Nociceptive and Non-nociceptive Responses of Neurons in the Medial Subthalamic Region and Lateral Hypothalamic Area of Cats and Their Relationship to the Effects of Morphine and Pentazocine

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Single neuronal activity was recorded extracellularly from the Forel's field (FH), subthalamic region immediately rostral to the FH (STRF), rostral end of the medial subthalamic region (RE) and lateral hypothalamic area (LHA) of the anesthetized cats. Many of the FR, RE and LHA neurons were excited by nociceptive stimulation such as pinching the skin with serrated forceps and/or intra-arterial injection of bradykinin. These nociceptive neurons were also excited by non-nociceptive stimulation such as tap of deep tissues, bending hairs with an air-puff and/or joint rotation. On the other hand, inhibition by both nociceptive and non-nociceptive stimuli was seen in and around the rostral end of the FH including STRF. Their receptive fields were large. After intravenous administration of either morphine or pentazocine, most nociceptive neurons became unresponsive to nociceptive stimuli, although they were driven by non-nociceptive stimuli. This suggests that morphine and pentazocine have a specific antinociceptive action on these nociceptive neurons. Intravenous naloxone reversed the antinociceptive action of morphine, but failed to reduce the action of pentazocine. This differentiation has an important functional significance.

bradykinin; morphine; pentazocine; naloxone; diencephalon

Previous studies established that the majority of neurons in the nucleus centralis lateralis (CL), ventralis lateralis (VL), and medialis dorsalis (MD) of the cat were excited by both nociceptive and non-nociceptive stimuli; that morphine and pentazocine had a specific antinociceptive action on these nociceptive neurons; and that a low dose of naloxone reversed the antinociceptive action of morphine, but failed to reduce the action of pentazocine (Nakahama et al. 1981). On the other hand, we have as yet very little information as to what is involved in peripheral somatic and visceral activation of neurons in the medial subthalamic region and the lateral hypothalamic area (LHA). Moreover, no experiments have

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yet been made which allow one to evaluate the effects of morphine and pentazocine on unit activities of these regions.

It is from this aspect that we are interested in the subject of nociceptive and non-nociceptive responses of neurons in the LHA as well as in the medial subthalamic region which was divided into 3 parts for convenience; Forel’s field (FH), the part immediately rostral to FH (STRF) and the rostral end (RE). Another point of interest is to investigate differences in the effects of morphine and pentazocine. To clarify this problem at the single neuron level in the cat, we have administered morphine, pentazocine and naloxone intravenously, and studied evoked firing of neurons of the FH, STRF, RE and LHA.

METHODS

Adult cats weighing 2.5 to 3.5 kg were used. The animals were initially anesthetized with sodium pentobarbital (initial dose, 40 mg/kg i.p., further small doses if necessary). The animals were then paralyzed with gallamine triethiodide (Flaxedil, 7 mg/kg i.v. with supplements as required) and anesthesia was subsequently maintained by artificial ventilation with 65% N₂O and 35% O₂. The fact that the animals were adequately anesthetized was shown by absence of pupillary and blood pressure changes upon nociceptive stimuli. End-tidal CO₂ concentration was kept at 4.0–4.5%. The blood pressure was maintained above 80 mmHg with i.v. 4% dextrose in saline. Rectal temperature was kept at about 38°C by a homeothermic blanket system.

Two openings were made in the skull for stereotaxic exploration of the bilateral target nuclei with stainless steel microelectrodes (Nishioka and Nakahama 1973). The electrode was connected to a source follower probe which fed conventional capacity-coupled amplifiers. Neuronal activity was displayed on a cathode-ray oscilloscope and the window discriminator was employed to assist with the identification of single neuron impulses. The neuronal discharges were also monitored through a loudspeaker. Two electrodes were advanced dorsoventrally into the different target nuclei bilaterally. Neuronal impulse sequences were stored into FM magnetic tape along with the record of stimulus and drug application.

