We have observed reflexive respiratory suppression, or “poststimulus respiratory suppression,” induced by electrical stimulation (ES) of thin-fiber muscular afferents [1–3]. This suppression is reversed by naloxone, suggesting the involvement of endogenous opioids. However, when the respiratory level was augmented by means of either hypercapnia, hypoxia, or naloxone administration, the magnitude of the poststimulus suppression was markedly attenuated without consistently altering the facilitatory response during the period of stimulation [4]. Although prestimulus respiratory activity is an important factor in determining the magnitude of the poststimulus suppression, a preliminary study had shown that morphine attenuated the magnitude of the poststimulus suppression when the prestimulus level of respiration was near the control level. Morphine, a common opioid agonist, is well known to depress ventilation [5]. Opioid receptors have been pharmacologically classified into at least three subtypes (μ, δ, and κ) on the basis of their interaction with opioid agonists and antagonists.

Morphine inhibits resting respiration, but it attenuates reflexive respiratory suppression in anesthetized cat through κ-receptor

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Abstract: Noxious stimulation of thin-fiber muscular afferents induces a reflexive respiratory suppression that we call “poststimulus respiratory suppression.” In anesthetized, vagotomized, paralyzed, and artificially ventilated cats, morphine depressed the level of resting respiration (inhibitory effect on resting respiration) and attenuated the magnitude of the poststimulus respiratory suppression (excitatory effect on the reflexively modified respiration). These two kinds of morphine effects were antagonized by naloxone, suggesting the participation of opioid receptors. To clarify the opioid receptor subtypes responsible for these effects of morphine, three type-selective opioid antagonists—naltrindole (δ antagonist), β-funaltrexamine (μ antagonist), and Mr2266 (κ antagonist)—were tested. The morphine-induced depression in the resting respiration was antagonized by pretreatment with the κ antagonist, not with the μ or δ antagonist. Furthermore, the morphine-induced attenuation in the magnitude of the poststimulus suppression was also blocked by the κ antagonist, but not by the μ or δ antagonist. In conclusion, (1) morphine inhibits resting respiration, but it attenuates the magnitude of the poststimulus respiratory suppression; (2) both these morphine effects are mediated by κ opioid receptors. The possibility that the κ3 receptor, one of the κ receptors subtypes, mediates the two kinds of morphine effects has been discussed. [Japanese Journal of Physiology, 50, 615–624, 2000]

Key words: morphine, respiration, reflexive respiratory depression, κ opioid receptor, Mr2266.
differences in apparent affinity for opioid ligands. Morphine is thought to be a preferential μ opioid agonist with some affinity for other subtype receptors [6, 7]. The aim of the current study is to determine the opioid receptor subtypes responsible for the actions of morphine on the resting respiration and on the magnitude of the poststimulus suppression by using three type-selective opioid antagonists.

MATERIALS AND METHODS

General procedure. Experiments were performed on 38 adult cats (body weight: 1.8–6.5 kg) anesthetized with a mixture of α-chloralose and urethane (initial doses: 60 and 300 mg/kg, i.p., respectively; supplemental doses: 1/12 of the initial doses, i.v., when necessary). Care and experiments on all animals used in this study were carried out according to the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan. Details of the general procedure are described in the preceding companion paper [4]. Briefly, the animals were vagotomized, glome-
tomized, paralyzed, and artificially ventilated by use of an air-oxygen mixture. The end-tidal CO2 and O2 concentrations (\(F_{ETO2}\) and \(F_{ETCO2}\)) were continuously monitored, and \(F_{ETCO2}\) was kept at about 20%.

