

Activation of Opioid μ Receptors in Caudal Medullary Raphe Region Inhibits the Ventilatory Response to Hypercapnia in Anesthetized Rats

Zhenxiong Zhang, Ph.D., Fadi Xu, M.D.,† Cancan Zhang, B.S.,‡ Xiaomin Liang, Ph.D.**

Background: **Opioids, extensively used as analgesics, markedly depress ventilation, particularly the ventilatory responsiveness to hypercapnia in humans and animals predominantly *via* acting on μ receptors. The medullary raphe region (MRR) contains abundant μ receptors responsible for analgesia and is also an important central area involving carbon dioxide chemoreception and contributing to the ventilatory responsiveness to hypercapnia. Therefore, the authors asked whether activation of μ receptors in the caudal, medial, or rostral MRR depressed ventilation and the response to hypercapnia, respectively.**

Methods: **Experiments were conducted in 32 anesthetized and spontaneously breathing rats. Ventilation and it response to progressive hypercapnia were recorded. The slopes obtained from plotting minute ventilation, respiratory frequency, and tidal volume against the corresponding levels of end-tidal pressure of carbon dioxide were used as the indices of the respiratory responsiveness to carbon dioxide. DAMGO ([d-Ala2, N-Me-Phe4, Gly-ol]-enkephalin), a μ-receptor agonist, was systemically administered (100 μg/kg) before and/or after local injection of CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH2) (100 ng/100 nl), a μ-receptor antagonist, into the caudal MRR, or locally administered (35 ng/100 nl) into the MRR subnuclei.**

Results: **The authors found that systemic DAMGO significantly inhibited ventilation and the response to carbon dioxide by 20% and 31%, respectively, and these responses were significantly diminished to 11% and 14% after pretreatment of the caudal MRR with CTAP. Local administration of DAMGO into the caudal MRR also reduced ventilation and the response to carbon dioxide by 22% and 28%, respectively. In sharp contrast, these responses were not observed when the DAMGO microinjection was made in the middle MRR or rostral MRR.**

Conclusions: **These results lead to the conclusion that μ receptors in the caudal MRR rather than the middle MRR or rostral MRR are important but not exclusive for attenuating the hypercapnic ventilatory response.**

FOR nearly two centuries, opioids have been among the most frequently used drugs to alleviate pain, coughing, and smooth muscle spasticity. But opioids cause respiratory depression, a particular problem when they are used as analgesics.[1] It is well documented that therapeutic doses of opioids substantially attenuated the ventilatory responsiveness to hypercapnia, one of the most important chemoreflexes for maintaining eupneic breathing, in both animals and humans[2–6] (see early references[7,8]). This depression is mainly due to a decrease in gain of the ventilatory response to carbon dioxide (*i.e.*, a decrease in the slope of ventilatory response to carbon dioxide).[8–13] The life-threatening impact on hypercapnic ventilatory reflex has markedly limited the use of opioids, especially in patients with breathing disorders such as obstructive sleep apnea and chronic obstructive pulmonary disease.[14] Opioids inhibit respiration mainly *via* stimulating central μ receptors,[12,15–20] although some early studies indicate involvement of both μ and δ receptors.[21,22] Intravenous administration of μ-receptor agonists significantly depressed ventilatory responsiveness to hypercapnia in a dose-dependent manner in awake and/or anesthetized human and animals.[2–6,12,16–18] Furthermore, administration of μ-receptor agonists did not produce this depression in μ receptor–deficient mice.[12,15,18] Currently, it is not fully understood which central sites are responsible for the opioid-induced depression of ventilatory responsiveness to hypercapnia.

The medullary raphe region (MRR) is an area critically involved in ventilatory responsiveness to hypercapnia and nociception. Numerous studies have shown that the MRR is important for regulating pain (see the review[23]). There are several lines of evidence that the MRR is one of the major contributors to respiratory modulation, especially to ventilatory response to hypercapnia. First, Nattie *et al.* reported that microinjection of acetazolamide, a carbonic anhydrase inhibitor, or carbon dioxide dialysis into the MRR to cause local tissue acidification significantly increases ventilation.[24,25] In contrast, a lesion or inhibition of the neurons limited to this region profoundly attenuated the ventilatory response to hypercapnia.[26,27] Second, in the *in vitro* studies, several investigators have demonstrated that 22% of primary cultured cells from the MRR respond to carbon dioxide/hydrion by increasing the neuronal firing rate,[28,29] demonstrating the local presence of central chemosensitive neurons. Third, fos immunoreactivity, a marker of neuronal excitation, was significantly increased in the MRR, particularly in the caudal MRR (cMRR) after exposure to hypercapnia.[30] Fourth, neurons in the MRR project extensively into the respiratory-related nuclei, including the ventral[31] and dorsal respiratory groups,[32] and innervate respiratory motoneurons.[33] Because the rat MRR has a rich distribution of μ receptors,[34] as identified by using immunohistologic approaches, we hypothesized that MRR μ receptors are involved in opioid-induced

* Postdoctoral Fellow, † Scientist, ‡ Technician.

Received from the Pathophysiology Program, Lovelace Respiratory Research Institute, Albuquerque, New Mexico. Submitted for publication December 12, 2006. Accepted for publication May 3, 2007. Supported by grant No. HL074183 from the National Heart, Lung, and Blood Institute, Bethesda, Maryland, and grant No. C-016N from the American Lung Association, New York, New York.

Address correspondence to Dr. Xu: Lovelace Respiratory Research Institute, Pathophysiology Program, 2425 Ridgecrest Drive Southeast, Albuquerque, New Mexico 87108. fxu@lrri.org.

depression of ventilation and the responses to hypercapnia. To examine this hypothesis, our experiments were conducted in anesthetized and spontaneously breathing rats. The baseline ventilation and ventilatory responses to progressive hypercapnia were compared before and after administering DAMGO ([d-Ala2, N-Me-Phe4, Gly-ol]-enkephalin), a selective μ-receptor agonist, systemically and locally into the MRR. Our preliminary data showed a specific involvement of cMRR μ receptors in the ventilatory responsiveness to carbon dioxide. Therefore, we also compared the effects of systemic DAMGO on the ventilatory responses before and after microinjection of CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH2), a μ-receptor antagonist, into the cMRR to explore the role of these receptors in the systemic DAMGO-induced ventilatory responses.

Materials and Methods

The experimental protocols were approved by the Institutional Animal Care and Use Committee in Lovelace Respiratory Research Institute, Albuquerque, New Mexico, accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, USA. The experiments were performed in tracheotomized and spontaneously breathing Sprague-Dawley adult male rats (350–450 g).

General Animal Preparation

Animals (n = 32) were initially anesthetized with sodium pentobarbital (30 mg/kg) and urethane (600 mg/kg, intraperitoneal). Supplemental urethane was administered, if needed, to reach an adequate level of anesthesia where rats exhibited neither an eye-blink nor limb-withdrawal reflex throughout the experiment. The right femoral vein and artery were cannulated, the former for drug administration and the latter for monitoring mean arterial blood pressure and heart rate (HR). The trachea below the larynx was exposed through a midline incision, tracheotomized by blunt dissection, and cannulated. The tracheal cannula was connected with a pneumotachograph to record airflow. The pneumotachograph had a linear flow–pressure relation in the range of 2–20 ml/s, a flow resistance of 0.046 cm H_2O ml/s, and a dead space of 0.2 ml. Another end of the pneumotachograph was placed (approximately 5 mm deep) in a plastic tube with a diameter fivefold greater than that of the pneumotachograph. A three-way stopcock was attached to the other side of the plastic tube and connected to a supplemental gases device through a gas-mixing flowmeter (GF-3MP; Cameron Instrument Co., Port Aransas, TX) with the flow volume at 1.0 l/min. By turning the switch, the gases to be inhaled from the different tanks were controlled. End-tidal pressure of carbon dioxide (P_{ETCO_2}) was measured *via* a carbon dioxide analyzer (MicroCapStar end-tidal carbon dioxide analyzer, model 15-10000; CWE, Inc., Ardmore, PA) connected to a side port of the tracheal cannula. Animals were placed into a rigid metal frame with their heads fixed and centered in a stereotaxic apparatus (model 1404; Kopf, Tujunga, CA). A hole (approximately 10 mm in diameter) was drilled at the midline of the skull in some rats for microinjection into the MRR. The animals' core temperature was monitored with a rectal probe and maintained at 36.5°–37.5°C by a heat pad and radiant heat lamp.