The following modes of non-nociceptive peripheral stimulation were used: bending hairs with an air-puff, touching of the skin, tapping circumscrip regions of the body, kneading of muscle groups, and joint angulation (Nishioka and Nakahama 1973). Nociceptive stimuli were applied by pinching the skin with serrated forceps, adjusted to be clearly noxious when applied to the investigator (Le Bars 1979; Nakahama et al. 1981). A small amount of bradykinin (3 μg in 0.5 ml saline) was injected into the right deep femoral artery through a polyethylene cannula inserted retrogradely for stimulation of the whole posterior limb (Lombard et al. 1975). This stimulus has been reported to be a most potent and specific one causing activation of nociceptive dorsal horn neurons (Besson et al. 1972; Belcher 1979). Bradykinin was administered within about 1 sec or less at intervals of 15–30 min. Saline injection produced no modification of the neuronal activity when this was examined in the early experiments in the series. Therefore this was not looked for subsequently. The cannula was washed with saline (0.2 ml) 1–2 min after each bradykinin injection.

Each animal received morphine (1 mg/kg) or pentazocine (2 mg/kg) intravenously only once. In some animals, naloxone (0.2 mg/kg) was given intravenously 30 min after the administration of morphine or pentazocine.

At the end of each experiment, the recording site was marked by passing anodal current. The animal was sacrificed under deep pentobarbital anesthesia and perfused with 10% formalin mixed with 2% potassium ferrocyanide. A prussian blue spot was obtained (Nishioka and Nakahama 1973). After proper fixation, frontal serial frozen sections of 25 μm thickness were stained with 0.2% cresylviolet. Thereafter the recording sites were determined by the use of the photographs and by the marking positions.
The neuronal impulse sequences recorded on magnetic tape were reproduced through the window discriminator, and processed for the purpose of data reduction by the PDP-11/34 computer.

RESULTS

Neuron type, location, and receptive field

Recordings were simultaneously made from 2 units in the different nuclei per animal for the purpose of the further drug study on the same units as well as of the elimination of chance variations. Plotted in Fig. 1 are the anatomical loci of 70 neurons in 35 cats which were responsive or irresponsive to nociceptive and non-nociceptive stimuli. Those units whose anterior-posterior (AP) positions lie between the sections illustrated (0.5–1.0 mm apart) were placed in the nearest section.

Out of 28 FH neurons, 7 (25%) were driven by non-nociceptive stimuli such as deep tissue tap, hair bending and joint angulation; 15 (54%), by both nociceptive and non-nociceptive stimuli; and 1 (4%), by nociceptive stimuli (Table 1). Of 11 STRF neurons, 2 (18%) responded to non-nociceptive stimuli; and 8 (73%), to both nociceptive and non-nociceptive stimuli. Of 28 LHA neurons, 4 (14%) were responsive to non-nociceptive stimuli; and 18 (64%), to both nociceptive and non-nociceptive stimuli.

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Fig. 1. Location of somesthetic neurons studied in the medial subthalamic region and the lateral hypothalamic area (LHA). Loci of 70 neurons are plotted on frontal planes that extend from AP 7.5 to 11.0. The recording sites of the target nuclei are conveniently plotted to the corresponding positions of the left side along with actual locations. Abbreviations: CL, nucleus centralis lateralis; CM, nucleus centrum medianum; FH, Forel’s fields; LP, nucleus lateralis posterior; MD, nucleus medialis dorsalis; Pul, pulvinar; RE, rostral end of the medial subthalamic region; STRF, subthalamic region immediately rostral to Forel’s field; VL, nucleus ventralis lateralis; VM, nucleus ventralis medialis; VPM, nucleus ventralis postero-medialis; ZI, zona incerta. e, excitatory; i, inhibitory neuron.
A majority of the FH (13/16), RE (2/2) and LHA (17/18) nociceptive neurons were excited by both nociceptive and non-nociceptive stimuli, and a few other neurons in the rostral-most part of FH (AP 9.0) and LHA were inhibited by these stimuli (Fig. 1). On the other hand, almost all STRF neurons (6/8) were inhibited and a few were excited by both nociceptive and non-nociceptive stimuli.

