN fibers on the dorsal horn of the spinal cord. Swanson et al.\textsuperscript{37,38)} demonstrated anatomically the presence of a nerve tract from the PVN to the spinal cord in the rat. Kawajiri and Satoh\textsuperscript{19)} suggested the existence of a descending pain suppression tract from the PVN to the spinal cord. Therefore, the analgesia obtained by PVN morphine injection may be the result of direct suppression of the dorsal horn neuron. However, fibers also project from the PVN to the brainstem\textsuperscript{13,38)} so the analgesia may be the result of suppression of the dorsal horn neuron indirectly through the brainstem.

This study also investigated the effect of hypophysectomy on the analgesic effect of PVN mor-
phine injection. Table 1 shows that there was no difference in pain threshold between the Hx and non-Hx rats. It appears that, when the pituitary gland is extracted, the feedback mechanism works and the hypothalamus becomes hyperfunctional. However, the analgesic effect of PVN morphine in Hx rats is about the same as in the non-Hx group. Therefore, the PVN is related to morphine analgesia, independent of the pituitary gland. Previous reports pointed out that hypophysectomy reinforced subcutaneous or intraperitoneal morphine analgesia, but none investigated its effect on the PVN morphine analgesic effect. When administered systemically, morphine acts simultaneously on a large number of receptors, including the PVN morphine receptors and analgesia occurs as a result. This may be different from the response caused by local morphine injection. Also, there may be other factors such as the different concentrations in cerebral tissues resulting from local injection and systemic administration, or variable sensitivity to morphine with individual brain sites. Kasson et al. found that hypophysectomy did not increase analgesia induced by subcutaneous morphine injection in the rat, and suggested that removal of the pituitary gland is not always closely related with morphine analgesia.

The presence of endogenous opioid peptides, for example β-endorphin, in the hypothalamus or pituitary gland is important, because β-endorphin in particular has a strong analgesic effect. Previously, β-endorphin formed in the pituitary gland was believed to return to the brain and induce analgesia. However, clinically analgesia is obtained even when the β-endorphin blood level is decreased after hypophysectomy. Also, the same opioid peptides present in the pituitary gland are also produced in brain nerve cells, and the existence of retrograde blood flow from the pituitary gland to the hypothalamus is doubtful. Therefore, β-endorphin formed in the pituitary gland is now not considered to cause analgesia. We did not investigate the endogenous opioid peptides, and future study is necessary on the relationship between the hypothalamo-hypophyseal system and the pain control mechanism. The present study shows that the PVN is an important action site above the brainstem level because low doses of morphine injected there induce remarkable analgesic effects.

Addendum

This study was performed with the support of the Japanese Ministry of Education under (C) No. 01570798 for Scientific Research and General Research.

References

1) Akaike A, Shibata T, Satoh M, Takagi H: Analgesia induced by microinjection of morphine into, and electrical stimulation of, the nucleus reticularis paragigantocellularis of rat medulla oblongata. Neuropharmacology 17: 775–778, 1978
Role of Hypothalamo-hypophyseal System in Morphine Analgesia

15) Jacquet YF, Lajtha A: Morphine action at central nervous system sites in rat: Analgesia or hyperalgesia depending on site and dose. Science 182: 490–492, 1973


38) Swanson LW, Sawchenko PE, Wiegand SJ, Price JL: Separate neurons in the paraventricular nucleus project to the median eminence and to the medulla or spinal cord. Brain Res 198: 190–195, 1980


41) Tseng LF, Wei ET, Loh HH, Li CH: β-endorphin: Central sites of analgesia, catalepsy and body temperature changes in rat. J Pharmacol Exp Ther 214: 328–332, 1980


Address reprint requests to: M. Hashimoto, M.D., Abashiri Neurosurgical Hospital, 4–7–48 Katsuramachi, Abashiri, Hokkaido 093, Japan.