To obtain an index of central respiratory activity, the neural respiratory output (RO) was calculated as average of the peak amplitude of integrated phrenic discharges (PK) and instantaneous respiratory rate (RR). To normalize data among cats, respiratory responses to inhaled CO2 (CO2 response) were measured by using a modified rebreathing method under artificial ventilation [4]. To examine the reproducibility of CO2 response curves of RO, CO2 responses were tested again 144 to 260 min after the first trials in 5 cats. The averaged data on the ratios of inflection points (standard RO values), x-axis intercepts, and initial rising slopes from apnea of the CO2 response curves to those of the first trials were 1.01 ± 0.01, 1.00 ± 0.02, and 1.02 ± 0.04, respectively. The averaged data of RO and PK per 30 s were normalized by assigning the standard RO and PK values obtained from the CO2 response curve as 100 units. The RO, PK, and RR were represented as the differ-
ences between the values during the 2 to 15 min pe-
riod after stopping ES and the values for a 1-min pe-
riod before the stimulus.

Drug administration. Naloxone hydrochloride (a nonselective antagonist) and naltrindole hydrochlo-
ride (a highly δ-selective antagonist) were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and β-funaltrexamine hydrochloride (β-FNA, a highly μ-selective irreversible antagonist) was from Research Biochemicals (Natick, MA, USA). Mr2266 base ((+)-5,9alphe-diethyl-2-(3-furylmethyl)-2'-hydroxy-6,7-
benzomorph, a relatively κ-selective antagonist) was a gift from Boehringer Ingelheim (Ingelheim am Rhein, Germany). Stock solutions of all drugs except Mr2266 were made in Ringer’s solution. Mr2266 was dissolved in 0.1 N hydrochloride solution and diluted with Ringer’s solution.

Morphine (0.3 mg/kg), naloxone (1 mg/kg), β-FNA (0.1 mg/kg), naltrindole (0.01 and 0.1 mg/kg), and Mr2266 (0.03 or 0.3 mg/kg), each in 0.7 ml of Ringer’s solution, were administered intravenously for 1 min. Higher doses of β-FNA could not be used because the dose of 0.3 mg/kg of β-FNA produced less effects on respiration than the lower dose of 0.1 mg/kg did, suggesting agonistic actions of β-FNA at higher doses [8]. Two doses (0.01 and 0.1 mg/kg) of naltrindole were sequentially tested. To produce sufficient effects by the reversible opioid antagonists, additive doses of naltrindole and Mr2266 were adminis-
tered before the administration of morphine (naltrindole, 0.1 mg/kg; Mr2266, 0.3 mg/kg). An injection of vehicle (0.7 ml) did not affect the resting respira-
tion.

Experimental design. We examined the following seven items: (1) morphine-induced respiratory depression at the control level of respiration and at about a twofold augmented level by decreasing ventilation and increasing \(F_{ETCO2}\); (2) effects of morphine on the CO2 responsiveness of respiratory activity; (3) augmentation of resting respiration induced by three type-selective opioid antagonists—β-FNA, naltrindole, and Mr2266—at the control level of respiration; (4) effects of the three type-selective opioid antagonists on the morphine-induced respiratory depression; (5) morphine-induced attenuation in the magnitude of the poststimulus respiratory suppression; (6) effects of the three type-selective opioid antagonists on the mag-
itude of the poststimulus respiratory suppression;
and (7) effects of the three type-selective opioid antagonists on the morphine-induced attenuation in the magnitude of the poststimulus respiratory suppression.

To minimize the number of animals, these examinations were performed serially as shown in Fig. 1. All animals were naive to the administration of morphine in the presence or absence of opioid antagonists.

Statistical analysis. Data are presented as mean±SE. They were analyzed by using either a Student’s t-test for unpaired or paired observations, or a one-way analysis of variance for repeated measures followed by the paired t-test. Differences were considered significant if p<0.05.

RESULTS

Effects of morphine on the resting respiration

Morphine (0.3 mg/kg) was administered to six animals when the resting respiration was near the control level and at about a twofold augmented level induced by decreasing ventilation (Group 1 in Fig. 1). Whether or not the respiratory levels were augmented, morphine markedly decreased the respiratory level, as seen in Fig. 2. The respiratory depression induced by morphine lasted for several hours. When the preinjection respiratory level was near the control, morphine depressed the level of respiration to apnea in three of the six animals. The averaged changes in the RO at 15 min after the injection of morphine were 291.1±15.9 units at the control level and 271.4±9.6 units at the augmented level (Fig. 5). The percentage change in the RO at the augmented level (−34.9±4.7%) was about half that at the control level (−70.9±13.4%) (p<0.001 by unpaired t-test).