Hypercapnic Exposure

The rats were exposed to four progressive, steady state concentrations of carbon dioxide, *i.e.*, 3, 5, 7, and 9% CO_2 (balanced with 40% O_2 and varied percentages of nitrogen). Each concentration lasted continuously for 2 min before switching to the next higher carbon dioxide concentration. It should be noted that the V_E and V_T responses to hypercapnia did not always reach the plateau during the 2-min exposure in the current study.

Microinjection into the MRR

A 25-gauge, 0.5-μl needle (Hamilton, Reno, NV), prefilled with saline or agents, was inserted into the given region of the MRR. According to the rat stereotaxic atlas of Paxinos and Watson,[35] the MRR, extended from 9–12 mm caudal to the bregma, was divided into three subregions, *i.e.*, the rostral, middle, and caudal MRRs (rMRR, mMRR, and cMRR, respectively), located at 9.0, 10.5, and 12.0 mm caudal to the bregma, respectively. The rMRR contained the magnus nucleus; the mMRR contained the magnus nucleus and its neighbor pallidus nucleus (RPa). The central sites for the mMRR and rMRR were localized 9 mm ventral to the cerebellar surface, whereas the central sites of the obscurus nucleus (ROb) and RPa that compose the cMRR were localized 8.3 and 9.3 mm ventral to the cerebellar surface. Each site of the rMRR and mMRR received 100 nl microinjection. Because the two nuclei in the cMMR were located separately and distantly, two injections (each 100 nl) were given. That is, the first was given when the needle was placed into the center of the ROb. After completion of the first injection, the needle was advanced 1 mm deeper, equivalent to the RPa, followed by the second injection. To test the unique role of the cMRR, DAMGO was purposely administered into the regions outside the cMRR in few rats, in which DAMGO was microinjected into the sites lateral (0.5 mm) to the cMRR.

Experimental Protocol

Hyperoxia (40% O_2, 1 l/min) was applied throughout the experiment to serve as a baseline. After stabilization of the cardiorespiratory baseline values for at least 10 min, effects of intravenous injection of DAMGO (0.3 ml, 100 μg/kg, intravenous; similar to the dose used in

previous studies[36]) on baseline ventilation were tested in 16 rats. Fourteen of these rats received the progressive hypercapnic exposure twice before systemic DAMGO injection and once 5 and 30 min after injection. The same protocols were repeated 2 h later in 2 of 14 rats to test the reproducibility of the DAMGO effect on the response to carbon dioxide over time. To determine the role of cMRR μ receptors in the ventilatory responses to systemic DAMGO, the effects of systemic DAMGO on ventilation and the responses to carbon dioxide were tested before and 2–3 min after local administration of CTAP (100 ng/100 nl) into cMRR in 5 of 14 rats.

The effects of stimulating the MRR μ receptors on cardiorespiratory activity were estimated by microinjection of DAMGO (35 ng/100 nl) into the cMRR, mMRR, and rMRR in three groups of rats (n = 7, 6, and 6, respectively), and the dose was chosen according to the previous studies.[37,38] Among these animals, three rats in each group came from those previously receiving an intravenous administration of DAMGO, and the remaining 10 rats underwent no previous systemic DAMGO. In those animals receiving previous intravenous DAMGO, a 2-h interval was allowed for recovery from systemic administration of DAMGO before microinjection into the MRR subregions, because this intravenously injected agent has a brief elimination half-life around 15 min in mammals.[39] Our preliminary studies showed that microinjection of DAMGO into the cMRR rather than mMRR or rMRR evoked remarkable respiratory changes, and the recovery time was longer as compared with the responses induced by intravenous injection. Therefore, progressive hypercapnia was performed twice before microinjection and once 5, 30, 60, and 90 min after microinjection.

Six other rats were also used to test whether systemic and local injection of vehicle (n = 3) or whether local injection of DAMGO into the regions outside the cMRR (n = 3) could alter the respiratory responses.

Identification of Microinjection Sites

After completion of the experiment, all animals were killed by an overdose of anesthetic and cervical dislocation. The brainstem was removed and fixed by soaking in 4% paraformaldehyde (pH 7.4) for at least 36 h at 4°C, and subsequently sectioned at a 40-μm thickness by a slicing machine (Leica, CM 1850; Microsystems GMbH, Nussioch, Germany). The area marked by Chicago sky blue was identified under a microscope, and the center of the stained area was used as the location of microinjection.

Preparation of μ-Receptor Agonist and Antagonist

DAMGO and CTAP (Sigma-Aldrich, St. Louis, MO) were dissolved in 0.9% saline containing 1% Chicago sky blue for central injection. DAMGO for systemic injection was prepared in 0.9% saline.

Data Acquisition and Statistical Analysis

Raw data of the airflow signal, blood pressure, HR, P_{ETCO_2}, and rectal temperature were digitized, monitored, and recorded by using a PowerLab/8sp (model ML 785; AD Instruments Inc., Colorado Springs, CO) connected to a computer using the PowerLab Chart 5 software. The airflow signals were integrated to generate tidal volume (V_T), respiratory frequency (f), and minute ventilatory volume (V_E). After stabilization, the cardiorespiratory baseline was determined by averaging the variables for 1 min immediately before administration of DAMGO. Because DAMGO injection into the femoral vein or the cMRR inhibited V_E and increased P_{ETCO_2}, this V_E was defined as hypercapnic baseline V_E. Three steps (isocapnic calculation) were taken to correct the DAMGO-induced hypercapnic effects on the baseline V_E. We calculated (1) ΔP_{ETCO_2} (the differences of P_{ETCO_2} before and after DAMGO administration), (2) ΔV_E (slope of $V_E - P_{ETCO_2} \times \Delta P_{ETCO_2}$), and (3) isocapnic baseline V_E after DAMGO administration (the differences between the hypercapnic baseline V_E and ΔV_E). The adjustment of baseline V_T and f after DAMGO administration was conducted by using the same approach. The responses to progressive hypercapnia were defined by averaging the variables for the last 10 s of the 2 min of given carbon dioxide exposure. Our preliminary studies showed linear correlations of respiratory (V_E, V_T, and f; $P < 0.05$) instead of cardiovascular variables, with P_{ETCO_2} as reported previously.[15,40] The linear correlation between V_E (V_T or f) and P_{ETCO_2} was tested by Pearson correlation with Microsoft Excel (Microsoft Corporation, Redmond, WA). Subsequently, if significant correlations existed, least squares analyses of linear regression were applied to obtain the values of the slopes. These slopes were used as the indices of ventilatory responsiveness to hypercapnia (chemosensitivity). The blood pressure and HR responses to carbon dioxide were presented as percent change from control (without hypercapnia). The control ventilatory responses to carbon dioxide were determined by averaging the relevant variables from the two tests before administration of DAMGO.

All data are presented as mean ± SE. A paired *t* test was used to test the significant differences of cardiorespiratory baseline variables obtained immediately before and after administration (systemic or local) of DAMGO. One-way analysis of variance for repeated measures was used to compare the differences of the cardiorespiratory responses to progressive hypercapnia: (1) before and 5 and 30 min after systemic or local administration of DAMGO into the rMRR and mMRR; (2) before and 5, 30, 60, and 90 min after microinjection of DAMGO into the cMRR; and (3) before and after systemic DAMGO with and without pretreatment of CTAP. The Fisher least significant difference posttest was used if the overall analysis of variance (an omnibus test) had a *P* value less than 0.05. The software Statistica 6.0 (StatSoft, Inc.,

Tulsa, OK) was used for statistical analysis. Differences are considered significant at a P value less than 0.05.

