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Biochemical Pharmacology, Vol. 27, pp. 2756–2758.

0006-2952/78/1201-2756$02.00/0

Microinjection of opioids into the nucleus reticularis gigantocellularis of the rat: Analgesia and increase in the normetanephrine level in the spinal cord

(*Received* 2 *February* 1978; *accepted* 9 *May* 1978)

In studies in which intracerebral microinjections of morphine were given, it was noted that several regions of the brain stem, e.g. the hypothalamus[1, 2], the periaqueductal gray matter[3–5], the floor of the fourth ventricle[6] and the ventral surface of the brain stem[7], are all involved in the antinociceptive action of this narcotic.

Recently, Takagi *et al.*[8, 9] found that, when they gave microinjections of morphine into the nucleus reticularis gigantocellularis (NRGC) of the medulla oblongata of rats, analgesia was produced in a dose-dependent manner. The ED_{50} value was 0.038 μg, and with even the small dose of 0.015 μg, a definite analgesia resulted[9]. These doses are considerably less than those (2–200 μg) used in other studies[1–7] in which intracerebral microinjections of morphine have been given.

In neurochemical studies carried out in our laboratory[10], it was noted that systemic injection of morphine increased the concentration of normetanephrine (NM), a metabolite of noradrenaline (NA), in the dorsal half (mainly the dorsal horn) of the rat whole spinal cord, but not in the ventral half (mainly the ventral horn). Moreover, it was observed that the NM increasing effect of morphine disappeared after transection of the spinal cord at C-1, but not after transection of the brain stem at the inter-collicular level. From these results it was concluded that the primary site of the NM increasing action of morphine is in the lower brain stem where noradrenergic cell bodies are located, and that morphine causes an increase in the neuronal impulse flow of the bulbospinal noradrenergic system which in turn enhances the release of NA, a possible inhibitory transmitter at the spinal dorsal horn [11, 12].

These observations prompted us to investigate the effect of microinjections of morphine into the NRGC of the medulla oblongata on the level of NM in the spinal cord of the rat.

Experiments were carried out on male Wistar rats (170–240 g). At least 1 week before testing, a guide cannula was unilaterally implanted into the cerebellum and positioned 4 mm above the intended site of injection as described previously [8]. Morphine HCl (0.5 μg in 0.5 μl of physiological saline) or methionine-enkephalin (10 μg in 0.5 μl of distilled water) was injected through the injection cannula which had been inserted into the guide cannula to protrude 4 mm beyond the end of it so that the tip was introduced into the NRGC. The injection cannula was withdrawn 10 sec after an injection period of 30 sec. The stereotaxic coordinates of the NRGC (AP, 10.0–10.5, L, 1.0, H, 9.7), including the nucleus reticularis paragigantocellularis, were determined according to the atlas of Fifková and Maršala [13]. The position of the injection site was verified histologically.

The antinociceptic action of morphine was evaluated by the tail-pinch method [8]. Hemostatic forceps (3 mm in width and 2 kg constant pressure) were applied at the base of the tail. Analgesic effects were scored as follows: 1, 0.5 and 0 scores were given if the latent periods of the biting response to the forceps were more than 10, 5–10 and less than 5 sec respectively.

The method of removal of the spinal cord was as described by Shiomi and Takagi [10]. The extraction and fluorimetric determinations of NM and NA in the whole spinal cord were carried out by the methods of Anton and Sayre [Refs. 14 and 15 respectively]. Statistical significance was determined by Student's t-test.

Although injection of the vehicle (0.5 μl of physiological saline) into the NRGC exerted no significant effect on the nociceptive response of the rat to tail-pinch, microinjection of morphine in a dose of 0.38 μg (0.5 μg as morphine HCl) produced a marked antinociceptive effect (Fig. 1b). The effect reached a maximum within 5 min and the nociceptive response to the tail-pinch recovered after 90 min. This is consistent with our previous reports[8, 9].

