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Occurrence of morphine tolerance and dependence in the nucleus paragigantocellularis neurons

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Abstract

The occurrence of morphine tolerance and dependence in the nucleus paragigantocellularis neurons was investigated. The spontaneous activity was recorded from the nucleus paragigantocellularis neurons of urethane-anesthetized rats, using single unit recording. Morphine microinjected (20 mg/ml, 120–200 nl) into the nucleus paragigantocellularis of control rats had both excitatory and inhibitory effects. These effects were reversed by microinjection of naloxone, revealing the possible involvement of μ receptors. Morphine microinjected into morphine-dependent rats failed to change the spontaneous activity of the nucleus paragigantocellularis neurons that accounts for the occurrence of tolerance to morphine in these neurons. Microinjection of naloxone (25 mg/ml, 120–200 nl) in control rats had no effect on the spontaneous firing rate of the nucleus paragigantocellularis neurons but in morphine-dependent rats, either alone or after morphine microinjection, naloxone increased neuronal activity significantly, indicating the occurrence of dependence on morphine in the nucleus paragigantocellularis neurons. These data show that the nucleus paragigantocellularis neurons may play a role in physical dependence on morphine. This conclusion is consistent with the finding, that activation of the nucleus paragigantocellularis by electrical stimulation in morphine-naive rats can elicit behaviors similar to those observed during naloxone-precipitated morphine withdrawal.

Keywords: Nucleus paragigantocellularis; Morphine tolerance; Morphine dependence; Naloxone; Single unit recording

1. Introduction

The principal effect of opiates is the alleviation of pain. They also produce a sense of well being and euphoria that, in some circumstances, lead to increased opiate use, which in turn can cause the development of tolerance and physical dependence.

Hyperactivity of the locus coeruleus neurons during antagonist-precipitated morphine withdrawal (Aghajanian, 1978; Aston-Jones et al., 1997; Rasmussen and Aghajanian, 1989; Akaoka and Aston-Jones, 1991; Valentino and Wehby, 1989) and its temporal correlation with withdrawal behavior (Rasmussen et al., 1990) suggest that activation of locus coeruleus neurons plays a role in at least some of the behavioral manifestations of opiate abstinence syndrome. This is consistent with other studies that show that: (1) systemic administration and local infusion of clonidine, an α_2-adrenoceptor antagonist, into the locus coeruleus suppresses the increased locus coeruleus unit activity (Aghajanian, 1978) and many behavioral symptoms (Gold et al., 1978; Taylor et al., 1988) seen during opiate withdrawal, and (2) destruction of the locus coeruleus decreases physical signs of opiate withdrawal (Maldonado and Koob, 1993). Withdrawal-induced activation of locus coeruleus neurons is only seen in vivo and not in brain slices taken from morphine-dependent rats (Andrade et al., 1983). Possibly, afferents to the locus coeruleus, which are disconnected in brain slices, are responsible for this withdrawal activation of locus coeruleus neurons (Rasmussen, 1991).

The nucleus paragigantocellularis located in the rostral ventrolateral medulla (Andrezik et al., 1981) is one of the two major afferents to the locus coeruleus (Ennis et al., 1992). The strong projection from the nucleus paragigantocellularis to the locus coeruleus has been confirmed with retrograde tracing using several tracers (Guyenet and Young, 1987; Pieribone et al., 1988), anterograde tracing with wheat germ agglutinin-conjugated horseradish peroxi-

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dase (WGA-HRP) (Aston-Jones et al., 1986) and *Phaseolus vulgaris* leucoagglutinin (Guyenet and Young, 1987), and also with electrophysiologic antidromic activation of the locus coeruleus (Ennis and Aston-Jones, 1987). Nucleus paragigantocellularis predominantly activates the locus coeruleus via an excitatory amino acid pathway (Ennis and Aston-Jones, 1988) and the nucleus prepositus hypoglossi in the dorsomedial medulla provides an inhibitory γ-aminobutyric acid-ergic input (Ennis and Aston-Jones, 1989). Also there is an adrenergic inhibitory projection from the nucleus paragigantocellularis to the locus coeruleus (Aston-Jones et al., 1992). The excitatory amino acid afferents from the nucleus paragigantocellularis to the locus coeruleus are thought to play a role in the withdrawal activation of the locus coeruleus neurons since: (1) non-selective excitatory amino acid antagonists, after both intraventricular injection or direct infusion into the locus coeruleus, attenuate the morphine withdrawal-induced activation of locus coeruleus neurons (Rasmussen and Aghajanian, 1989; Rasmussen et al., 1991; Akaoka and Aston-Jones, 1991); (2) opiate withdrawal increases glutamate and aspartate efflux in the locus coeruleus (Aghajanian et al., 1994; Zhang et al., 1994); (3) radiofrequency lesions of the nucleus paragigantocellularis greatly attenuate naltrexone withdrawal-induced activation of neurons in the locus coeruleus ipsilateral to the nucleus paragigantocellularis lesion (Rasmussen and Aghajanian, 1989); and (4) activation of the nucleus paragigantocellularis by electrical stimulation in morphine-naive rats can elicit behaviors similar to those observed during opioid withdrawal (Liu et al., 1999). Haghparast et al. (1998) showed that there is a significant enhancement of the spontaneous activity of the nucleus paragigantocellularis neurons following subcutaneous administration of naloxone in morphine-dependent rats as an opiate withdrawal-induced activation of the nucleus paragigantocellularis neurons. Therefore, we microinjected naloxone into the nucleus paragigantocellularis to elucidate whether this withdrawal-induced activation is the result of dependence of the nucleus paragigantocellularis neurons on morphine or is mediated by the nucleus paragigantocellularis afferents originating from other brain areas.