The morphine-induced respiratory depression was clearly antagonized by naloxone in five animals tested, which had been treated with morphine at the augmented respiratory level 54 to 216 min earlier. At 7 min after the injection of naloxone (1 mg/kg), the RO increased by 51.4±15.9 units over the value before the injection of morphine.

Attenuation in the CO₂ responsiveness of respiratory activity by morphine

The CO₂ response curves were determined before and 48 to 192 min after the administration of morphine in five animals (Group 1 in Fig. 1). A representative CO₂ response curve of RO is shown in Fig. 3. In each animal, the apnea threshold (x-axis intercept) and slope (rising from apnea) of the CO₂ response curve were examined. After an administration of morphine (0.3 mg/kg), the CO₂ sensitivity of the RO was significantly reduced: Morphine elevated the apnea threshold to 6.0±0.2%, from 5.1±0.1%, and reduced the slope to 72.6±17.8 units/% CO₂, from 133.9±21.2 units/% CO₂ (p<0.05 by paired t-test, respec-
When the PK was plotted against CO₂ values, morphine similarly reduced the slope to 70.2 ± 12.3 units/% CO₂, from 100.3 ± 9.7 units/% CO₂. This morphine-induced attenuation in the CO₂ responsiveness was antagonized by naloxone, when CO₂ response curves were determined 44 to 133 min after the injection of naloxone (1 mg/kg), following morphine. The apnea threshold and slope changed to 4.5 ± 0.6% and 166.9 ± 26.2 units/% CO₂, respectively, which were near the values before the injection of morphine.

Augmentation of the resting respiration by the type-selective opioid antagonists

The effects on the resting respiration of the reversible antagonists naltrindole (δ antagonist) and Mr2266 (κ antagonist) and the irreversible antagonist β-FNA (μ antagonist) were examined.

Naltrindole was sequentially administered in two doses; first 0.01 mg/kg (first injection) and second 0.1 mg/kg (second injection) at intervals of 14 to 17 min in five animals (Group 2 in Fig. 1). The respiratory levels clearly increased after the first injection and remained almost unchanged after the second. At 7 min after the second injection, the RO had increased by 27.5 ± 5.0 units over the prefirst injection value (p<0.01 by a paired t-test) (Fig. 4A).

Mr2266 was administered at a dose of 0.3 mg/kg in five animals (Group 4 in Fig. 1). At 15 min after the injection, the RO increased by only 15.9 ± 4.6 units, but significantly (p<0.01 by paired t-test) (Fig. 4B). In three of the five animals, the short-term decrease in the RO was observed at a few minutes after the injection, possibly a result of some agonistic effect of Mr2266. In another two animals, the lower dose of 0.03 mg/kg of Mr2266 was administered. At 15 min after the injection, the RO had increased by 12.1 and 14.5 units, respectively, which were similar to the rises produced by the dose of 0.3 mg/kg.

The effects of β-FNA (0.1 mg/kg) were estimated over 100 min after the injections in five animals (Group 3 in Fig. 1), because reportedly the μ-specific irreversible binding of β-FNA reached a plateau within 60 min [9, 10] and lasted for at least 4 d [10]. At 100 min after the injection of β-FNA, the RO had...
increased by 76.2 ± 26.9 units (p < 0.05 by a paired t-test), as shown in Fig. 4C.

Effects of morphine on the resting respiration in the presence and absence of the type-selective opioid antagonists

Morphine was administered when the respiratory level was augmented by the pretreatment with either naltrindole (n = 5), β-FNA (n = 5) or Mr2266 (n = 6). Figure 5 shows the RO values before and 15 min after the injection of morphine, where the RO was administered more than 2 h before the injection of morphine. The data in the absence of the antagonists (cont. and high) are also illustrated in Fig. 2. Open column: the RO for a 1-min period before the injection of morphine; closed column: the RO for a 1-min period at 15 min after administration of morphine. Each column and bar represent the mean and SE. * , **, and *** indicate statistically significant differences at levels of p < 0.05, p < 0.01, and p < 0.001, respectively, and n.s. indicates no significance, by paired t-test.