The measurement of NM was used as an index of the activity of noradrenergic neurons, since NM is considered to be formed by catechol - *O* - methyltransferase from NA after its neuronal release [16]. Microinjection of morphine in a dose of 0.38 μg into the NRGC increased the level of NM in the spinal cord (Fig. 1a) with no effect on that of NA. This indicates the increased activity of the noradrenergic system in the spinal cord. The increase in the level of NM in the spinal cord reached a peak 5 min after injection of morphine and disappeared after 30 min. This time course corresponds relatively with that of morphine analgesia (Fig. 1, panels a and b). Thus, the NM enhancing action and analgesia of morphine injected into the NRGC were more rapid in onset and shorter in duration than that injected systemically[10]. The NM levels after microinjection of morphine, in doses of 0, 0.076, 0.15 and 0.38 μg, into the NRGC were dose-dependent: 23.4 ± 2.1 ($n = 15$), 29.9 ± 4.4 ($n = 13$), 32.7 ± 3.7 ($n = 18$) and 37.7 ± 5.1 ng/g (mean ± S. E. M.) ($n = 14$) respectively. Injection of the vehicle (0.5 μl) into the NRGC had no significant effect on the NM level in the spinal cord (Fig. 1a). Pretreatment of rats with naloxone HCl (1 mg/kg, s.c.) 10 min before microinjection of morphine (0.38 μg) into the NRGC completely prevented the NM increase induced by morphine.

Endogenous opioid peptides, when injected into the NRGC of the rat, have a potent analgesic action[17]. We examined the effect of methionine-enkephalin, injected into the NRGC, on the level of NM in the spinal cord of the rat. As shown in Fig. 2, methionine-enkephalin (10 μg) significantly increased the level of NM 5 min after the microinjection. The vehicle (0.5 μl) injected into the NRGC produced no significant effect on the level of NM in the spinal cord. The NM-increasing effect of methionine-enkephalin was blocked by naloxone HCl (1 mg/kg, s.c.) administered 10 min before microinjection of the peptide. These results indicate that both morphine and methionine-enkephalin injected into the NRGC ele-

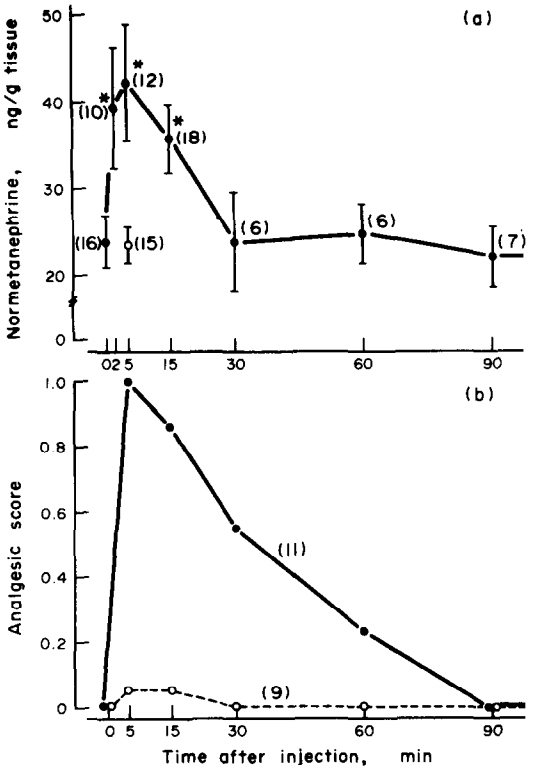

Fig. 1. Effects of morphine injected into the nucleus reticularis gigantocellularis (NRGC) of rats on the level of normetanephrine in the spinal cord (a) and the nociceptive response to tail-pinch (b). Either 0.38 μg morphine (●) or 0.5 μl vehicle (○) was injected into the NRGC at time 0. Ordinate: the level of normetanephrine in the whole spinal cord (a) and the mean analgesic score (see text) (b). Figures in parentheses indicate the number of rats used. Vertical bars represent S. E. M. An asterisk (*) indicates $P < 0.05$ vs control, the value at time 0.