2. Materials and methods

2.1. Animals

Thirty-five male NMRI rats (250–350 g) were used in these experiments in two major groups: the control and the dependent groups. They were housed at controlled temperature (22 ± 2) and under a 12-h light–dark cycle. Animals were allowed access to water and food ad libitum.

2.2. Chronic morphine administration

Dependent rats received morphine sulfate and sucrose 3% w/v in tap water as their drinking fluid. Sucrose was used to mask the bitter taste of morphine. Morphine was given in increasing concentrations of 0.1, 0.2, 0.3 mg/ml for every 48 h and 0.4 mg/ml during the next days (Badawy et al., 1982; Mansouri et al., 1997). The mean amount of received drugs was 80 mg/kg/day. No correction was made for possible spillage of morphine solution. Control rats received only tap water since dependence on and tolerance to morphine does not occur in the nucleus paragigantocellularis of control (receiving 3% w/v sucrose) and sham-operated (receiving tap water) rats (Haghparast et al., 1998). Development of physical dependence on morphine was determined by elicitation of the precipitated withdrawal syndrome by intraperitoneal administration of naloxone HCl (2 mg/kg) (Mansouri et al., 1997). Naloxone was administered to one rat from every group (5–10 rats) that was treated with morphine for at least 3 weeks. In all cases, a number of behavioral symptoms of the withdrawal syndrome, such as wet-dog shakes, head-shakes, diarrhea, ptosis, teeth chattering and writhing, were produced that indicated the occurrence of dependence on morphine (Badawy et al., 1982).

2.3. Nucleus paragigantocellularis microinjection

Animals were anesthetized with urethane (1.2 g/kg i.p.) and additional doses (0.15 g/kg) were administered every hour for a maximum of two times to maintain the level of anesthesia. After tracheal cannulation (tracheal ventilation occurred by spontaneous ventilation), rats were placed in a stereotaxic instrument. Using a thermistor-controlled heating pad, body temperature was maintained at 35.5–36.8°C.

The neck tissue at the caudal skull margin was reflected and the occipital bone over the caudal cerebellum was partially removed to reveal the caudal apex of the IVth ventricle. A 23-gauge stainless steel guide cannula was positioned at a point 9 mm caudal from Lambda and 1.6 mm lateral from the midline and then lowered slowly by 9.3 mm from the brain surface at an angle of 30°. A 27-gauge multibarrel injection cannula (2 mm lower than guide cannula) consisting of three separate barrels was connected to three 1-μl Hamilton syringes and then inserted through the guide cannula. Then 120–200 nl of normal saline, morphine sulfate (Temad, Iran, 20 mg/ml in isotonic saline) (Pan and Fields, 1996) and naloxone hydrochloride (Sigma, 25 mg/ml in isotonic saline) were injected over 3 min. If there was a change in spontaneous firing during injection due to a volume effect or body movement, the animal was discarded.

2.4. Nucleus paragigantocellularis recordings

A 2-mm hole was drilled in the skull above nucleus paragigantocellularis (3.26 caudal to lambda and 1.6 lateral to midline) and the dura was reflected. Extracellular recordings from individual neurons were obtained with glass micropipettes (2–5 MΩ impedance) filled with 2%

pontamine sky blue dye in 0.5 M sodium acetate. Micropipettes were stereotaxically advanced into the nucleus paragigantocellularis (9.6–10.1 mm ventral to skull surface). Micropipette recordings were amplified by a microelectrode amplifier (Nihon Kohden), displayed continuously on a storage oscilloscope (Tektronix) as unfiltered and filtered (100 Hz–5 kHz bandpass) signals, and monitored with an audio monitor. Action potentials were isolated from background activity with a window discriminator (WPI), which generated output pulses for signals that crossed a lower voltage gate and peaked below an upper voltage gate. The discriminator output pulses were led to a computer for on-line data collection. Spontaneous firing rates were defined as the average frequency (in spikes per second). Time setting for data collection was 180 s with 500-ms bin size.