Mr2266 was also observed when it was applied after an administration of morphine. In another two animals, which were treated with Mr2266 at 95 and 205 min after morphine administration, respectively, Mr2266 increased the RO by 37.8 and 15.0 units over the value before morphine, respectively. Reportedly Mr2266 has some affinity for μ receptors [7]; however, the antagonistic effect of Mr2266 was not mediated by μ receptors. Because Mr2266 completely antagonized the morphine-induced respiratory depression in the β-FNA–pretreated animals (Group 3 in Fig. 1), the RO shifted to 200.2 ± 52.4 units, from 103.5 ± 11.0 units, at 15 min after the injection of Mr2266. Thus it can be concluded that the morphine-induced respiratory depression is mediated by κ receptors, but not by μ or δ receptors.

Effects of morphine on the magnitude of the poststimulus suppression

The effects of morphine on the magnitude of the poststimulus suppression were examined in eight animals (Group 1 in Fig. 1), and a representative example and the averaged results of the effects are shown in Figs. 6 and 7. Before the administration of morphine, a clear poststimulus suppression was induced at the control level of respiration (Fig. 6A). After the administration of morphine, the poststimulus suppression was barely discernable when the prestimulus level of respiration was near the control level (Fig. 6B). Following a further injection of naloxone, a clear post-
stimulus suppression was observed again (Fig. 6C). The time courses of the averaged PK responses during and after ES are shown in Fig. 7A. The PK increment during the stimulation period after morphine (19.7 ± 4.5 units) was similar to that before morphine (13.6 ± 2.9 units) (Fig. 7A). However, the magnitudes of the poststimulus suppression in the RO and PK were significantly attenuated compared with those in the control (p < 0.05 by paired t-test) (Fig. 7A, B). The percentage changes of the magnitude of the poststimulus suppression after morphine were attenuated (RO, -4.5 ± 3.4%; PK, -2.0 ± 1.5%) compared with those in the control (RO, -25.0 ± 5.2%; PK, -19.3 ± 4.8%). Naloxone effectively reversed this morphine-induced attenuation in the magnitude of the poststimulus suppression (RO, -13.8 ± 4.9%; PK, -10.3 ± 4.4%) without altering the PK increment during the stimulation period (15.8 ± 3.2 units) (Fig. 7A, B).

Effects of the type-selective opioid antagonists on the magnitude of the poststimulus suppression and on the morphine-induced attenuation in the magnitude of the poststimulus suppression

The effects of pretreatment with naltrindole, β-FNA, or Mr2266 on the magnitude of the poststimulus suppression and on the morphine-induced attenuation in the magnitude of the poststimulus suppression were examined in five animals each (Groups 2–4, Fig. 1). Figure 8 shows the averaged changes in the RO compared with the results in the absence of the antagonists (data shown in Fig. 7). The averaged changes in the PK and RR were influenced by morphine and the antagonists in the same manner as in the RO (data not shown).

First, on the magnitude of the poststimulus suppression, naltrindole, β-FNA, and Mr2266 (Groups 2–4 in Fig. 1) had no significant effects (Fig. 8). After the administration of β-FNA (Group 3 in Fig. 1), the magnitude of the poststimulus suppression in four of the five animals became larger than the control, but the changes varied widely among animals.

