Effects of Morphine on Responses of Nociceptive Ventrobasal Thalamic Neurons in Diabetic Rats

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ABSTRACT—The influence of diabetes on the effects of morphine on the responses of ventrobasal (VB) thalamic neurons to mechanical noxious stimuli were studied in chloral hydrate-anesthetized rats. Animals were rendered diabetic by an injection of streptozotocin (60 mg/kg, i.v.). Morphine (0.3 mg/kg), administered i.v., produced a reduction in the responsiveness of VB thalamic neurons to noxious stimulation in control rats. This effect was reversed by naloxone. In contrast, the inhibitory effects of morphine on the nociceptive responses of VB thalamic neurons were significantly attenuated in diabetic rats, as compared with the controls. However, there were no significant differences in inhibitory potency between diabetic and control rats when morphine (30 nM) was administered intrathecally. It seems likely that these changes in the sensitivity of VB thalamic neurons to morphine are, to some extent, the source of the reduction in the analgesic efficacy of morphine in diabetic rats.

Keywords: Diabetes, Ventrobasal thalamic neuron, Morphine, Analgesia

The ventrobasal (VB) thalamic complex in the rat has been shown to contain neurons that respond exclusively to noxious stimulation (1, 2). It was demonstrated previously that neuronal responses to nociceptive stimuli in the rat VB thalamic neurons were highly sensitive to low doses of morphine (3–5). Since the doses of morphine required to inhibit the response of VB thalamic nociceptive neurons are lower than those that induce a significant depression in the responses of dorsal horn neurons elicited by activation of cutaneous C-fibers, Benoist et al. (3) suggested that the depressant effect of low doses of morphine at the thalamic level may be mainly supraspinal in origin.

Several studies have demonstrated that rats and mice with streptozotocin-induced diabetes, as well as spontaneously diabetic mice, are significantly more sensitive to the antinociceptive effects of morphine than controls (6–8). Both the selectivity of \( \mu \)-receptors and their distribution in regions of the brain that play a putative role in the regulation of the perception of pain strongly suggest that the \( \mu \)-receptor is a major factor in the supraspinal mediation of opioid analgesia (9, 10). We suggested previously that the reduction in the antinociceptive potency of morphine in diabetic mice is due to the dysfunction of supraspinal, and not spinal, \( \mu \)-opioid receptor-mediated analgesia (8). To test this hypothesis, we evaluated the effects of systemic administration of a low dose of morphine on the response of VB thalamic nociceptive neurons to noxious stimulation of the hindpaw and their response to iontophoretically applied glutamate in diabetic rats in a comparison with control rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Tokyo Animal Laboratory, Inc., Tokyo, Japan), initially 8 weeks old, weighing about 250 g at the beginning of the experiments, were used. Animals were housed in groups of six per cage under a 12-hr light-dark cycle with food and water continuously available. The room temperature was maintained at 22 ± 1°C. After a one-week adaptation period, animals were rendered diabetic by an injection of streptozotocin (STZ; 60 mg/kg, i.v.) prepared in 0.1 N citrate buffer at pH 4.5. Age-matched control rats were injected with the vehicle alone. The experiments were conducted 8 weeks after injection of vehicle or STZ. Rats with a serum glucose concentration above 400 mg/dl were considered diabetic.

Animals received an i.p. injection of chloral hydrate
in a volume of 10 μl and subsequently flushed through the cannula with 10 μl of saline by use of a microinjection pump (10 μl/min).

RESULTS

Forty-eight neurons (24 neurons in 24 control rats and 24 neurons in 24 diabetic rats) responding to noxious mechanical stimulation, but not to weak forms of mechanical stimulation such as brushing, were studied. All neurons responded with an increase in firing rate. No significant differences were observed between control and diabetic rats in the percentage increase in the number of spikes, in response to the noxious mechanical stimulation (control rats, 807.0 ± 178.1%, n = 16; diabetic rats, 915.6 ± 140.0%, n = 16) or in response to iontophoretic application of glutamate (control rats, 1242.3 ± 335.2%, n = 8; diabetic rats, 876.6 ± 225.1%, n = 8).