2.5. Experimental protocol

In both control and dependent rats, after stable recording from isolated single neurons in the nucleus paragigantocellularis (20–30 min) for baseline activity, saline was microinjected into the nucleus paragigantocellularis to distinguish between the effect of volume pressure on the unit activity of the nucleus paragigantocellularis neurons from the pharmacologic effect of a drug.

Nine minutes later, other drugs were administered according to the protocol. In the first subgroup of control rats ($n = 4$), morphine sulfate was microinjected into the nucleus paragigantocellularis and recording was continued for nearly 65 min to show the time course of morphine effects in the nucleus paragigantocellularis before morphine was washed out by circulating blood. In the second subgroup of control rats ($n = 10$), 12 min after morphine administration, naloxone was microinjected into the nucleus paragigantocellularis, then recordings were continued for 30 min. In our pilot study, the peak of the morphine effect occurred nearly 12 min after injection, so we selected this time for naloxone administration. In the third subgroup of control rats ($n = 6$), only naloxone was microinjected to determine the effect of naloxone on baseline activity (30-min recordings). Drug administration and recording for the first ($n = 7$) and second ($n = 8$) subgroups of dependent rats were just the same as for the second and third subgroups of control rats. The length of time from the last morphine administration to the time of recording was less than 2 h. Therefore, the time frame from the start of the surgical procedures to the end of the experiments was 190–225 min. According to the work of Gellert and Holtzman (1978), plasma levels of morphine are not reduced to levels which can lead to abstinence syndrome in this time frame.

2.6. Data analysis

After a unit was isolated and its stability was determined, the pre-injection spontaneous firing rate was determined. From every rat, only one neuron was recorded. Then drugs were microinjected into the nucleus paragigantocellularis and effect of their administration on the unit activity of the nucleus paragigantocellularis neurons was observed. A change in activity was defined as an increase or decrease in firing rate by mean baseline activity $\pm$ two standard deviations (2 S.D.), respectively. The obtained results are expressed as means $\pm$ standard error of mean