Results

Effects of Systemic DAMGO on Cardiorespiratory Activities

In the current study, baseline P_{ETCO_2}, V_E, f, V_T, blood pressure, and HR in the 32 anesthetized rats were 43.2 ± 2.6 mmHg, 198.6 ± 8.4 ml/min, 103 ± 4 breaths/min, 1.9 ± 0.1 ml, 122 ± 4 mmHg, and 367 ± 11 beats/min, respectively, which are similar to those reported previously.[41] Systemic DAMGO significantly increased baseline P_{ETCO_2} (from 41.3 ± 2.4 to 48.4 ± 2.6 mmHg; n = 16; $P < 0.01$) and decreased baseline V_E by 20% due to the depression of f, and lowered blood pressure by 18% with no change in HR (fig. 1). The baseline V_E, f, and V_T before and after systemic DAMGO were 201 ± 10 *versus* 161 ± 6 ml/min ($P < 0.01$), 105 ± 5 *versus* 76 ± 4 breaths/min ($P < 0.01$), and 2.1 ± 0.2 *versus* 2.2 ± 0.2 ml ($P > 0.05$), respectively. After isocapnic calculation, the adjusted baseline V_E, f, and V_T after systemic DAMGO were significantly reduced to 56 ± 8 ml/min, 72 ± 6 breaths/min, and 1.2 ± 0.3 ml, respectively (P values < 0.01 as compared with the data obtained before DAMGO). As compared with baseline, 9% CO_2 increased the responses of V_E, f, and V_T by 234 ± 16, 18 ± 3, and 182 ± 14%, respectively. The representative recordings of the cardiorespiratory responses to progressive hypercapnia before and after intravenous DAMGO are shown in figure 2, whereas the group data of respiratory responsiveness to hypercapnia are presented in figure 3. Intravenous administration of DAMGO significantly depressed the V_E and V_T responsiveness to carbon dioxide by 31% and 33%, respectively, with little effect on f responsiveness (figs. 2 and 3). The ventilatory changes disappeared approximately 30 min after DAMGO administration. With respect to cardiovascular activity, no significant blood pressure (−0.3 ± 1.1%) or

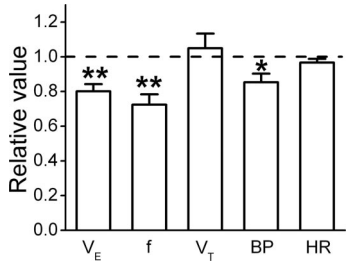

Fig. 1. Effect of intravenous injection of DAMGO (100 μg/kg) on cardiorespiratory baseline variables in anesthetized and spontaneously breathing rats. All variables are presented as relative values normalized to control represented by the *horizontal dashed line*. Data are presented as mean ± SE; n = 16. * $P < 0.05$, ** $P < 0.01$ between the data obtained before (control) and after DAMGO administration by paired *t* test. BP = mean arterial blood pressure; f = respiratory frequency; HR = heart rate; V_E = minute ventilation; V_T = tidal volume.

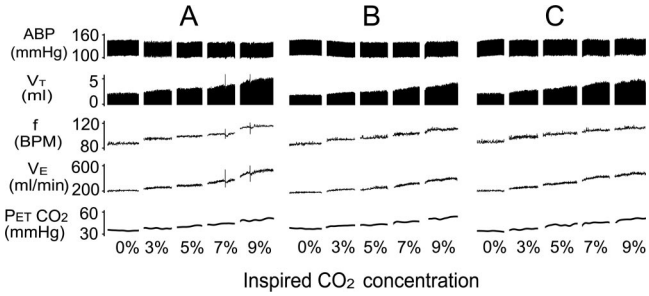

Fig. 2. Experimental recordings of the cardiorespiratory responses to progressive hypercapnia obtained before (*A*), 5 min after (*B*), and 30 min after (*C*) DAMGO injection (100 μg/kg, intravenous). Traces from top to bottom are arterial blood pressure (ABP), tidal volume (V_T), respiratory frequency (f), minute ventilation (V_E), and end-tidal pressure of carbon dioxide (P_{ETCO_2}). A depressed ventilatory (V_E and V_T) responsiveness to carbon dioxide appeared 5 min after DAMGO injection, and this depression almost disappeared 30 min later. BPM = beats/min.

HR responses (−2.9 ± 1.9%) to hypercapnia were found, as they were not affected by intravenous DAMGO (0.2 ± 0.9% and 0.3 ± 0.8%; n = 14; $P > 0.05$). It should be noted that these cardiovascular data were averaged from the responses to four degrees of hypercapnia because DAMGO did not alter the cardiovascular response to each hypercapnic level. This was the same for the

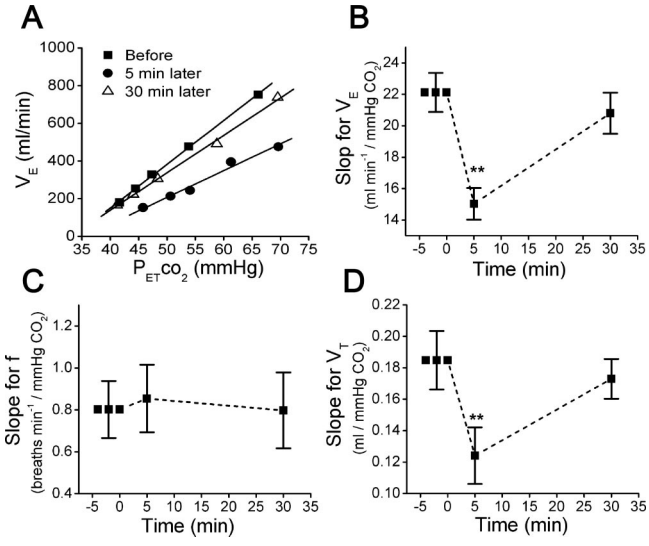

Fig. 3. Effect of systemic DAMGO on the responsiveness of minute ventilation (V_E), respiratory frequency (f), and tidal volume (V_T) to progressive hypercapnia. *A* represents the examples of the V_E responsiveness to end-tidal pressure of carbon dioxide (P_{ETCO_2}) before, 5 min after, and 30 min after DAMGO administration in an anesthetized rat, and the linear regression equations before, 5 min after, and 30 min after intravenous injection of DAMGO: (1) for V_E, y = 23.2x − 778.8, y = 14.2x − 503.1, and y = 19.8x − 655.1, respectively; (2) for f, y = 0.85x + 62.02, y = 0.95x + 50.16, and y = 0.78x + 60.04, respectively; and (3) for V_T, y = 0.19x − 5.29, y = 0.13x − 3.58, and y = 0.17x − 4.43, respectively. The range of R^2 in all equations was 0.94–0.99. *B–D* illustrate the group data of the slope for V_E, f, and V_T, respectively. "0" on the x-axis indicates the onset of intravenous administration of DAMGO. Data are presented as mean ± SE; n = 14. ** $P < 0.01$ compared with control by repeated one-way analysis of variance.

subsequent experiments. Intravenous injection of the vehicle, 0.9% saline alone, produced no effect on the cardiorespiratory variables.

Influence of Blocking cMRR μ Receptors on Systemic DAMGO-induced Respiratory Inhibition

To evaluate the role of cMRR μ receptors in the ventilatory responses to systemic DAMGO, the effects of systemic DAMGO on ventilation and the responses to carbon dioxide were tested before and 2–3 min after local blockade of cMRR μ receptors in five rats. As illustrated in figure 4, the systemic DAMGO-induced inhibition of baseline ventilation (−20%) and the response to carbon dioxide (−31%) was substantially diminished by pretreatment of the cMRR with CTAP (100 ng/100 nl). After the pretreatment, the systemic DAMGO only inhibited the baseline ventilation by 11% *via* acting on f and the response to carbon dioxide by 14% *via* changing V_T response. That is, local CTAP pretreatment in the cMRR diminished the systemic DAMGO-induced inhibitions for baseline ventilation and the response to carbon dioxide by 45% and 55%, respectively. Because the interval between the first and second systemic DAMGO (after the local injection of CTAP) was approximately 2 h, we tested whether the respiratory responses to the systemic DAMGO were changed over this period in two rats. We found no differences between the first and second systemic DAMGO-induced reduction of ventilation (−19 ± 2 *vs.* −20 ± 3%) and the response to carbon dioxide (−30 ± 3 *vs.* −31 ± 2%).

