Effects of a κ -Receptor Agonist U-50488 on Bulbar Respiratory Neurons and Its Antagonistic Action Against the μ Receptor-Induced Respiratory Depression in Decerebrate Cats

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ABSTRACT—The function of κ receptor-mechanisms in bulbar respiratory network was investigated in decerebrate cats. Intravenous injection of U-50488 (0.3–3.0 mg/kg) dose-dependently decreased the phrenic nerve discharge and shortened inspiration and expiration. U-50488 caused hyperpolarization, and decreased input resistance and the action potential discharge in respiratory neurons. The effects of U-50488 were antagonized by nor-binaltorphimine. DAMGO (0.3 mg/kg, i.v.) decreased the phrenic discharge and prolonged inspiration and expiration. U-50488 partially reversed the respiratory depression induced by DAMGO. These results suggest that the activation of κ receptors by itself depresses the central respiratory activity, while it opposes the μ receptor-mediated respiratory depression.

Keywords: Respiratory neuron, Phrenic nerve discharge, κ Receptor-mechanism

Opioid receptors are the primary sites of actions of opiates and endogenous opioid peptides, which have a variety of physiological and pharmacological effects. It is accepted that these effects are largely mediated by μ receptors and subsidiarily by δ and κ receptors (1). In addition to analgesia, respiratory depression is one of the main central effects of opioids (2). μ Receptors were shown to decrease the respiratory magnitude, and μ and/or δ receptors were shown to modify the respiratory cycle (3, 4). The κ agonists appear to have little effect on respiration (2, 5). Only larger doses cause a rapid breathing pattern (6, 7). However, the function of κ receptors on the bulbar respiratory network is still unclear. To address this, the present study was performed to investigate the effects of a κ agonist U-50488 on the phrenic nerve discharge and membrane potential of bulbar respiratory neurons in decerebrate cats. Furthermore, the antagonizing action of κ receptors against the μ receptor-mediated responses has been demonstrated in the brain, including analgesia, tolerance, reward and memory (8). Although U-50488 is reported to reverse the μ receptor-induced hypercapnia and hypoxia (9), the interaction between κ and μ receptors in the central respiratory system needs more investigation. Therefore, we

studied the antagonism between U-50488 and a μ agonist DAMGO in the neuronal respiratory activity.

The present study was conducted in accordance with Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. Adult cats of either gender (body weight 2.7–4.0 kg) were anesthetized with halothane. The femoral artery and vein were cannulated, respectively, for recording arterial blood pressure and for administration of drugs. The adequate depth of anesthesia during surgery was assessed by monitoring a stable blood pressure and heart rate and a total absence of nociceptive reflex. Mid-collicular decerebration, occipital craniotomy and pneumothorax were performed. The cervical vagus and carotid sinus nerves were cut bilaterally. The animals were paralyzed with pancuronium bromide, and the lungs were artificially ventilated with a positive end-expiratory pressure of 1–2 cmH₂O. The end-tidal concentrations of O₂ and CO₂ were maintained at 29–31% and 4.0–4.5%, respectively. Rectal temperature was kept at 36.5–38.0°C by external heating. After the surgery, halothane anesthesia was discontinued and a minimum of 3 h elapsed before respiratory activities were recorded. Efferent discharge of the desheathed phrenic nerve was recorded and integrated by a leaky integrator (0.1-s time constant) for monitoring the central respiratory activity. Membrane potential of respira-

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tory neurons was recorded with single micropipettes filled with 2 M K-citrate (20–50 M Ω) from the ventral respiratory group (3, 10). Augmenting inspiratory (aug-I), postinspiratory (post-I) and augmenting expiratory (aug-E) neurons were identified by the temporal relationship of the membrane potential trajectory to phrenic nerve discharge (3, 4, 10). Input resistance was measured by injecting a negative constant current (1.0 nA, 100 ms, 2 Hz) through the recording pipette. The selective agonist of κ receptors U-50488 hydrochloride (*trans*-($\pm$)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrol-idinyl)-cyclohexyl]-benzeneacetamide, 0.3–3.0 mg/kg; TOCRIS, St. Louis, MO, USA), the antagonist of κ receptors nor-binaltorphimine dihydrochloride (nor-BNI, 1.0 mg/kg; TOCRIS), the selective agonist of μ receptors DAMGO (Tyr-D-Ala-Gly-NMePhe-Gly-ol-enkephalin, 0.3 mg/kg; RBI, Natick, MA, USA) and naloxone hydrochloride (NLX, 0.03 mg/kg; RBI) were dissolved in physiological saline and administered intravenously. At the end of the experiments, the animals were killed with pentobarbital (>100 mg/kg, i.v.). All recordings were monitored on computer display using a signal processing software (PowerLab/4s; ADInstruments, Castle Hill, Australia) and