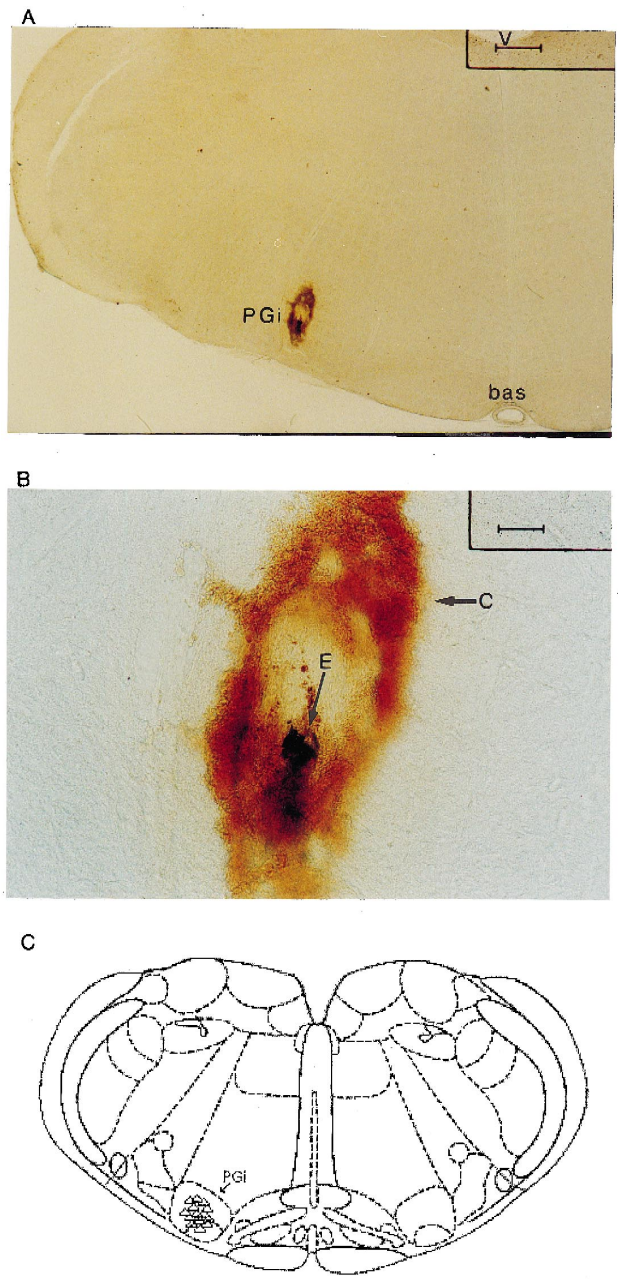

Fig. 1. A typical photomicrograph shows the site of recording electrode and injection cannula in a coronal section of the nucleus paragigantocellularis. (A) Photomicrograph ($\times 25$), bas = basilar artery, V = IVth ventricle, PGi = nucleus paragigantocellularis, scale bar = 350 μm. (B) Photomicrograph ($\times 200$), E = recording electrode site, C = injection cannula site, scale bar = 45 μm. (C) The reconstruction of the recording sites in the dependent group. Open triangles denote the recording sites.

(S.E.M.). The data were subjected to one-way or two-way analysis of variance (ANOVA) (repeated measures for comparison between the various times within each group and completely randomized for comparison between different groups) and followed by protected Tukey's test for multiple comparisons, as needed.

2.7. Histological verification

Micropipette penetrations were marked by iontophoretic ejection of dye with negative current pulses (7 μA, 10 min) (Ennis and Aston-Jones, 1988). At the end of the recording session, injection sites were marked by injection of neutral red (Fig. 1A). Animals were then deeply anesthetized and perfused with 0.9% saline followed by 10% formalin in phosphate buffer. The brains were removed and stored in the same buffer. Selected brain regions were cut in 50-μm-thick sections with a vibrotome and mounted on gelatinized glass slides. Locations of recording and injection sites were identified according to the atlas of Paxinos and Watson (1986) (Fig. 1A). All reported data are from the animals in which recording and injection sites were confirmed.

3. Results

3.1. Morphine effects on spontaneous activity of nucleus paragigantocellularis neurons of control rats

In control rats, of 14 identified nucleus paragigantocellularis neurons, morphine decreased the spontaneous firing rate of seven neurons (50%). In the remainder, morphine increased the unit activity of the nucleus paragigantocellularis neurons (Fig. 2A and C). In the following, these two subgroups are called control$^-$ and control$^+$, respectively. One-way ANOVA showed significant differences in the firing rate before and after morphine injections in both control$^-$ [$F(9,54) = 6.9$, $P < 0.0001$] and control$^+$ [$F(9,54) = 7.77$, $P < 0.0001$] subgroups. Multiple comparison test (Tukey) revealed that in the control$^-$ subgroup there were significant differences in unit activity 6, 9, and 12 min after morphine injection ($P < 0.01$). Also the same test showed that in the control$^+$ subgroup unit activity 6, 9, and 12 min after morphine microinjection was significantly different from that before morphine injection ($P < 0.01$) (Fig. 2C). The onset of morphine suppression or enhancement occurred nearly 1 min after injection and its maximum effect was observed 12–15 min after injection.