Second, on the morphine-induced attenuation in the magnitude of the poststimulus suppression, only Mr2266 had significant effects. In the animals pretreated with naltrindole and β-FNA (Groups 2 and 3 in Fig. 1), morphine still attenuated the magnitude of the poststimulus suppression (p < 0.05 by paired t-test after a one-way analysis of variance, respectively; Fig. 8). In contrast, the pretreatment with Mr2266 (Group 4)
Two Kinds of Effects of Morphine on Respiration

Fig. 8. The morphine-induced attenuation in the post-stimulus suppression in the presence and absence of the type-selective opioid antagonists. ES was performed at the respiratory level near the control in the presence (five animals each) and absence (eight animals) of the antagonists. The data in the absence of the antagonists are also illustrated in Fig. 7. The magnitudes of the poststimulus suppression (PS-Suppression) in the respiratory output (RO) are represented as the differences between values during the 2 to 15 min period after stopping ES and the values for a 1-min period before the stimulus. Open column: in the control; closed column: after the antagonist; dotted column: after morphine in the presence of an antagonist. NTI, 26 to 32 min after naltrindole (FETCO2: 4.1±0.4%) and 20 to 102 min after morphine (FETCO2: 5.0±0.5%); FNA, 122 to 197 min after β-FNA (FETCO2: 4.2±0.1%) and 20 to 58 min after morphine (FETCO2: 4.8±0.1%); Mr2266, 28 to 33 min after Mr2266 (FETCO2: 3.7±0.3%) and 20 to 35 min after morphine (FETCO2: 3.8±0.2%). Each column and bar represent the mean and SE. Significant effects of morphine were noted in the naltrindole group (F(2,8)=7.569, p=0.0143) and in the β-FNA group (F(2,8)=6.569, p=0.0198) by a one-way analysis of variance, then a paired t-test. *Statistically different (p<0.05) between before and after administrations of morphine in the presence of antagonists by a paired t-test.

In the present study, morphine depressed the resting respiration and reduced the CO2 responsiveness of respiratory activity, which is consistent with previous reports [5]. In contrast, morphine of the same dosage attenuated the magnitude of the poststimulus suppression, which was a kind of facilitatory effect on the reflexively modified respiration. These two kinds of morphine effects were blocked by Mr2266, but not by β-FNA or naltrindole, suggesting the participation of κ opioid receptors.

Morphine is a preferential µ-selective opioid agonist with some affinity for other receptor subtypes [6, 11]. It has been reported that morphine induces analgesia through µ or µ1 [12–14] and acts on various physiological functions besides respiration, including thermoregulation [15], cardiovascular regulation [13, 16], and gastrointestinal motility [14, 17], through various opioid receptor subtypes. Morphine is thought to induce respiratory depression and analgesia through different receptor subtypes because of the significant difference between apparent pA2 values for these two effects [18] and the failure of a µ-selective antagonist to antagonize the respiratory depression [12, 19]. Furthermore, since the morphine-induced depression in respiratory rates was not antagonized by β-FNA under a less stressed condition [20], it may be that morphine-induced respiratory depression is mediated by opioid receptors other than µ.

Under stressful conditions, the endogenous opioid system may tonically suppress the respiratory activity, because naloxone facilitated the respiratory activity under a stressful condition [21], but not under less stressed conditions [22, 23]. We have previously demonstrated that the concentrations of endogenous β-endorphin, which has a broad affinity for µ, δ, and κ receptors [6, 11], in cerebrospinal fluid were reciprocally correlated with respiratory rates after surgical stress [24]. In the present study and under the same experimental conditions as the previous study, nalox-
one and the type-selective antagonists for \( \mu \), \( \delta \), and \( \kappa \) receptors facilitated the resting respiration to a variable extent, depending on the receptor subtypes, suggesting that surgical stress induced tonic suppression of the resting respiration through either of \( \mu \), \( \delta \), and \( \kappa \) opioid receptors.