The somatic receptive fields of the majority of the nociceptive and non-nociceptive neurons studied were large, and often encompassed the entire body. A few neurons were found to have purely ipsilateral or contralateral receptive areas. Their margins were not sharply defined. A somatotopic organization of neurons was not found within the nuclei. These observations correspond well to those made in the cat medial thalamus (Bong et al. 1978), the CL, VL and MD (Nakahama et al. 1981). The responsiveness of almost a half of neurons in each of the nuclei studied was weak as compared to that of the CL, VL and MD neurons; and that of the remainder of neurons was similar to that of the thalamic neurons (Nakahama et al. 1981).

**Effect of bradykinin**

In the 7 FH neurons, the mean latency of excitatory effects of bradykinin was 10.9±2.3 sec (mean and s.d.) and the mean duration was 14.7±10.0 sec; and in the 5 LHA neurons, the mean latency was 6.9±0.9 sec and the mean duration was 12.1±6.3 sec. The mean latency was longer in the FH neurons than in the LHA neurons (p<0.01, t-test), while the duration was not different (p>0.05) between these neuron groups. In the 3 STRF inhibitory neurons, the latency was 10.8±8.9 sec and the duration was 14.0±2.2 sec.

**Effects of morphine and pentazocine**

The antinociceptive action of morphine and pentazocine upon the nociceptive neurons is summarized in Table 2. Approximately 60% of the FH and LHA neurons examined became unresponsive to nociceptive stimuli such as skin pinch and bradykinin injection after the analgesic administration; while all of the STRF and RE neurons examined became unresponsive to nociceptive stimuli. In contrast, there was no discernible change of responsiveness to non-nociceptive stimuli.
Effects of Morphine and Pentazocine

TABLE 2. Effects of morphine and pentazocine upon nociceptive neurons

<table>
<thead>
<tr>
<th>Location of neurons</th>
<th>Nociceptive neurons</th>
<th>Morphine</th>
<th>Pentazocine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-</td>
<td>No effect</td>
<td>Anti-</td>
</tr>
<tr>
<td></td>
<td>nociception</td>
<td></td>
<td>nociception</td>
</tr>
<tr>
<td>FH</td>
<td>16(2)</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>STRF</td>
<td>2(6)</td>
<td>2(4)</td>
<td>0</td>
</tr>
<tr>
<td>RE</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>LHA</td>
<td>17(1)</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

The values without parentheses are numbers of the excitatory neurons; those in parentheses, the inhibitory neurons.

Fig. 2. Effects of morphine and naloxone on the single FH excitatory neuron activity to natural stimulation. Marks at the upper part of each tracing indicate the time of stimuli applied to the peripheral receptive fields. Naloxone was given 30 min after morphine administration.

Fig. 3. Effects of morphine and naloxone on the STRF inhibitory neuron discharges.
compared with the responses before the analgesic administration (cf., Figs. 2 and 3). The antinociceptive action was observed at 10 min intervals over 90 min, although it was not tested within the first 10 min. This observation suggests that morphine and pentazocine have a specific antinociceptive action.

All of the 7 FH, 2 STRF, 1 RE and 4 LHA non-nociceptive neurons were unaffected by analgesic administration.

**Effects of naloxone**

The effects of naloxone 30 min after analgesic administration were studied on the nociceptive neurons.

In the 4 FH, 1 STRF, 1 RE and 3 LHA excitatory neurons, naloxone administration reversed the antinociceptive action of morphine (Fig. 2). Similar results were obtained in the 3 STRF inhibitory neurons (Fig. 3). However, naloxone did not reverse the antinociceptive action of morphine in the 1 LHA excitatory neuron.

