NMDA receptors mediate peripheral chemoreceptor afferent input in the conscious rat

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[1]*Department of Physical Therapy and Exercise Science, State University of New York at Buffalo, Buffalo, New York 14214; and* [2]*Constance S. Kaufman Pediatric Pulmonary Research Laboratory, Departments of Pediatrics and Physiology, and Tulane University School of Medicine, New Orleans, Louisiana 70112*

Ohtake, Patricia J., José E. Torres, Yair M. Gozal, Gavin R. Graff, and David Gozal. NMDA receptors mediate peripheral chemoreceptor afferent input in the conscious rat. *J. Appl. Physiol.* 84(3): 853–861, 1998.—*N*-methyl-D-aspartate (NMDA) glutamate receptors mediate critical components of cardiorespiratory control in anesthetized animals. The role of NMDA receptors in the ventilatory responses to peripheral and central chemoreceptor stimulation was investigated in conscious, freely behaving rats. Minute ventilation ($\dot{V}_E$) responses to 10% O_2, 5% CO_2, and increasing intravenous doses of sodium cyanide were measured in intact rats before and after intravenous administration of the NMDA receptor antagonist MK-801 (3 mg/kg). After MK-801, eupcapnic tidal volume (V_T) decreased while frequency increased, resulting in a modest reduction in $\dot{V}_E$. Inspiratory time (T_I) decreased, whereas expiratory time remained unchanged. The $\dot{V}_E$ responses to hypercapnia were qualitatively similar in control and MK-801 conditions, with slight reductions in respiratory drive (V_T/T_I) after MK-801. In contrast, responses to hypoxia were markedly attenuated after MK-801 and were primarily due to reduced frequency changes, whereas V_T was unaffected. Sodium cyanide doses associated with significant $\dot{V}_E$ increases were 5 and 50 μg/kg before and after MK-801, respectively. Thus 1-log shift to the right of individual dose-response curves occurred with MK-801. Selective carotid body denervation reduced $\dot{V}_E$ during hypoxia by 70%, and residual hypoxic ventilatory responses were abolished after MK-801. These findings suggest that, in conscious rats, carotid and other peripheral chemoreceptor-mediated hypoxic ventilatory responses are critically dependent on NMDA receptor activation and that NMDA receptor mechanisms are only modestly involved during hypercapnia.

hypoxia; hypercapnia; glutamate receptors; carotid body denervation; blood pressure

THE IMPORTANCE of excitatory amino acid neurotransmission in central mechanisms of cardiorespiratory control is becoming increasingly evident. *N*-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors are involved in peripheral and central chemoreception (2, 27, 29), respiratory pattern generation (8, 10, 33), transmission of bulbospinal drive to spinal cord motoneurons (9), and cardiovascular control (26).

Eucapnic minute ventilation ($\dot{V}_E$) was increased after central application of NMDA in anesthetized cats (26), and comparable results were also observed after glutamate microinjections in anesthetized rats (40). Conversely, ventilatory reductions occurred after intracisternal or topical administration of an NMDA receptor antagonist in spontaneously breathing, lightly anesthetized dogs (2, 20), anesthetized cats (1), and anesthetized rats (8).

NMDA receptor activation is involved in respiratory pattern generation and pneumotaxic mechanisms in the anesthetized rat (8) and cat (10). Activity of neurons in the dorsal and ventral respiratory areas of the cat medulla displays intrinsic phasic respiratory characteristics that are NMDA and non-NMDA glutamate receptor dependent (35). Intravenous administration of NMDA receptor antagonists elicited significant prolongation of inspiratory duration (T_I) in anesthetized and awake intact cats (10), and vagotomy further accentuated this effect, leading to apneusis (11).