stored on a hard disk. The amplitude of integrated phrenic discharge and the duration of inspiration (Ti) and expiration (Te) were measured. Membrane potential of respiratory neurons was measured at the active and inactive phases. Data were expressed as the mean $\pm$ S.D. Significant difference was evaluated by the Student's paired *t*-test or the Dunnett multiple comparison test following one-way ANOVA at $P < 0.05$.

The phrenic nerve displayed an augmenting discharge during inspiration, a small decrementing after-discharge during early expiration and a complete arrest of discharge during late expiration (Fig. 1). The average values of Ti and Te in 14 animals were 1.6 ± 0.5 and 3.2 ± 0.9 s, respectively. U-50488 at doses of 1.0 and 3.0 mg/kg decreased the phrenic discharge and shortened Ti and Te in a dose-dependent manner. The lowest dose (0.3 mg/kg) had no effect. The response reached a peak 3–5 min after the injection and recovered between 15–20 min. Changes in the respiratory subphases and phrenic activity are shown in Fig. 1, C and D, respectively.

Stable intracellular recordings were achieved in 8 (3 aug-I, 2 post-I, 3 aug-E) neurons. As illustrated previously

Fig. 1. Effects of U-50488 on the phrenic nerve activity. U-50488 was intravenously injected at a dose of 1.0 mg/kg (A) and of 3.0 mg/kg (B). Abbreviations: PN, phrenic nerve discharge; int. PN, integrated phrenic nerve discharge. Traces were taken before and 5 min after the injection. C: Percent changes in the inspiratory time (Ti) and expiratory time (Te). D: Percent changes in the peak amplitude of phrenic neurogram. Values are the mean $\pm$ S.D. ($n = 4-6$). * $P < 0.05$, ** $P < 0.01$ vs the pre-drug value (Dunnett's test).

(3, 4, 10), aug-I neurons (Fig. 2A) and aug-E neurons (Fig. 2B) displayed an incrementing depolarization during inspiration and during expiration, respectively, and post-I neurons exhibited a decrementing depolarization during early expiration. All neurons discharged action potentials during the depolarizing phase and hyperpolarized during the remaining phase. The membrane potential was -55.9 ± 4.3 mV at the active phase and -68.8 ± 3.7 mV at the inactive phase. The amplitude of membrane potential fluctuations was 12.9 ± 2.5 mV. U-50488 (3.0 mg/kg) produced membrane hyperpolarization of 2.3 ± 0.9 mV ($P < 0.05$) at the active phase and 1.9 ± 0.6 mV ($P < 0.05$) at the inactive phase, but no change in the membrane potential fluctuation (Fig. 2A). The burst discharge of action potentials was decreased. Nor-BNI antagonized the effects of U-50488, while the agent itself had no significant effect. Input resistance was measured in 4 (2 aug-I, 1 post-I, 1 aug-E) neurons (Fig. 2B). Control values of membrane resistance were 6.1 ± 2.5 M Ω during the active phase and 11.7 ± 3.8 M Ω during the inactive phase. U-50488 decreased the input resistance by $13 \pm 6\%$ ($P < 0.05$) of the control throughout the respiratory cycle.

DAMGO (0.3 mg/kg) significantly decreased the phrenic nerve discharge and prolonged the respiratory cycle, due

to lengthening of Ti and Te (Fig. 3A). This depression gradually developed and became stable 20–30 min after the drug injection. In some cases it was followed by apnea. The response did not recover for 2 h. The effects of U-50488 against the DAMGO-induced respiratory depression were examined in 5 animals (Fig. 3B). U-50488 reversed significantly, but not completely, the reduced phrenic nerve discharge and prolonged Ti and Te. An additional injection of nor-BNI returned the pattern to that before U-50488. Naloxone completely antagonized the DAMGO's effects. Figure 3C summarizes the changes in the respiratory sub-phases and phrenic nerve discharge induced by DAMGO and by addition of U-50488.