In the 5 FH, 1 RE and 4 LHA excitatory neurons naloxone failed to reduce the antinociceptive action of pentazocine (Fig. 4). The same results were obtained in the 1 FH and 2 STRF inhibitory neurons.

**DISCUSSION**

The FH receives direct ascending input from the medial magnocellular region of the medullary and pontine reticular formation (Nauta and Kuypers 1958) and from the midbrain reticular formation (Bowsher 1975) belonging to the paleospinothalamic nociceptive system (Bowsher 1957; Kaelber and Smith 1979; Kerr 1979). On the other hand, this area receives input directly from the dorsal column nuclei of the medulla (Boivie 1971) which is known as belonging to the lemniscal non-nociceptive system (Mountcastle and Darian-Smith 1968). When these anatomical reports are considered, it is of interest that most of the FH neurons
were excited by nociceptive and/or non-nociceptive stimulation. The lesions of the FH blocked nociceptive escape responses (Kaelber 1977), and the stimulation induced ocular and head rotation (Hyde and Toczek 1962; Kaelber and Smith 1979). This would suggest that the FH contributes to the motor reactions to nociception as a part of non-pyramidal circuitry (Bowsher 1975).

This study indicates that most of the LHA neurons were excited by nociceptive and non-nociceptive stimulation. Stimulation of the LHA of cats elicited affective attack (Huang and Flynn 1975; Smith and Flynn 1980a, b). Lesions of the LHA of rats decreased responses to presentation of visual, olfactory and tactile as well as nociceptive mechanical stimuli (Marshall et al. 1971). Preterminal degeneration was seen in the LHA after lesions of the reticular formation at all levels rostral to the medulla (Nauta and Kuypers 1958; Wolf 1971; Bowsher 1975); and the cuneiform nucleus of the midbrain reticular formation projects fibers on the LHA (Edwards and de Olmos 1976) belonging to the paleospinothalamic nociceptive system (Kerr 1979). The significance of these results is that the LHA may be involved in some affective aspects of the nociceptive response.

The bradykinin effect is mediated by small fibers which innervate deep somatic tissues and visceral organs (Krauthamer et al. 1977). Large amounts of intra-arterial bradykinin (30–70 µg) appear much less specific and activate cells fired by non-nociceptive stimuli as well as cells fired by nociceptive stimuli (Belcher 1979). Since we used a small amount of bradykinin (3 µg), the nature of the bradykinin-induced pain resembles deep, visceral or protopathic pain more than it does superficial, cutaneous pain (Krauthamer et al. 1977).

Systemically applied morphine induces a long lasting depression of activities of dorsal horn cells involved in the transmission of nociceptive messages in rats while responses to the activation of large myelinated fibers conveying non-nociceptive impulses are not affected (Le Bars et al. 1980). The depressive effect of morphine is reversed by naloxone. It is also indicated that intravenous morphine depresses nociceptive reactions without affecting tactile responses in man, and this depressing effect is completely reversed by naloxone (Willer and Bussel 1980). Under a discrete trial schedule of shock titration in squirrel monkeys, the effects of morphine are antagonized by a low dose (0.01–0.3 mg/kg) of naloxone (Dykstra 1979). These data accord with the present study showing that morphine has a specific antinociceptive action on the neurons in the medial subthalamic region and the LHA of cats; and that naloxone (0.2 mg/kg, i.v.) reversed the antinociceptive action of morphine. This suggests that specific opiate receptors are involved in the observed effect of morphine (Le Bars et al. 1980; Nakahama et al. 1981).

Pentazocine is a weak narcotic antagonist and a potent analgesic with substantially less dependence liability than morphine (Keats and Telford 1964; Beaver 1980). This may be supported by the present study, which indicates that intravenous naloxone failed to reduce the antinociceptive effects of pentazocine on the neurons in the medial subthalamic region and the LHA. Further studies are
needed for the relation between pentazocine and opiate receptors.

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References