Cardiorespiratory Responses to Microinjection of DAMGO into the cMRR

As shown in figure 5, microinjection of DAMGO into the cMRR in seven rats significantly increased P_{ETCO_2} (from 43.3 ± 2.5 to 47.6 ± 3.1; n = 7; $P < 0.05$) and reduced baseline V_E by 22%, mainly due to depressing baseline V_T (by 18%), whereas the baseline values for f, blood pressure, and HR were not significantly affected. As compared with control, microinjection of DAMGO into the cMRR significantly diminished the baseline V_E (212 ± 12 *vs.* 166 ± 11 ml/min; $P < 0.01$) and V_T (2.1 ± 0.2 *vs.* 1.7 ± 0.2 ml; $P < 0.05$), with little effect on f (98 ± 8 *vs.* 98 ± 10 breaths/min; $P > 0.05$). After isocapnic calculation, a greater decrease in V_E (105 ± 8 ml/min) and V_T (1.3 ± 0.2 ml) was observed as compared with those without isocapnic calculation. It should be noted that the adjusted baseline f (97 ± 8 breaths/min) was still not significantly different from the control ($P > 0.05$). Microinjection of DAMGO into the cMRR significantly depressed V_E chemosensitivity to carbon dioxide by 24% and 28% 5 and 30 min after microinjection, respectively. This inhibitory effect was due to the depression of both V_T and f responsiveness. The animals' ventilatory responses to hypercapnia were recovered 60 min after DAMGO microinjection. The typical experimental recordings and the group data showing the cardiovascular and/or respiratory responses to progressive hypercapnia before and after microinjection are displayed in figures 6 and 7, respectively. The absence of cardiovascular response to hypercapnia was not altered by microinjection of DAMGO into the cMRR (blood pressure, 3.75 ± 1.98% and HR, −2.21 ± 1.88%; n = 7; $P > 0.05$). Microinjection of vehicle into the cMRR

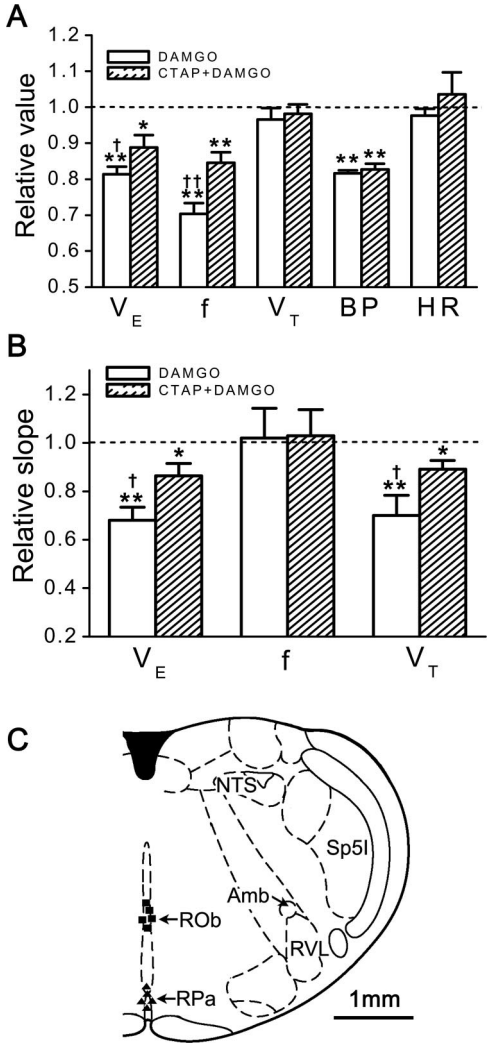

Fig. 4. Comparison of the systemic DAMGO-induced inhibition of baseline ventilation (*A*) and the responsiveness to carbon dioxide (*B*) before and after microinjection of CTAP into the caudal medullary raphe region. All variables are presented as relative values normalized to control (before intravenous DAMGO) marked by the *horizontal dashed line*. Data are presented as mean ± SE; n = 5. * $P < 0.05$ and ** $P < 0.01$ between the control and DAMGO/CTAP-induced changes, whereas † $P < 0.05$ and †† $P < 0.01$ between DAMGO-induced changes before and after microinjection of CTAP by repeated one-way analysis of variance. The coronal slice (*C*) shows locations of the microinjections 12.0 mm caudal to the bregma and either 8.3 mm (*squares*) or 9.3 mm (*triangles*) ventral to the cerebellar surface. Amb = ambiguus nucleus; BP = mean arterial blood pressure; f = respiratory frequency; HR = heart rate; NTS = nucleus of solitary tract; ROb = raphe obscurus nucleus; RPa = raphe pallidus nucleus; RVL = rostroventrolateral reticular nucleus; Sp5I = spinal 5 nucleus, interpolar part; V_E = minute ventilation; V_T = tidal volume.

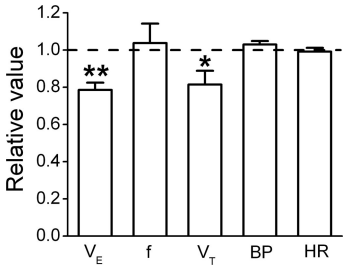

Fig. 5. Effect of microinjection of DAMGO into the caudal medullary raphe region on cardiorespiratory baseline variables in anesthetized and spontaneously breathing rats. All variables are presented as relative values normalized to the control represented by the *dashed line*. Data are presented as mean ± SE; n = 7. * $P < 0.05$, ** $P < 0.01$ between the data obtained before (control) and after DAMGO microinjection by paired *t* test. BP = mean arterial blood pressure; f = respiratory frequency; HR = heart rate; V_E = minute ventilation; V_T = tidal volume.

produced no remarkable effect on baseline respiration and the response to carbon dioxide in three rats (table 1). In addition, microinjections were given in the regions outside the cMRR in three rats as marked in figure 7D. These injections did not significantly change the baseline ventilation and the response to carbon dioxide (table 1). In these cases, absence of significant changes of baseline blood pressure (129 ± 12 *vs.* 128 ± 13 mmHg) and HR (345 ± 12 *vs.* 347 ± 13 beats/min) in response to DAMGO were also observed.

Cardiorespiratory Responses to Microinjection of DAMGO into the mMRR and rMRR

As shown in figures 8 and 9, microinjection of DAMGO into the mMRR produced no significant effect on baseline cardiorespiratory variables or the respiratory response to progressive hypercapnia. The same results were also observed when DAMGO was microinjected into the rMRR (figs. 10 and 11). Similar to microinjection

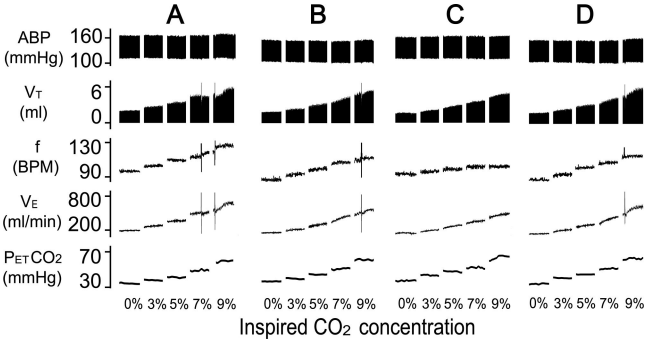

Fig. 6. Experimental recordings of the cardiorespiratory responses to progressive hypercapnia obtained before (*A*), 5 min after (*B*), 30 min after (*C*), and 90 min after (*D*) microinjection of DAMGO into the caudal medullary raphe region in an anesthetized and spontaneously breathing rat. Traces from top to bottom are arterial blood pressure (ABP), tidal volume (V_T), respiratory frequency (f), minute ventilation (V_E), and end-tidal pressure of carbon dioxide (P_{ETCO_2}). Note that the response of V_E and f to progressive hypercapnia was inhibited at 5–30 min after microinjection of DAMGO, but recovers 90 min after the microinjection.