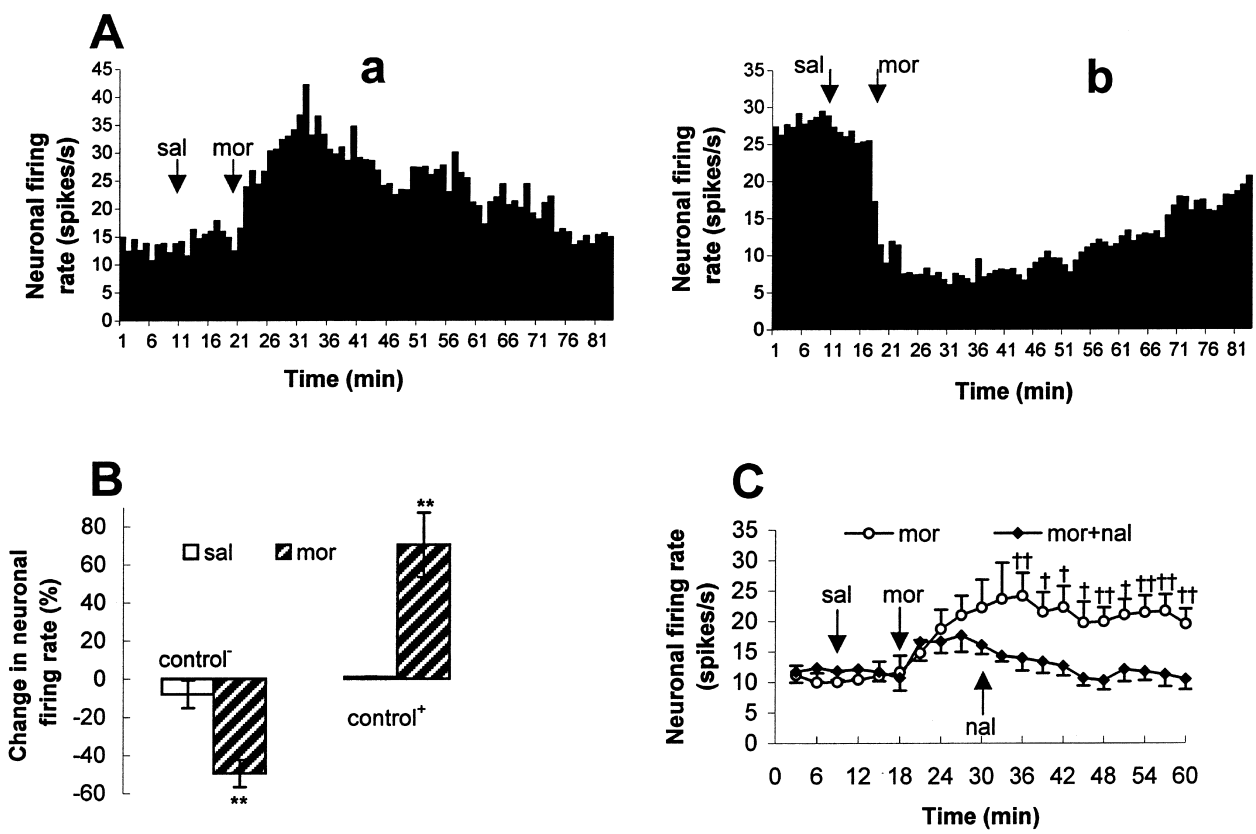

Fig. 2. (A) Typical peristimulus time histogram (PSTH) of excitatory (a) and inhibitory (b) effects of morphine (20 mg/ml, 120–200 nl) on nucleus paragigantocellularis neurons. (B) Effects of morphine on spontaneous activity of nucleus paragigantocellularis neurons ($n = 14$). In 7 of 14 neurons morphine had an excitatory effect (control$^+$) and in the rest morphine decreased the spontaneous activity (control$^-$). (C) Reversing effect of naloxone (25 mg/ml, 120–200 nl) on excitatory action of morphine ($n = 4$). $^{**}P < 0.01$, repeated measures ANOVA followed by Tukey's test. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, completely randomized ANOVA followed by Tukey's test. sal = saline, mor = morphine, nal = naloxone, PGi = nucleus paragigantocellularis.

In three rats of the control$^+$ subgroup and in one rat of the control$^-$ subgroup only morphine was microinjected to determine the latency of the morphine effect in the nucleus paragigantocellularis before morphine was washed out by circulating blood. As can be seen in Fig. 2A, the effect of morphine on the nucleus paragigantocellularis neurons lasted for at least 45 min.

In the control$^-$ subgroup, the spontaneous activity of the nucleus paragigantocellularis neurons was decreased $49.6 \pm 7.2\%$ and in the control$^+$ subgroup it was increased $70.3 \pm 16.9\%$ after morphine microinjection (Fig. 2B). The mean effect of morphine 3–12 min after injection was compared with the spontaneous activity before injection.

3.2. Naloxone effects on spontaneous activity of nucleus paragigantocellularis neurons of control rats

In control rats ($n = 6$), neither saline nor naloxone microinjections had a significant effect on the spontaneous firing rate of the nucleus paragigantocellularis neurons (Fig. 3C). One-way ANOVA did not show significant differences in the firing rate before and after injections.

In six rats of the control$^-$ subgroup in which morphine had a decreasing effect, and in four rats of the control$^+$ subgroup, naloxone was microinjected and in all cases reversed the effects of morphine. In other words, there was no significant difference between the unit activity before morphine and after naloxone injections. To determine the reversing effect of naloxone after morphine injection, two subgroups of control$^+$ rats (in the first subgroup only morphine, and in the second one morphine and naloxone have been injected) were compared (see Fig. 2C). Two-way ANOVA showed significant differences in the unit activity of neurons after naloxone injection between the two groups ($F(1,70) = 27.1$, $P < 0.01$). The data were subjected to protected Tukey's test for multiple comparisons. From six to 30 min after naloxone injection, its effect on spontaneous activity appeared to be significant (Fig. 2C).