The present study demonstrated for the first time i) a weak but significant depressive effect of a κ agonist and ii) μ -opposing action on the central respiratory activity in vivo.

U-50488 decreased the central respiratory activity and shortened Ti and Te. However, the effects were weaker and shorter, when compared with those of DAMGO. These results are consistent with the previous in vivo findings using rats (7) and rhesus monkeys (6), where similar doses of U-50488 decreased tidal volume and increased respiratory frequency. However, in vitro experiments using neo-

Fig. 2. Effects of U-50488 on an aug-I neuron (A) and an aug-E neuron (B). A: A set of traces of membrane potential (MP) and phrenic nerve discharge (PN) were taken before, 5 min after U-50488 and 5 min after nor-binaltorphimine (nor-BNI). B: Input resistance was measured by injecting a negative constant current (-1 nA, 100 ms, 2 Hz) through the recording pipette.

Fig. 3. Antagonistic effects of U-50488 against the respiratory depression induced by DAMGO. **A:** Effects of DAMGO on the phrenic nerve activity and antagonism of naloxone (NLX). **B:** Antagonistic effects of U-50488 against the DAMGO-induced respiratory depression. U-50488 was administered 20 min after DAMGO injection, which was followed by nor-BNI injection (5 min after U-50488). Naloxone (NLX) was injected 5 min after nor-BNI injection. Abbreviations: PN, phrenic nerve discharge; int. PN, integrated phrenic nerve discharge. **C:** Percent changes against the pre-drug value in the inspiratory time (Ti), the expiratory time (Te) and the peak amplitude of phrenic neurogram (PN). Values are the mean $\pm$ S.D. ($n = 5$). * $P < 0.05$ (Student's paired t -test).

nate rats showed no change (5) or a decrease in respiratory frequency (11) after U-50488. This discrepancy may be due to differences in the experimental conditions and animals used. In the present study, U-50488 hyperpolarized the membrane throughout the respiratory cycle and decreased the discharge activity in three types of bulbar respiratory neurons. Since nor-BNI completely antagonized the effects of U-50488, these changes are mediated by κ receptors, probably κ_1 receptors (12). Moreover, the possibility that high doses of κ agonists might exert non-selective stimulating actions on other opioid receptors (2) is excluded. It has been demonstrated that the activation of κ receptors causes hyperpolarization by increasing K^+ conductances in the postsynaptic membrane through a direct G-protein coupled mechanism (13), or inhibits transmitter release from the presynaptic terminals by decreasing Ca^{2+} conductances (14). Since input resistance decreased while the membrane potential fluctuations remained unchanged after U-50488,

the agent is more likely to increase the postsynaptic K^+ conductances in respiratory neurons. Together, the decreased activity in three types of respiratory neurons induced by κ receptors may lead to shortening of the respiratory cycle and depression of the central respiratory activity.

Respiratory depression caused by DAMGO was partially but significantly reversed by U-50488. This antagonizing effect of U-50488 was blocked by nor-BNI. The result indicates that κ receptors have an opposing effect on the μ receptor-mediated respiratory inhibition. Interaction between κ and μ receptors is demonstrated for analgesia in the bulbospinal tract, dopamine release in the nucleus accumbens and long-term potentiation in the hippocampus (8). In those cases, the antagonizing interaction can be attributed to distinct cellular locations of the two receptors, i.e., μ and κ receptors are differentially localized in the physiologically different types of neurons or terminals. This may be a case in the central respiratory system, since

three subtypes of opioid receptors are reported to distribute widely in the respiratory-related area in the brainstem (15, 16). Activation of μ receptors by intravenous DAMGO or morphine decreased the membrane potential fluctuation and increased input resistance in respiratory neurons by decreasing both excitatory and inhibitory transmissions (A. Haji et al., unpublished observation). Therefore, it seems likely that the κ and μ mechanisms act at the different sites independently.

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