Effects of morphine on the responses of VB neurons elicited by noxious mechanical stimulation

Typical recordings of the effect of morphine (0.3 mg/kg, i.v.) on the responses of VB thalamic nociceptive neurons to noxious mechanical stimulation of the hindpaw are shown in Fig. 1 (control rat) and Fig. 2 (diabetic rat). At a dose of 0.3 mg/kg, i.v., morphine markedly depressed the evoked responses in all 10 neurons examined in control rats. The effect of morphine developed rapidly, lasted for more than 20 min and was abolished by naloxone injected i.v. at a dose of 0.5 mg/kg (Figs. 1 and 3). However, the i.v.-administration of morphine at 0.3 mg/kg failed to depress the evoked responses of the VB thalamic nociceptive neurons in all cases examined in diabetic rats (Fig. 2). Indeed, as shown in Fig. 3, the morphine-induced percentage inhibition of evoked responses was significantly reduced in diabetic rats as compared with that in control rats (maximal effect in control rats, 88.1 ± 3.2%, n = 10; maximal effect in diabetic rats, 27.3 ± 7.9%, n = 10, P < 0.01). On the other hand, morphine had no significant effect on the spontaneous activities (e.g., spike number or height) of VB thalamic neurons in both control and diabetic rats (data not shown).

The effects of intrathecal injection of morphine on the responses of VB thalamic nociceptive neurons were examined both in control and diabetic rats. As shown in Fig. 4, at a dose of 30 nM, i.t., morphine markedly depressed the evoked responses in all nociceptive neurons examined both in control and diabetic rats. The maximal effect of morphine on evoked responses in diabetic rats was similar to that in control rats (control rats, 74.9 ± 6.5% inhibition, n = 6; diabetic rats,
Fig. 1. The effects of intravenous administration of morphine at 0.3 mg/kg on the nociceptive responses of thalamic VB neurons in control rats. The filled triangles beneath the trace indicate the application of noxious mechanical stimulation to the hindpaw.

Fig. 2. The effects of intravenous administration of morphine at 0.3 mg/kg on the nociceptive responses of thalamic VB neurons in diabetic rats. The filled triangles beneath the trace indicate the application of noxious mechanical stimulation to the hindpaw.
Fig. 3. Changes with time in the effects of intravenous administration of morphine (0.3 mg/kg) on the nociceptive responses of thalamic VB neurons elicited by noxious mechanical stimulation of the hindpaw in control (●) and diabetic (○) rats. Naloxone (0.5 mg/kg, i.v.) was injected 20 min after the administration of morphine. Each point represents the mean with S.E. of the results from 10 experiments. Significant differences from the values for control rats are indicated by ** (P < 0.01) and * (P < 0.05).

Fig. 4. Changes with time in the effects of intrathecal administration of morphine (30 nM) on the nociceptive responses of the thalamic VB neurons elicited by the noxious mechanical stimulation of the hindpaw in control (●) and diabetic (○) rats. Naloxone (5 nM, i.t.) was injected 20 min after the administration of morphine. Each point represents the mean with S.E. of the results from 6 experiments.
76.3 ± 9.3% inhibition, n = 6). Both in the control and diabetic rats, the depressant effect of morphine became apparent within 5 min after administration and lasted for more than 20 min. Furthermore, the effect of morphine was abolished by i.t.-administration of naloxone (5 nM).

Effects of morphine on the responses of VB neurons elicited by iontophoretically applied glutamate

Figure 5 shows typical recordings of the effects of morphine (0.3 mg/kg, i.v.) on the responses of VB thalamic nociceptive neurons to iontophoretically applied glutamate in control rats. The evoked responses to iontophoretically applied glutamate were markedly depressed by morphine in all the cases examined in control rats. The time course of the depressant effect of morphine on the response to iontophoretically applied glutamate was similar to that of the effect of morphine on the response to noxious mechanical stimulation of the hindpaw (Fig. 7). However, the i.v.-administration of morphine at 0.3 mg/kg had no obvious effect on the evoked responses of the VB thalamic nociceptive neurons to iontophoretically applied glutamate in diabetic rats (Fig. 6). Indeed, as shown in Fig. 7, the morphine-induced percentage inhibition of evoked responses was significantly reduced in diabetic rats compared with that in control rats (maximal effect in control rats, 78.4 ± 6.4%, n = 8; maximal effect in diabetic rats, 25.8 ± 6.8%, n = 8, P < 0.01).

DISCUSSION

There is evidence that nociceptive transmission is enhanced in animals with STZ-induced diabetes (12–14). Indeed, the threshold for pain perception in response to noxious mechanical stimuli, but not noxious thermal stimuli, is reduced in diabetic animals (14). However, the analgesic effect of morphine is defective in diabetic mice as determined with both noxious mechanical and thermal stimuli (8). Therefore, we postulated that the reduction in analgesic potency of morphine in diabetic mice is not caused by a reduction in the threshold for perception of pain (8). This hypothesis is strongly supported by the results of the present study in which we found that the effects of morphine on the responses of VB thalamic neurons to noxious mechanical stimulation of the hindpaw were significantly reduced in diabetic rats as compared with those in control rats, whereas there is no difference between control and diabetic mice in terms of the responsiveness of VB thalamic neurons in the absence of morphine. It is unclear at the present time why the responsiveness of VB thalamic neurons to noxious mechanical stimuli in diabetic rats was not altered as compared to that in control rats. Further studies are needed to resolve this problem.