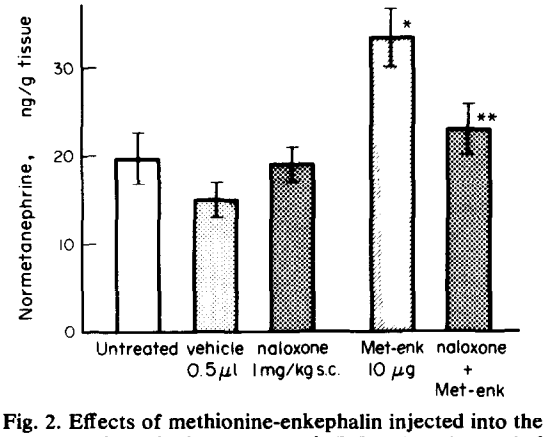

Fig. 2. Effects of methionine-enkephalin injected into the nucleus reticularis gigantocellularis (NRGC) on the level of normetanephrine in the rat spinal cord. Either 10 μg methionine-enkephalin or 0.5 μl vehicle was injected into the NRGC 5 min before death. Naloxone HCl (1 mg/kg) was subcutaneously injected 15 min before death. Columns show mean values from ten to twelve experiments. Vertical bars represent S. E. M. A single asterisk (*) indicates $P < 0.01$ vs untreated control; $P < 0.005$ vs vehicle. A double asterisk (**) indicates $P < 0.05$ vs methionine-enkephalin (Met-enk).

vate the level of NM in the spinal cord by binding to opiate specific receptors.

In contrast with the NRGC, no significant changes in the NM level in the spinal cord were produced with microinjection of morphine (0.38 μg) into the periaqueductal gray matter, the nucleus raphe magnus and the nucleus reticularis pontis caudalis, all sites less sensitive to morphine than the NRGC in producing analgesia[9]. Thus, the NRGC of the medulla oblongata may be an important site to the morphine-induced activation of noradrenergic neurons in the spinal cord as well as the morphine-induced analgesia after systemic injection.

To demonstrate whether or not there are noradrenergic cell bodies in the NRGC, the effect of a microinjection of 6 - hydroxydopamine (6-OHDA) into the NRGC on the NA level in the spinal cord was determined. Four days after the injection of 6-OHDA hydrobromide (10 μg in 0.5 μl of physiological saline containing 0.020% ascorbic acid), no significant changes in the NA level in the spinal cord were observed; controls: 0.380 ± 0.031 ($n = 7$), and 6-OHDA: 0.355 ± 0.020 μg/g (mean ± S. E. M.) ($n = 4$). Thus, there are apparently no cell bodies of NA-containing neurons in the NRGC. This result is in accord with histofluorescence findings of Palkovits and Jacobowitz[18]. Those suggest that some NA-containing cell bodies in the lower brain stem are relay nuclei for the bulbospinal inhibitory system in the case of NM-increasing action of morphine injected into the NRGC. Cell bodies of noradrenergic neurons in the spinal cord are located mainly in the nucleus reticularis lateralis (NRL) and the locus coeruleus (LC)[19]. The NRL gives rise to noradrenergic nerve terminals only in the dorsal horn, in contrast with the LC which projects to the ventral as well as the dorsal horn[19]. Moreover, the NM-increasing effect of morphine has been observed in the dorsal half of the spinal cord, including the dorsal horn, but not in the ventral half[10]. Therefore, the NRL probably plays a more responsible role as a relay for the NM-enhancing effect of morphine than the LC.

Acknowledgements—Thanks are due to M. Ohara, Kyoto University, for assistance with the manuscript. This study was supported in part by a Scientific Research Fund (No. 148116) from the Ministry of Education, Science and Culture of Japan.

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606, Japan

YASUSHI KURAISHI
KIYOSHI FUKUI
HIROHITO SHIOMI
AKINORI AKAIKE
HIROSHI TAKAGI

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