3.3. Morphine effects on spontaneous activity of nucleus paragigantocellularis neurons of dependent rats

In the dependent group ($n = 7$) morphine microinjection failed to change the unit activity of the nucleus paragigantocellularis neurons ($2.54 \pm 2\%$). This effect of morphine was significantly different [$F(2,68) = 6.76$, $P < 0.01$] from the effect of morphine in the control subgroups. The Tukey's test revealed that the differences were signifi-

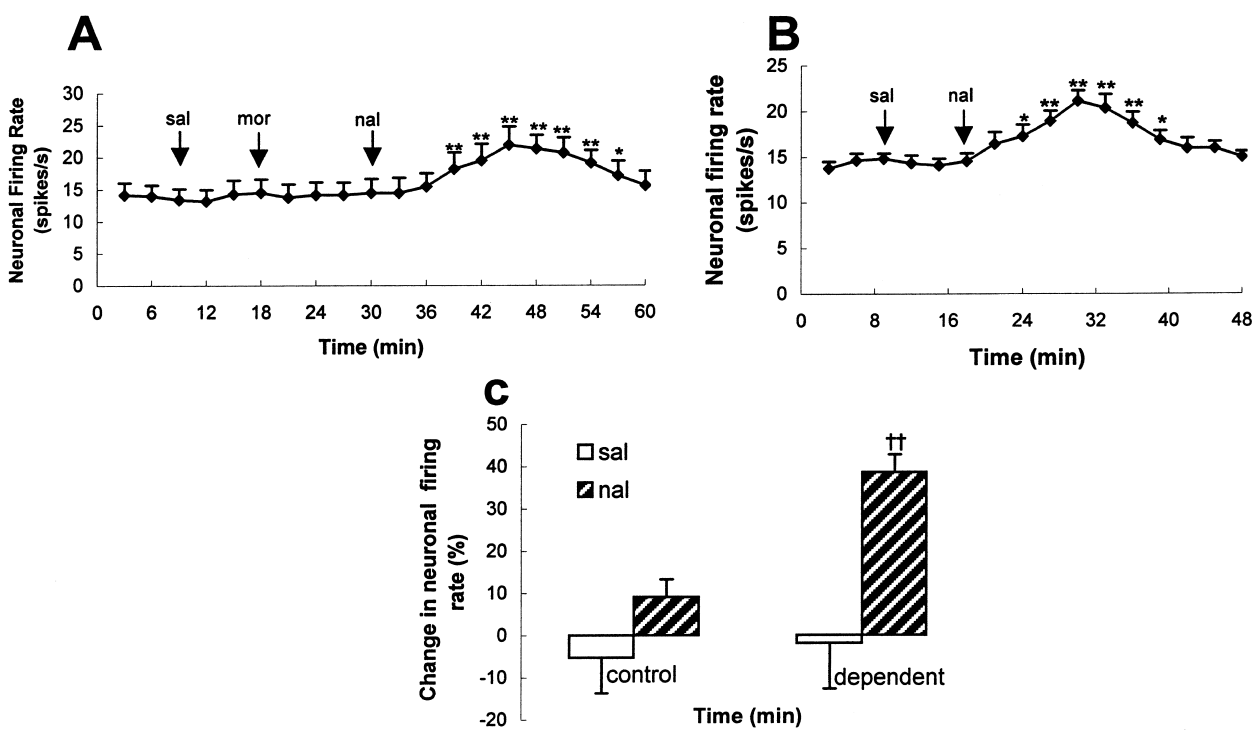

Fig. 3. Effects of morphine (20 mg/ml, 120–200 nl) and naloxone (25 mg/ml, 120–200 nl) on the spontaneous activity of nucleus paragigantocellularis neurons in dependent rats. (A) Morphine ($n = 7$) had no effect on the spontaneous activity of nucleus paragigantocellularis neurons while naloxone increased the unit activity of nucleus paragigantocellularis neurons in the presence of morphine. (B) Effect of naloxone on the spontaneous activity of nucleus paragigantocellularis neurons of morphine-dependent rats in the absence of morphine ($n = 8$). Notice that naloxone increased the spontaneous activity of nucleus paragigantocellularis neurons. (C) Effect of naloxone in control ($n = 6$) and dependent ($n = 8$) rats. Naloxone had no significant effect on the spontaneous activity of nucleus paragigantocellularis neurons of control rats while it significantly increased the unit activity of nucleus paragigantocellularis neurons from dependent rats. $^*P < 0.05$, $^{**}P < 0.01$, repeated measures ANOVA followed by Tukey's test. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, completely randomized ANOVA followed by Tukey's test. PGi = nucleus paragigantocellularis, sal = saline, mor = morphine, nal = naloxone.

cant between the dependent group and the control^{+} subgroup ($P < 0.05$) or the control^{-} subgroup ($P < 0.01$).