Ventilatory responses to hypoxia were markedly attenuated after NMDA receptor blockade in anesthetized dogs (2), conscious rats (27), and piglets (22). In anesthetized dogs, carotid chemoreceptor-mediated excitation of expiratory bulbospinal neurons was dependent on NMDA receptors (9), whereas phrenic nerve nucleus responses to peripheral chemoreceptor stimulation showed NMDA and non-NMDA receptor dependency in anesthetized rats (6). In contrast to hypoxia, the ventilatory response to hypercapnia was not altered by NMDA receptor antagonism in anesthetized cats (33). However, in decerebrate cats, NMDA and non-NMDA receptors reportedly mediate aspects of the phrenic nerve response to hypercapnia (29).

Synaptic activation of excitatory amino acid receptors within the medulla is important in the central control of blood pressure. Hypertension and tachycardia were observed after NMDA or non-NMDA receptor antagonism within the nucleus of the solitary tract (NTS) in anesthetized rats (37).

All these studies demonstrate that NMDA glutamate receptors mediate critical components of cardiorespiratory control. However, the majority of these findings have been determined in anesthetized or reduced preparations. Recently, it has been shown that the role of NMDA receptors is dependent on the state of the animal as well as the degree of afferent input (5). Indeed, NMDA receptor blockade was not sufficient to induce apneustic breathing in conscious rodents, whereas administration of small doses of anesthetics and/or vagotomy eliminated the NMDA-dependent inspiratory off-switch mechanism (5). In addition, central NMDA receptor antagonism, which produces hypertension in anesthetized rats, had no pressor effect in conscious animals (7). Therefore, the major aim of our current study was to extend current information of the role of NMDA receptors in cardiorespiratory responses

of awake rats to peripheral and central chemoreceptor stimulation.

We hypothesized that, in intact, conscious, freely behaving rats, systemic administration of the NMDA receptor antagonist MK-801, which readily crosses the blood-brain barrier (41), would attenuate the ventilatory response to hypoxia but not to hypercapnia. Because hypoxia exerts peripheral excitatory and central inhibitory effects, it is difficult to evaluate the role of NMDA receptors in the ventilatory response to carotid chemoreceptor stimulation using hypoxia as the only stimulus. Therefore, we chose to use pharmacological [intravenous administration of sodium cyanide (NaCN)] stimulation of carotid chemoreceptors to further assess the peripheral component of the hypoxic ventilatory response during NMDA receptor antagonism. In addition, to elucidate the role of NMDA receptors in chemoreceptor afferent pathways that do not involve carotid body activation, hypoxic ventilatory responses were evaluated in animals after selective carotid body denervation (CBD) before and after NMDA receptor antagonism.

METHODS

Animals

Experimental protocols were approved by the Institutional Animal Use and Care Committee. Survival experiments were performed on 57 male Sprague-Dawley adult rats (300–450 g). In a preliminary stage, anesthesia was induced by pentobarbital sodium (Nembutal, 50 mg/kg ip), and atropine sulfate (0.4 mg/kg) was administered for reduction of secretions. Rectal temperature was monitored with a Harvard thermal probe to maintain core temperature at 37.5°C by a servo-controlled heating pad. After a 1-cm incision of the skin was performed, indwelling polyethylene catheters (PE-50, 0.56 mm ID, 0.88 mm OD) were surgically placed in the femoral artery and vein and advanced 5–7 cm to reach the abdominal aorta and inferior vena cava for subsequent blood pressure measurements, arterial blood sampling, and fluid or drug administration. Catheters were secured, tunneled subcutaneously, exteriorized in the dorsal aspect of the neck, flushed with a heparin-containing solution (1,000 U/ml saline), sealed with heat, and stored in a plastic cap sutured to the skin. To assess the role of NMDA receptors in mediating the hypoxic ventilatory response originating from peripheral chemoreceptors other than carotid bodies, selective carotid chemodenervation was performed in a subset of animals. Bilateral neck incisions were made, the bifurcation of the common carotid artery was identified, and the singular carotid body artery was ligated. Chemodenervation was confirmed by marked attenuation of the ventilatory response to intravenous administration of NaCN (20 μg/kg).