The present study strongly suggests that morphine modifies the respiration through \( \kappa \) receptors in the stressful experimental condition. The \( \kappa \) receptors have been subdivided into \( \kappa_1 \), \( \kappa_2 \), and \( \kappa_3 \) subtypes [11, 25], although the \( \kappa_2 \) receptor has not been adequately examined. It has been thought that \( \kappa \) receptors are not involved in opioid-induced respiratory depression [26], since the most selective \( \kappa_1 \) agonist, U-50488, had only modest effects on respiration in vivo, if any [26, 27], except that it depressed respiratory activity in in vitro preparations [28]. Some other \( \kappa \) agonists, such as ethylketazocine, IC1197067, and U69593, however, depressed respiration [29–31]. Thus, the respiratory depression induced by \( \kappa \) agonists may be mediated by other \( \kappa \) receptors than \( \kappa_1 \). It is reported that some agents that had been considered \( \mu \) selective also retain a relatively high affinity for \( \kappa_3 \) receptors [7, 11], and \( \kappa_3 \) receptors exist with a relatively high density in the brain stem [32], which includes the area playing an important role in respiratory activity. Morphine is a \( \mu \)-selective ligand that can bind to \( \kappa_3 \) receptors, though its affinity at the \( \kappa_3 \) site is tenfold lower than at the \( \mu \) site [11]. As \( \kappa \) the antagonist in this experiment, we used Mr2266, which is rather unsselective among the different \( \kappa \) receptors [33, 34], because nor-binaltorphimine, a well-known \( \kappa \) antagonist, is a highly selective \( \kappa_1 \) antagonist, but it has a very low affinity for \( \kappa_3 \) receptor [7, 11, 33, 35]. Furthermore, Mr2266 has some affinity for \( \mu \) receptors; however, it is conceivable that the two kinds of morphine effects on respiration demonstrated in the present study is mediated by \( \kappa_3 \) receptors, because it was confirmed that Mr2266 completely antagonized the effects of morphine also in the presence of \( \beta \)-FNA.

The magnitude of the poststimulus suppression was attenuated by raising the prestimulus respiratory level [4]. However, when that level was near the control level, morphine markedly attenuated the magnitude of the poststimulus suppression, and this attenuation was antagonized by naloxone. It is possible that the effects of morphine on the magnitude of the poststimulus suppression is modulated by some specific opioid mechanism, although all three type-selective antagonists tested did not affect the magnitude of the poststimulus suppression in the absence of morphine. The morphine-induced attenuation in the magnitude of the poststimulus respiratory suppression appears to be the attenuation of opioid inhibitory potency, or tolerance. Crain and Shen postulated that opioid receptors might be linked to inhibitory (\( G_i \)) and excitatory G (\( G_o \)) proteins and that they switch their coupling from inhibitory to excitatory mode during chronic opioid treatment, resulting in tolerance [36]. The present findings that the two kinds of effects of morphine on respiration are mediated by \( \kappa \) opioid receptors may be consistent with the model in which opioid receptors are linked to \( G_i-G_o \).

The next interesting questions to be asked may be on the mechanisms of opioid receptor-mediated modulation in the respiratory neuronal circuits, although this is beyond the scope of the present paper. There are only a few reports on this line, which clearly show that \( \mu \)-opioid receptors in the pre-Bötzinger complex are involved in the depression of frequency of neonatal rat respiration [37, 38]. In our present study, however, morphine influenced both RR and PK via opioid receptors other than \( \mu \) (probably \( \kappa_3 \)); therefore it seems unlikely that the pre-Bötzinger complex is implicated in these morphine effects. At present we have no definite answer to the above-mentioned question, since there are no works concerned with the modulation of other respiratory neuronal circuits through identified opioid receptors. One possible site we can suppose is the parabrachial region. In our previous study, the microinjection of a very low dose of morphine into the parabrachial region (NPB) immediately caused a sustained, naloxone-reversible depression of respiration, suggesting a direct action of morphine on the NPB [39]. Moreover, a microinjection of kainic acid into the medialis and lateralis regions of the NPB revealed that (1) the NPB plays a critical role in the poststimulus respiratory suppression; (2) the medialis and lateralis regions of the NPB have facilitatory and inhibitory effects, respectively, on respiration; and (3) the magnitude of the poststimulus suppression is very likely to be controlled through the counterbalancing effects of facilitatory and inhibitory activities in these two regions of the NPB [40], as we have discussed in the preceding companion paper [4].

In summary, the present study first reveals that morphine inhibited resting respiration and attenuated the magnitude of the poststimulus respiratory suppression via \( \kappa \) receptor, possibly the \( \kappa_3 \) receptor. However, the neuronal circuits and receptor subtypes responsible for these phenomena remain to be identified in future experiments.

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