3.4. Naloxone effect on spontaneous activity of nucleus paragigantocellularis neurons of dependent rats

After baseline recording, isotonic saline was microinjected and 9 min later naloxone was administered into the nucleus paragigantocellularis and recording was continued for 30 min ($n = 8$). Naloxone administration increased the nucleus paragigantocellularis unit activity ($38.45 \pm 4.19\%$) (Fig. 3C). The effect of naloxone was calculated as the average of its effect at 9, 12, 15 and 18 min after administration. As for morphine, the onset of the naloxone effect was seen 1 min after microinjection and its maximum effect occurred 12 min after administration. One-way ANOVA showed significant differences in unit activity before and after naloxone microinjection [$F(15, 105) = 10.16$, $P < 0.0001$]. Multiple comparison test showed that, at 9, 12, 15, and 18 min after naloxone administration, unit activity was significantly different from that before or 21–30 min after naloxone microinjection ($P < 0.01$) (Fig. 3B).

Naloxone, which had a reversing effect in controls, significantly increased the nucleus paragigantocellularis firing rate ($53.98 \pm 7.49\%$) in dependent rats (Fig. 3A). This effect was calculated as the average effect of naloxone at 12, 15, 18, 21, and 24 min after its administration. The peak of the naloxone effect was 15 min after its microinjection into the nucleus paragigantocellularis. One-way ANOVA showed a significant difference in firing rate before and after naloxone administration [$F(19,114) = 14.64$, $P < 0.0001$). Multiple comparison test (Tukey) revealed that the spontaneous activity of the nucleus paragigantocellularis neurons at 9, 12, 15, 18, 21, and 24 min after naloxone microinjection was significantly different from that before naloxone administration ($P < 0.01$) (Fig. 3A).

A two-way ANOVA showed a significant difference in the nucleus paragigantocellularis firing rates between dependent and control groups after naloxone microinjection [$F(1, 36) = 4.48$, $P < 0.05$] (Fig. 3C). Also, the same analysis showed that naloxone (after morphine administration) increased the firing rate of the nucleus paragigantocellularis neurons significantly ($n = 7$), compared to both control subgroups [$F(2, 68) = 6.76$, $P < 0.01$].

4. Discussion

There are several protocols for the induction of dependence on morphine, such as using subcutaneous pellets (Rasmussen et al., 1996; Rasmussen, 1995), daily intraperitoneal (i.p.) injection (Leung et al., 1986), and administration of morphine in drinking water (Badawy et al., 1982). We selected the last protocol since the stress of handling and injection or the stress of the surgical procedure for morphine pellet implantation would be avoided. With this protocol, it takes more time to induce dependence and the daily consumption of morphine is greater than with the other methods. Since the animals could adjust the amount of the drug received, this method of dependence induction is more similar to human dependence and addiction.

In our experiments, there was no significant difference between the baseline firing rates in control and dependent rats despite dependence, which was confirmed by the fact that subcutaneous administration of naloxone in all treated rats produced some withdrawal signs.

In control rats, morphine had significant effects on the unit activity of the nucleus paragigantocellularis neurons (either attenuation or enhancement). This result is consistent with those reported by Satoh et al. (1979). They showed that iontophoretic administration of morphine (100–200 nA, 2–3 min) had both excitatory and inhibitory effects on the nucleus paragigantocellularis neurons. Also Azami et al. (1981) iontophoretically administered morphine into the nucleus paragigantocellularis, which contrary to our results, in most cases had no effect on the nucleus paragigantocellularis neuron activity, but when effects were seen, large currents of morphine (150 nA) tended to be excitant and smaller currents (50 nA) depressant. The second part of their results supports our data that morphine can have different effects on probably different nucleus paragigantocellularis cells. Rosenfeld (1994) showed the nucleus paragigantocellularis-injected opiates depress on-cell activity and enhance off-cell activity. Our present results are inconsistent with those reported by Kaplan and Fields (1991) and Bederson et al. (1990). They found that the cells inhibited by morphine were excited during naloxone-induced abstinence and that the cells that were excited by morphine were inhibited during abstinence. In fact, our present results are consistent with the in vitro work of Pan et al. (2000), who have reported that naloxone produces a membrane depolarization in both κ-sensitive primary cells and μ-sensitive secondary cells recorded in morphine-treated slices.