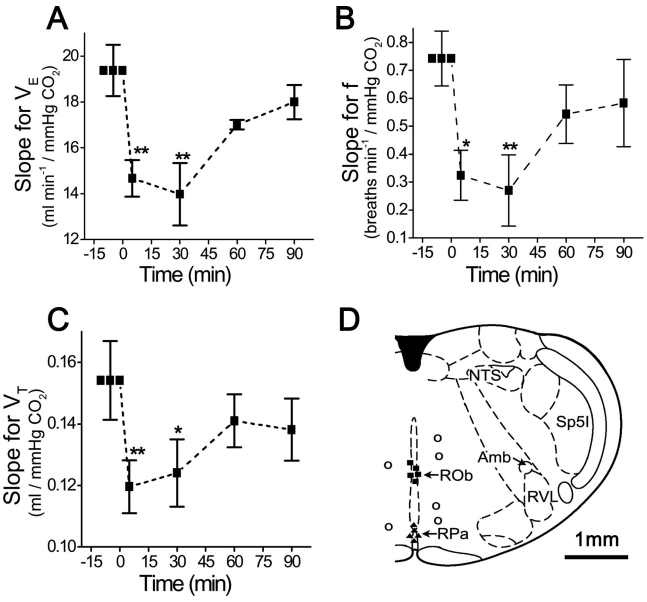

Fig. 7. Group data showing the effects of microinjection of DAMGO into the caudal medullary raphe region on the minute ventilation (V_E; *A*), respiratory frequency (f; *B*), and tidal volume (V_T; *C*) responsiveness to progressive hypercapnia and the locations of the microinjections (*D*). The linear regression equations before and 5, 30, 60, and 90 min after microinjection of DAMGO were (1) for V_E, y = 19.9x − 433.2, y = 14.1x − 272.2, y = 13.3x − 350.5, y = 17.1x − 371.5, and y = 18.8x − 406.8, respectively; (2) for f, y = 0.78x + 73.66, y = 0.38x + 94.21, y = 0.30x + 101.01, y = 0.54x + 82.80, and y = 0.61x + 90.16, respectively; and (3) for V_T, y = 0.15x − 2.89, y = 0.11x − 2.01, y = 0.12x − 3.41, y = 0.14x − 3.07, and y = 0.14x − 2.52, respectively. The range of R^2 in all equations was 0.94–0.99. "0" on the x-axis indicates the onset of microinjection of DAMGO. Data are presented as mean ± SE; n = 7. * $P < 0.05$, ** $P < 0.01$ compared with the control. The coronal slice (*D*) shows locations of the microinjections 12.0 mm caudal to the bregma and either 8.3 mm (*squares*) or 9.3 mm (*triangles*) ventral to the cerebellar surface. There were six microinjections outside the caudal medullary raphe region that are represented by an *open circle* in three rats. Amb = ambiguus nucleus; NTS = nucleus of solitary tract; ROb = raphe obscurus nucleus; RPa = raphe pallidus nucleus; RVL = rostroventrolateral reticular nucleus; Sp5I = spinal 5 nucleus, interpolar part.

into the cMRR, the absence of cardiovascular response to hypercapnia was not affected by DAMGO microinjected into the mMRR (blood pressure, 2.47 ± 1.81% and HR, 0.86 ± 0.57%; n = 6; $P > 0.05$) or the rMRR (blood pressure, 2.97 ± 1.12% and HR, 1.62 ± 0.71%; n = 6; $P > 0.05$).

Discussion

In the current study, we found that intravenous administration of DAMGO significantly inhibited baseline ventilation by 20% mainly due to depressing f, associated with an increase in P_{ETCO_2}. After isocapnic adjustment, a much greater inhibition was uncovered. A large number of previous studies has demonstrated that opioids administered systemically decrease breathing mainly through slowing f in humans and animals, especially in

Table 1. Effect of Microinjection of Vehicle into the cMRR (A) and DAMGO into the Regions outside the cMRR (B) on Respiratory Baseline and the Response to Carbon Dioxide

	Baseline			Slope		
Response	V_E, ml/min	f, breaths/min	V_T, ml	$\Delta V_E/\Delta P_{ETCO_2}$, ml · min^{-1}/mmHg CO_2	$\Delta f/\Delta P_{ETCO_2}$, breaths · min^{-1}/mmHg CO_2	$\Delta V_T/\Delta P_{ETCO_2}$, ml/mmHg CO_2
A (n = 3)						
Before	160 ± 18	89 ± 3	1.86 ± 0.28	22.2 ± 1.1	0.66 ± 0.50	0.22 ± 0.03
After	153 ± 13	87 ± 3	1.79 ± 0.22	22.1 ± 1.1	0.71 ± 0.42	0.21 ± 0.04
B (n = 3)						
Before	181 ± 17	95 ± 6	2.05 ± 0.08	20.2 ± 2.2	0.85 ± 0.31	0.21 ± 0.02
After	183 ± 19	93 ± 5	2.09 ± 0.11	21.6 ± 2.7	0.91 ± 0.42	0.20 ± 0.03

Data are presented as mean ± SE.

cMRR = caudal medullary raphe region; f = respiratory frequency; P_{ETCO_2} = end-tidal pressure of carbon dioxide; V_E = minute ventilation; V_T = tidal volume.

the anesthetized condition[3,8,12,42–44] (see the review[8]). In the current study, we found that as compared with control, hypercapnia increased the V_E responses by 234% mainly *via* increasing V_T (182%), which is similar to the results reported previously.[8] Systemic administration of DAMGO attenuated the ventilatory responsiveness to progressive hypercapnia by 31% owing to a reduction of V_T rather than f responsiveness. In agreement, it has been indicated that the slopes of the hypercapnic ventilatory responses were decreased and shifted to the right after systemic delivery of opioid agonists in humans, monkeys, rats, and mice.[9–13] Among these studies, activation of μ receptor–induced attenuation of V_T (up to 29%) is dominant as compared with the slowing of f.[11,13,45,46]

The MRR is an area responsible for the ventilatory responsiveness to hypercapnia[24–27] and nociception.[23] Although in rats the MRR has a rich distribution of μ receptors,[34] it is unknown whether MRR μ receptors are involved in opioid-induced depression of ventilation and the ventilatory responses to hypercapnia. Our major finding in the current study is that microinjection of DAMGO into the cMRR, but not the mMRR or rMRR, decreased baseline ventilation by 22%, with an associated increase in P_{ETCO_2}. After isocapnic calculation, this inhibition became 50%, predominantly *via* reducing V_T. More important, microinjection of DAMGO into the cMRR attenuated the hypercapnic ventilatory responsiveness by 28% due to a decrease in both responses of V_T and f. This is the first evidence to show that among the MRR, the cMRR μ receptors are uniquely inhibitory to baseline ventilation and the ventilatory response to hypercapnia. To estimate the contribution of cMRR μ receptors to the systemic DAMGO-induced ventilatory responses, we compared the baseline ventilatory responses and hypercapnic ventilatory responses to systemic DAMGO before and after blockade of cMRR μ receptors by local injection of CTAP. We found that the inhibition of baseline (−20%) and carbon dioxide ventilatory responses (−31%) to systemic DAMGO was diminished to −11% and −14%, respectively. That is, blockade

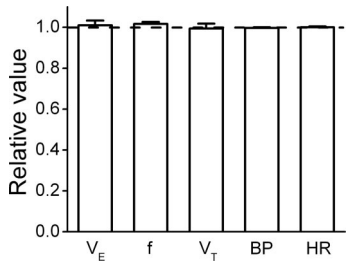

Fig. 8. Baseline effect of microinjection of DAMGO into the middle medullary raphe region on the cardiorespiratory variables. All variables are presented as relative values normalized to control represented by the *horizontal dashed line*. Data are presented as mean ± SE in six rats. BP = mean arterial blood pressure; f = respiratory frequency; HR = heart rate; V_E = minute ventilation; V_T = tidal volume.