The present results indicate that the effect of systemic administration of a low dose of morphine on the responses of VB thalamic neurons to noxious stimulation of the hindpaw was significantly attenuated in dia-

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Fig. 6. The effects of intravenous administration of morphine of 0.3 mg/kg on the responses of thalamic VB neurons elicited by the iontophoretic application of glutamate (Glu) with a current of 10 nA (filled horizontal bars) in diabetic rats.

Fig. 7. Changes with time in the effects of intravenous administration of morphine (0.3 mg/kg) on the nociceptive responses of thalamic VB neurons elicited by the iontophoretic application of glutamate with a current of 10 nA in control (●) and diabetic (○) rats. Each point represents the mean with S.E. of the results from 8 experiments. Significant differences from the values for control rats are indicated by ** (P < 0.01).
betic rats as compared with control rats. Low doses of morphine (< 1000 μg/kg, i.v.) have been shown to have a strongly depressant effect on the responses of specific nociceptive neurons in the VB complex of the normal rats (3–5). The studies employing the intracerebral and intracerebroventricular injection technique have demonstrated multiple analgesic sites of morphine: for example, the periaqueductal gray matter of the midbrain (PAG), the nucleus reticularis giganto-cellularis (NRGC), the nucleus reticularis paragiganto-cellularis (NRPG), the nucleus raphe magnus (NRM), the locus coeruleus (LC), and the spinal dorsal horn (15–19). The suppression of noxious evoked activity of thalamic neurons by systemically administered morphine may, of course, be secondary to a reduction in their excitatory afferent input via activation of opioid receptors (probably, μ-opioid receptors) in these regions. Previously, we observed that mice rendered diabetic for 2 weeks had a lower sensitivity than controls to s.c.- or i.c.v.-administration of morphine, whereas the antinociceptive potency of i.t.-administered morphine was not significantly reduced in diabetic rats (8, 20). Several studies have indicated that the dose of morphine for inhibition of the VB neuronal nociceptive responses in rats is very much lower than that for inhibition of responses of spinal dorsal horn neurons (21, 22). Furthermore, in the present study, there were no significant differences in the potency of i.t.-administered morphine with respect to the responses of VB thalamic neurons to noxious stimulation between diabetic and control rats. Thus, the present electrophysiological data agree with our previous behavioral observations (8, 20) and confirm that the reduction in the antinociceptive potency of morphine in diabetic rats is due to dysfunction of the supraspinal (e.g., PAG, NRGC, NRPG, NRM and LC) mediation of opioid analgesia.

Recently, several authors have reported that an ascending pain-modulation system may exist between PAG and the thalamus (23–26). Indeed, the nociceptive neurons in the VB thalamic complex can be prevented from responding to noxious stimulation by electrical stimulation of the PAG in the rat (24) and the cat (23, 26). Furthermore, it has been proposed that morphine-induced analgesia is mediated predominantly supraspinally, with the PAG being a key site of action (18, 27). In the present study, a low dose of morphine (0.3 mg/kg, i.v.) also was found to antagonize the responses of nociceptive neurons in the VB thalamic complex to iontophoretically applied glutamate in control rats. However, the effect of morphine on the responses of VB thalamic neurons to iontophoretically applied glutamate was significantly attenuated in diabetic rats as compared with control rats. It has also been reported that opiate receptors are present in some thalamic areas of the rat (28–30). If morphine does directly act at sites in the thalamus, one might predict that i.v.-morphine would depress the spontaneous firing of VB neurons. However, in the present study, i.v.-morphine was able to suppress the responses of nociceptive VB neurons without affecting their spontaneous firing. Furthermore, Hill and Pepper (31) reported that the suppressant effect of i.v.-morphine on the excitation of nociceptive thalamic neurons by iontophoretically applied glutamate was reversed by i.v.-naloxone, but not by iontophoretically applied naloxone. It seems likely, therefore, that the observed depressant effect on responses of the nociceptive VB thalamic neurons to iontophoretically applied glutamate by morphine may be secondary to a reduction in their excitatory afferent input via the activation of an ascending pain-modulating system which may exist between PAG and the thalamus. Thus, it is possible that dysfunction of an ascending pain-modulating system from the PAG to the thalamus may occur in diabetic animals.

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