Such different actions of morphine could be attributed to descending pain inhibitory systems that are affected by opioid administration. It has been hypothesized that in supraspinal regions including the ventrolateral medulla, noxious stimulation increases on-cell firing and inhibits the spontaneously active off-cell, thus reducing the inhibitory modulation of pain conduction at the spinal cord. Administration of opioids inhibits the on-cell, causing the firing rate of the off-cell to increase, thus inhibiting nociceptive transmission in the spinal cord (Fields et al., 1991). Therefore, the opioid activation of off-cells seems to be indirect via inhibition of an inhibitory input (Heinricher et al., 1992). This suggestion is consistent with the finding of Barbaro et al. (1989) that, in barbiturate-anesthetized rats, periods of on-cell activity are associated with decreased

off-cell activity. Among the inhibitory projections to the nucleus paragigantocellularis, those originating from the periaqueductal gray seem to have a prominent role, since [Met^5]enkephalin in the nucleus paragigantocellularis is without its usual profound analgesic effect if the periaqueductal gray is simultaneously blocked with tetracaine (Rosenfeld, 1994). Besides all recording sites were at the caudal nucleus paragigantocellularis, which are not at the level of the facial nucleus (Van Bockstaele et al., 1989). In our experiments, the excitatory or inhibitory effects of morphine were seen nearly 1 min after the injection, which is consistent with the 20–60 s reported by Satoh et al. (1979), but the effects of morphine lasted for 45–50 min after injection, which is different from the 300 s after termination of iontophoresis. Probably different morphine injection procedures (pressure injection vs. iontophoresis) are responsible for this difference, especially the amount of administered morphine was higher in our experiments, which could account for its longer existence in the nucleus paragigantocellularis until it was washed out by circulating blood.

Naloxone microinjected into the nucleus paragigantocellularis of control rats at the peak of the morphine effect antagonized the effect completely, showing an opioid receptor-mediated effect that is likely mediated by μ-opioid receptors. Such a result was also reported by others (Satoh et al., 1979; Azami et al., 1981; Haghparast et al., 1998).

In dependent rats, morphine failed to cause any change in the spontaneous firing of the nucleus paragigantocellularis neurons as the result of complete tolerance to morphine. Previously, partial tolerance to morphine had been reported in the nucleus paragigantocellularis neurons of dependent rats (Haghparast et al., 1998). Naloxone, which had a reversing effect in control rats, significantly increased the nucleus paragigantocellularis firing rate in the dependent rats after morphine injection. Also, naloxone microinjection in dependent rats (without morphine injection) enhanced the nucleus paragigantocellularis neuronal activity while in control rats it had no effect. Such results could explain the occurrence of tolerance to and dependence on morphine in the nucleus paragigantocellularis neurons, although the relatively long delay in the maximum effect of morphine and naloxone (12–15 min) may suggest the possibility of involvement of other brain nuclei whose projections enter the nucleus paragigantocellularis.

The occurrence of tolerance to and dependence on morphine in the nucleus paragigantocellularis neurons was first suggested by Rasmussen and Aghajanian (1989), who showed that local opiate withdrawal in the locus coeruleus was attenuated by the nucleus paragigantocellularis lesions. Their suggestion was consistent with the findings of Andrade et al. (1983), who showed that the withdrawal activation of the locus coeruleus neurons seen in vivo is not seen in brain slices taken from morphine-dependent rats. The existence of locus coeruleus afferents from the nucleus paragigantocellularis has been reported frequently (Astier et al., 1990; Ennis et al., 1992; Ennis and Aston-Jones, 1987, 1988). These afferents, which are mostly excitatory, can cause hyperactivity of locus coeruleus neurons during naloxone-precipitated withdrawal. The temporal correlation between this hyperactivity and behavioral symptoms of withdrawal syndrome may suggest a role for the nucleus paragigantocellularis in such behaviors. This hypothesis is strengthened by the findings of Liu et al. (1999), who showed that electrical stimulation of the nucleus paragigantocellularis in morphine-naive rats can elicit behaviors similar to those observed during naloxone-precipitated withdrawal, which last 30 min after the nucleus paragigantocellularis stimulation (Rockhold et al. 2000). The results of Haghparast et al., who showed the enhancement of the nucleus paragigantocellularis spontaneous activity after subcutaneous naloxone injection in morphine-dependent rats, and our present results support the concept that the nucleus paragigantocellularis is one of the brain areas involved in locus coeruleus activation and in the behavioral symptoms of naloxone-precipitated withdrawal in dependent rats.

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