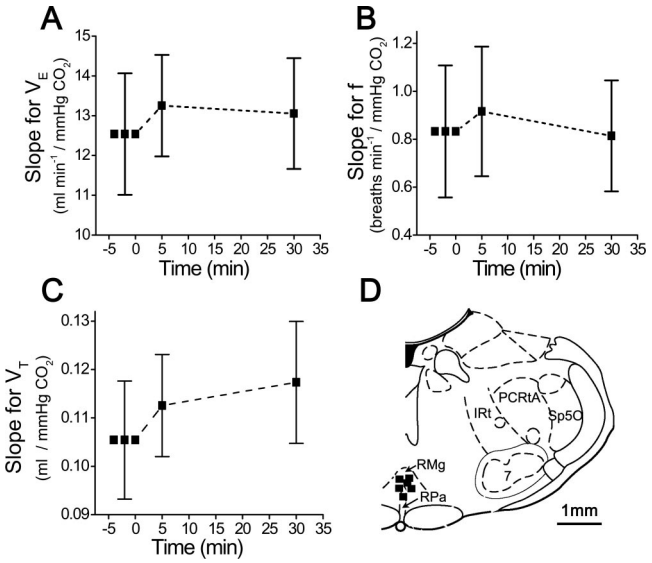

Fig. 9. Effects of microinjection of DAMGO into the middle medullary raphe region on the minute ventilation (V_E; *A*), respiratory frequency (f; *B*), and tidal volume (V_T; *C*) responsiveness to progressive hypercapnia in the rats and the locations of the microinjections (*D*). "0" on the x-axis represents the onset of microinjection of DAMGO. Data are presented as mean ± SE; n = 6. *Squares* in *D* show the locations of the microinjections 10.5 mm caudal to the bregma and 9 mm ventral to the cerebellar surface. 7 = facial nucleus; IRt = intermediate reticular nucleus; PCRtA = parvicellular reticular nucleus, alpha; RMg = raphe magnus nucleus; RPa = raphe pallidus nucleus; Sp5O = spinal 5 nucleus, oral part.

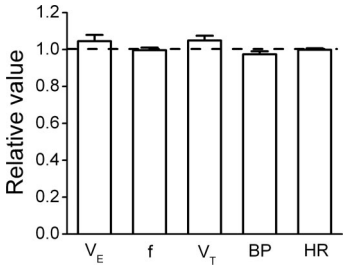

Fig. 10. Baseline effect of microinjection of DAMGO into the rostral medullary raphe region on the cardiorespiratory variables. All variables are presented as relative values normalized to control represented by the *horizontal dashed line*. Data are presented as mean ± SE in six rats. BP = mean arterial blood pressure; f = respiratory frequency; HR = heart rate; V_E = minute ventilation; V_T = tidal volume.

of cMRR μ receptors diminished the systemic DAMGO-induced inhibitions for baseline ventilation and the response to carbon dioxide by 45% and 55%, respectively, suggesting that these μ receptors play a major role in inhibiting ventilation and attenuating the ventilatory responses to carbon dioxide. Although opioids inhibit respiration mainly *via* stimulating central μ receptors,[12,15–20] some early studies indicated involvement of both μ and δ receptors[21,22] and even showed that δ receptors could modulate or counteract the respiratory depression induced by μ receptors.[16] Our data cannot rule out the possible involvement of MRR δ receptors in the respiratory modulations. Directly answering whether the hypercapnia-recruited cMRR neurons containing μ receptors are chemosensitive and/or respiratory modulated is beyond the scope of the current study. The presence of chemosensitive neurons in the cMRR has been well established.[24,25,28] On the other hand, investigators have also shown that the cMRR contains respiratory-modulated neurons in cats,[47,48] and the firing patterns of these neurons are altered during fictive cough.[47] Ultimately, further studies are required to clarify which types of cMRR neurons contain μ receptors (chemosensitive and/or respiratory-modulated neurons), and how stimulation of these receptors alters the ventilatory responsiveness to hypercapnia.

The significance of our study is evident. The most adverse effect of opioids, even at therapeutic doses, is the substantial depression of breathing that could be lethal. In fact, the rate of opioid-induced deaths in 1999 was 122 per million in Australia.[49] Opioids markedly depress the ventilatory response to hypercapnia that is essential for maintaining a normal eupneic breathing and critical for survival in awake and/or anesthetized humans and animals.[2–6,12,16–18] To date, however, how morphine depresses ventilation, leading to respiratory failure in the clinic, remains unclear. Our finding that cMRR μ receptors play an important role in the systemic DAMGO-induced inhibition of ventilation and the response to carbon dioxide benefits our understanding of central morphine depression of ventilation, especially the ventilatory response to carbon dioxide. In fact, there is evidence to suggest some functional and anatomical similarities in the MRR between humans and rats. MRR serotonergic neurons have been believed to be involved in chemosensitivity to carbon dioxide *in vivo* [24,25] and *in vitro*.[28,29] A lesion or inhibition of these neurons limited in the MRR profoundly attenuates the ventilatory response to hypercapnia in animals.[26,27] In infants, serotonergic neurons are heavily distributed in the MRR that is homologous in position to chemosensitive neurons in rats.[50] The abnormality of medullary serotonergic system (the MRR) has been linked to some respiratory disorders such as sudden infant death syndrome.[51,52] The important role of cMRR μ receptors in morphine-induced attenuation of the ventilatory response to carbon dioxide also builds a foundation for further defining whether cMRR μ receptors modulate the ventilatory chemosensitivity *via* local serotonergic mechanism.

Mu receptors in the MRR are not critical for cardiovascular regulation. In this study, intravenous DAMGO can significantly depress blood pressure by 18% (fig. 1), similar to the previous studies reported in anesthetized rats.[53] Unexpectedly, this cardiovascular modulation was not observed when DAMGO was microinjected into the MRR subregions we tested. There are three reasons that may account for the absence of cardiovascular effect in our study. First, the depressed cardiovascular effect by systemic administration of opioids was blocked by naloxone methiodide, an opioid antagonist restricted to the periphery,[53] suggesting peripheral μ receptors' cardiovascular modulation. Second, instead of the MRR, μ

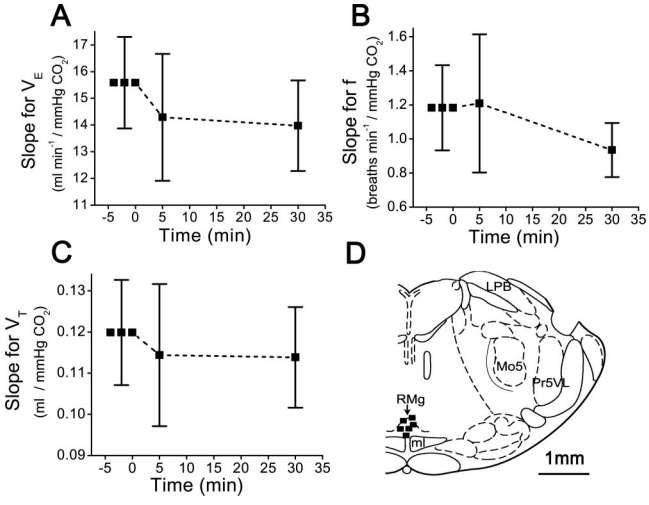

Fig. 11. Effect of microinjection of DAMGO into the rostral medullary raphe region on the minute ventilation (V_E; *A*), respiratory frequency (f; *B*), and tidal volume (V_T; *C*) responsiveness to progressive hypercapnia in the rats. "0" on the x-axis presents the onset of microinjection of DAMGO. Data are presented as mean ± SE; n = 6. *Squares* in *D* show the locations of the microinjections 9 mm caudal to the bregma and 9 mm ventral to the cerebellar surface. LPB = lateral parabrachial nucleus; ml = medial lemniscus; Mo5 = motor 5 nucleus; Pr5VL = principal sensory 5 nucleus, ventrolateral part; RMg = raphe magnus nucleus.

receptors are also located within other brainstem regions responsible for cardiorespiratory modulation.[54] Therefore, their influence on cardiovascular modulation cannot be ruled out. Third, it is possible that activation of the whole MRR rather than only one of its subnuclei is required for involvement of MRR μ receptors in cardiovascular regulation.

There are several limitations in this study. As mentioned in the Materials and Methods section, the V_E and V_T responses to hypercapnia did not always reach the plateau during the 2-min exposure in the current study. This suggests that the ventilatory sensitivity reported here seems not to be obtained in a full hypercapnic steady state condition. However, it would not invalidate our finding that DAMGO inhibits ventilation and the responsiveness to hypercapnia because the rats treated without and with DAMGO had undergone the same hypercapnic exposure. Moreover, the ventilatory responses to progressive hypercapnia by rebreathing carbon dioxide have been widely used to reflect the central ventilatory chemosensitivity in animals and humans.[13,55–57] It was reported that the systemic administration of opioids had greater potency and efficacy in male than in female rodents[58–60] although debatable in humans.[61] No attempt was made in the current study to delineate whether the sex difference also exists in the mice. Urethane was chosen in the current study because of its minimal cardiorespiratory depression compared with other anesthetics. We cannot rule out the possible interaction between the anesthetic and DAMGO in changing ventilation and the response to carbon dioxide. We did not directly measure the diffused size of microinjection of DAMGO. However, because injecting the same volume of DAMGO (100 nl) into the regions outside the cMRR (0.5 mm lateral to the cMRR) or into the mMRR (1.5 mm rostral to the cMRR) did not alter ventilation and the response to hypercapnia, the spread should be considerably limited. In addition, because of the cylinder-shaped ROb in the cMRR, the microinjection used in this study was not expected to block all μ receptors in this region. Unlike the systemic administration of DAMGO, the effects of microinjection into the cMRR on the ventilatory response to carbon dioxide lasted much longer (5 min *vs.* 30 min), similar to application of morphine on the medullary ventral surface in anesthetized cats.[7] It is unknown why the effects elicited by local microinjection are prolonged. However, the prolonged respiratory depression after injection into the cMRR may be related to the diffusion of the injection from each other of the ROb and RPa or a longer downstream effect of DAMGO.

In summary, it is well documented that opioids centrally inhibit respiration and depress the ventilatory responsiveness to hypercapnia in anesthetized humans and animals. However, the mechanisms related to this depressed response remain unknown. Our results show that microinjection of DAMGO into the cMRR rather than mMRR or rMRR significantly depressed ventilation and the response to hypercapnia in anesthetized rats. Moreover, blockade of cMRR μ receptors diminished the systemic DAMGO-induced inhibitions for ventilation and the response to carbon dioxide by 45% and 55%, respectively. We conclude that cMRR μ receptors are at least one of the major contributors to opiate-induced depression of the ventilatory response to hypercapnia.

The authors thank Jianguo Zhuang, M.D., Ph.D. (Associate Research Scientist, Pathophysiology Program, Lovelace Respiratory Research Institute, Albuquerque, New Mexico), for his assistance with the experiment setup and statistics.

References

1. McCrimmon DR, Alheid GF: On the opiate trail of respiratory depression. Am J Physiol Regul Integr Comp Physiol 2003; 285:R1274–5
2. Jaffe JH, Martin WR: Opioid agonists and antagonists, Goodman and Gilman's The Pharmacological Basis of Therapeutics. Edited by Gilman AG, Rall TW, Nies AS, Taylor P. New York, Pergamon, 1990, pp 485–521
3. Gutstein HB, Akil H. Opioid analgesics, Goodman & Gilman's The Pharmacological Basis of Therapeutics 10th Edition. Edited by Hardman JG, Limbird LE, Goodman AG. New York McGraw-Hill, 2001, pp 569–619.
4. Shook JE, Watkins WD, Camporesi EM: Differential roles of opioid receptors in respiration, respiratory disease, and opiate-induced respiratory depression. Am Rev Respir Dis 1990; 142:895–909
5. Yeadon M, Kitchen I: Opioids and respiration. Prog Neurobiol 1989; 33: 1–16
6. Putnam RW, Filosa JA, Ritucci NA: Cellular mechanisms involved in CO(2) and acid signaling in chemosensitive neurons. Am J Physiol Cell Physiol 2004; 287:C1493–526
7. Hurle MA, Mediavilla A, Florez J: Differential respiratory patterns induced by opioids applied to the ventral medullary and dorsal pontine surfaces of cats. Neuropharmacology 1985; 24:597–606
8. Santiago TV, Edelman NH: Opioids and breathing. J Appl Physiol 1985; 59:1675–85
9. Bourke DL, Warley A: The steady-state and rebreathing methods compared during morphine administration in humans. J Physiol 1989; 419:509–17
10. Weil JV, McCullough RE, Kline JS, Sodal IE: Diminished ventilatory response to hypoxia and hypercapnia after morphine in normal man. N Engl J Med 1975; 292:1103–6
11. Vivian JA, Kishioka S, Butelman ER, Broadbear J, Lee KO, Woods JH: Analgesic, respiratory and heart rate effects of cannabinoid and opioid agonists in rhesus monkeys: Antagonist effects of SR 141716A. J Pharmacol Exp Ther 1998; 286:697–703
12. Romberg R, Sarton E, Teppema L, Matthes HW, Kieffer BL, Dahan A: Comparison of morphine-6-glucuronide and morphine on respiratory depressant and antinociceptive responses in wild type and mu-opioid receptor deficient mice. Br J Anaesth 2003; 91:862–70
13. Eager KR, Robinson BJ, Galletly DC, Miller JH: Endogenous opioid modulation of hypercapnic-stimulated respiration in the rat. Respir Physiol 1994; 96:13–24
14. Kelly HW: Drug-induced pulmonary diseases, Pharmacotherapy: A Pathophysiological Approach. Edited by Dipiro JT, Talbert RL, Hayes PE, Yee GC, Posey LM. Norwalk, Appleton & Lange, 1993, pp 482–93
15. Dahan A, Sarton E, Teppema L, Olievier C, Nieuwenhuijs D, Matthes HW, Kieffer BL: Anesthetic potency and influence of morphine and sevoflurane on respiration in μ-opioid receptor knockout mice. ANESTHESIOLOGY 2001; 94:824–32
16. Su YF, McNutt RW, Chang KJ: Delta-opioid ligands reverse alfentanil-induced respiratory depression but not antinociception. J Pharmacol Exp Ther 1998; 287:815–23
17. Matthes HW, Smadja C, Valverde O, Vonesch JL, Foutz AS, Boudinot E, Denavit-Saubie M, Severini C, Negri L, Roques BP, Maldonado R, Kieffer BL: Activity of the delta-opioid receptor is partially reduced, whereas activity of the kappa-receptor is maintained in mice lacking the mu-receptor. J Neurosci 1998; 18:7285–95
18. Matthes HW, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, Befort K, Dierich A, Le Meur, M Dolle P, Tzavara E, Hanoune J, Roques BP, Kieffer BL: Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. Nature 1996; 383:819–23
19. Christrup LL: Morphine metabolites. Acta Anaesthesiol Scand 1997; 41: 116–22
20. Pasternak GW, Bodnar RJ, Clark JA, Inturrisi CE: Morphine-6-glucuronide, a potent mu agonist. Life Sci 1987; 41:2845–9
21. Morin-Surun MP, Boudinot E, Gacel G, Champagnat J, Roques BP, Denavit-

Saubie M,: Different effects of mu and delta opiate agonists on respiration. Eur J Pharmacol 1984; 98:235–40

22. Yeadon M, Kitchen I: Multiple opioid receptors mediate the respiratory depressant effects of fentanyl-like drugs in the rat. Gen Pharmacol 1990; 21: 655–64

23. Narita M, Tseng LF: Evidence for the existence of the beta-endorphin-sensitive "epsilon-opioid receptor" in the brain: The mechanisms of epsilon-mediated antinociception. Jpn J Pharmacol 1998; 76:233–53

24. Bernard DG, Li A, Nattie EE: Evidence for central chemoreception in the midline raphe. J Appl Physiol 1996; 80:108–15

25. Nattie EE, Li A: CO2 dialysis in the medullary raphe of the rat increases ventilation in sleep. J Appl Physiol 2001; 90:1247–57

26. Nattie EE, Li A, Richerson G, Lappi DA: Medullary serotonergic neurones and adjacent neurones that express neurokinin-1 receptors are both involved in chemoreception *in vivo*. J Physiol 2004; 556:235–53

27. Taylor NC, Li A, Nattie EE: Medullary serotonergic neurones modulate the ventilatory response to hypercapnia, but not hypoxia in conscious rats. J Physiol 2005; 566:543–57

28. Wang W, Pizzonia JH, Richerson GB: Chemosensitivity of rat medullary raphe neurones in primary tissue culture. J Physiol 1998; 511(pt 2):433–50

29. Wang W, Tiwari JK, Bradley SR, Zaykin RV, Richerson GB: Acidosis-stimulated neurons of the medullary raphe are serotonergic. J Neurophysiol 2001; 85:2224–35

30. Pete G, Mack SO, Haxhiu MA, Walbaum S, Gauda EB: CO(2)-induced c-Fos expression in brainstem preprotachykinin mRNA containing neurons. Respir Physiol Neurobiol 2002; 130:265–74

31. Connelly CA, Ellenberger HH, Feldman JL: Are there serotonergic projections from raphe and retrotrapezoid nuclei to the ventral respiratory group in the rat? Neurosci Lett 1989; 105:34–40

32. Voss MD, De Castro, D Lipski J, Pilowsky PM, Jiang C: Serotonin immunoreactive boutons form close appositions with respiratory neurons of the dorsal respiratory group in the cat. J Comp Neurol 1990; 295:208–18

33. Zhan WZ, Ellenberger HH, Feldman JL: Monoaminergic and GABAergic terminations in phrenic nucleus of rat identified by immunohistochemical labeling. Neuroscience 1989; 31:105–13

34. Ding YQ, Kaneko T, Nomura S, Mizuno N: Immunohistochemical localization of mu-opioid receptors in the central nervous system of the rat. J Comp Neurol 1996; 367:375–402

35. Paxinos G, Watson C: The Rat Brain in Stereotaxic Coordinates, 4th edition. San Diego, a division of Harcourt Brace, 1998, pp 56–69

36. Haji A, Takeda R: Effects of a kappa-receptor agonist U-50488 on bulbar respiratory neurons and its antagonistic action against the mu receptor-induced respiratory depression in decerebrate cats. Jpn J Pharmacol 2001; 87:333–7

37. Nason MW Jr, Mason P: Medullary raphe neurons facilitate brown adipose tissue activation. J Neurosci 2006; 26:1190–8

38. Hurley RW, Hammond DL: Contribution of endogenous enkephalins to the enhanced analgesic effects of supraspinal mu opioid receptor agonists after inflammatory injury. J Neurosci 2001; 21:2536–45

39. Szeto HH, Lovelace JL, Fridland G, Soong Y, Fasolo J, Wu D, Desiderio DM, Schiller PW: *In vivo* pharmacokinetics of selective mu-opioid peptide agonists. J Pharmacol Exp Ther 2001; 298:57–61

40. Dahan A, DeGoede J, Berkenbosch A, Olievier IC: The influence of oxygen on the ventilatory response to carbon dioxide in man. J Physiol 1990; 428:485–99

41. Wang R, Xu F, Zhuang J, Zhang C: Carotid sinus nerve is involved in cardiorespiratory responses to intracarotid injection of capsaicin in the rat. J Appl Physiol 2006; 100:60–6

42. Howard RS, Sears TA: The effects of opiates on the respiratory activity of thoracic motoneurones in the anaesthetized and decerebrate rabbit. J Physiol 1991; 437:181–99

43. Ling GS, Spiegel K, Lockhart SH, Pasternak GW: Separation of opioid analgesia from respiratory depression: Evidence for different receptor mechanisms. J Pharmacol Exp Ther 1985; 232:149–55

44. Ferguson LM, Drummond GB: Acute effects of fentanyl on breathing pattern in anaesthetized subjects. Br J Anaesth 2006; 96:384–90

45. Camporesi EM, Nielsen CH, Bromage PR, Durant PA: Ventilatory CO2 sensitivity after intravenous and epidural morphine in volunteers. Anesth Analg 1983; 62:633–40

46. Modalen AO, Quiding H, Frey J, Westman L, Lindahl S: A novel molecule with peripheral opioid properties: The effects on hypercarbic and hypoxic ventilation at steady-state compared with morphine and placebo. Anesth Analg 2006; 102:104–9

47. Baekey DM, Morris KF, Nuding SC, Segers LS, Lindsey BG, Shannon R: Medullary raphe neuron activity is altered during fictive cough in the decerebrate cat. J Appl Physiol 2003; 94:93–100

48. Hosogai M, Matsuo S: Inspiratory neurons with decrementing firing pattern in raphe nuclei of feline medulla. Auton Neurosci 2002; 99:13–7

49. Hall WD, Degenhardt LJ, Lynskey MT: Opioid overdose mortality in Australia, 1964-1997: Birth-cohort trends. Med J Aust 1999; 171:34–7

50. Kinney HC, Belliveau RA, Trachtenberg FL, Rava LA, Paterson DS: The development of the medullary serotonergic system in early human life. Auton Neurosci 2007; 132:81–102

51. Kinney HC, Filiano JJ, White WF: Medullary serotonergic network deficiency in the sudden infant death syndrome: Review of a 15-year study of a single dataset. J Neuropathol Exp Neurol 2001; 60:228–47

52. Panigrahy A, Filiano J, Sleeper LA, Mandell F, Valdes-Dapena M, Krous HF, Rava LA, Foley E, White WF, Kinney HC: Decreased serotonergic receptor binding in rhombic lip-derived regions of the medulla oblongata in the sudden infant death syndrome. J Neuropathol Exp Neurol 2000; 59:377–84

53. Champion HC, Zadina JE, Kastin AJ, Hackler L, Ge LJ, Kadowitz PJ: Endomorphin 1 and 2, endogenous ligands for the mu-opioid receptor, decrease cardiac output, and total peripheral resistance in the rat. Peptides 1997; 18: 1393–7

54. Xia Y, Haddad GG: Ontogeny and distribution of opioid receptors in the rat brainstem. Brain Res 1991; 549:181–93

55. Greenwald MK: Effects of opioid dependence and tobacco use on ventilatory response to progressive hypercapnia. Pharmacol Biochem Behav 2004; 77:39–47

56. Tafil-Klawe M, Klawe JJ, Sikorski W, Drzewiecka B: Reflex respiratory responses to progressive hyperoxic hypercapnia and normocapnic hypoxia in normocapnic and hypercapnic obstructive sleep apnea patients. J Physiol Pharmacol 2004; 55 (suppl 3):135–8

57. Suzuki S, Watanuki Y, Yoshiike Y, Okubo T: Effects of fenoterol on ventilatory response to hypercapnia and hypoxia in patients with chronic obstructive pulmonary disease. Thorax 1997; 52:125–9

58. Cicero TJ, Nock B, Meyer ER: Gender-related differences in the antinociceptive properties of morphine. J Pharmacol Exp Ther 1996; 279:767–73

59. Baamonde AI, Hidalgo Andres-Trelles FA: Sex-related differences in the effects of morphine and stress on visceral pain. Neuropharmacology 1989; 28:967–70

60. Kavaliers M, Innes DG: Developmental changes in opiate-induced analgesia in deer mice: Sex and population differences. Brain Res 1990; 516:326–31

61. Sarton E, Olofsen E, Romberg R, den Hartigh J, Kest B, Nieuwenhuijs D, Burm A, Teppema L, Dahan A: Sex differences in morphine analgesia: An experimental study in healthy volunteers. ANESTHESIOLOGY 2000; 93:1245–54