Animals were allowed to recover for at least 48–72 h, as demonstrated by return to normal feeding and sleep-wakefulness schedules. Animals were provided free access to water and rat chow and maintained on a 12:12-h light-dark cycle (light onset at 0630) and 22 ± 1°C ambient temperature. For habituation purposes, animals spent at least 1–2 h/day in a whole body plethysmograph chamber.

Ventilatory and Cardiovascular Recordings

Cardiorespiratory measurements were continuously acquired in the freely behaving, unrestrained animal placed in a previously calibrated 3-liter barometric chamber (Buxco Electronics, Troy, NY). A reference chamber of equal size, in which temperature was measured using a T-type thermocouple, was used to minimize the effect of signal drift due to temperature and pressure changes outside the chamber. Environmental temperature was maintained within the thermoneutral range (24–28°C). Calibration volumes of air were repeatedly introduced into the chamber before and on completion of recordings. At least 60 min before the start of each protocol, animals were allowed to acclimate to the chamber, through which humidified air (90% relative humidity) was passed at a rate of 8 l/min, using a precision-flow pump-reservoir system. Pressure changes in the chamber due to the inspiratory and expiratory temperature changes were measured using a high-gain differential pressure transducer (model MP45-1, Validyne). Analog signals were continuously digitized and analyzed on-line by a microcomputer software program (Buxco Electronics). A rejection algorithm was included in the breath-by-breath analysis routine and allowed for accurate rejection of motion-induced artifacts. T_I, expiratory time (T_E), tidal volume (V_T), respiratory frequency (f), and $\dot{V}_E$ were computed and stored for subsequent off-line analysis.

Systemic arterial pressure was measured from the arterial femoral catheter connected to a calibrated pressure transducer via a custom-designed swivel apparatus in the recording chamber (Buxco Electronics). Physiological signals were digitized, and a beat-to-beat peak-trough analysis routine allowed computation of heart rate (HR), systolic and diastolic pressures, and mean arterial pressure (MAP).

Protocol

Four separate groups of animals were used in this study. Intact animals received normal saline (2 ml iv) before hypoxic (n = 22) or hypercapnic (n = 14) exposure, during which ventilatory responses were measured. Ventilatory challenges of 30-min duration were performed with 5% CO_2-balance room air or with 10% O_2 in N_2 by rapidly bleeding the premixed gas mixture into the recording chamber. Steady-state responses were assessed during the last 5 min of each challenge. After the ventilatory challenge, dizocilpine maleate [(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclopenten-5,10-imine hydrogen maleate, MK-801; RBI, Natick, MA] dissolved in 2 ml of normal saline (3 mg/kg) was administered intravenously over a period of 1 min. Thirty minutes after MK-801 administration, ventilatory challenges were repeated. Hypoxic ventilatory challenges were also performed in selectively chemodenervated animals (n = 7) before and 30 min after MK-801 administration.

In a fourth group of animals (n = 14), 0.0, 2.5, 5, 10, 25, 50, and 100 μg/kg NaCN were rapidly infused before and 30 min after administration of MK-801, as described above. The volume of each infusion and corresponding flush was kept constant at 0.8 ml. Each NaCN dose was administered in triplicate.

Measurement of Blood-Gas Values

After withdrawal of 75–100 μl of blood from the dead space of the implanted catheter, another 150 μl were sampled for immediate analysis of P_{O_2}, P_{CO_2}, and pH with a blood-gas analyzer (model 178, Ciba Corning). Measurements were always performed in room air and during the last minute of each ventilatory challenge.

Data Analysis

Values are means ± SD. For ventilatory challenges, steady-state responses were assessed during the last 5 min of each challenge. Statistical significance of the difference in data before and after each drug administration was assessed within each group by paired *t*-tests. Differences in data between the two treatment groups were compared by analysis of variance (1-way analysis of variance) and the multiple comparison method using Bonferroni's correction of the *t*-test.

Ventilatory and cardiovascular measurements for challenges with each NaCN dose were averaged within animals. Comparisons between responses measured before and after MK-801 administration were performed by two-way analysis of variance for repeated measures followed by Tukey's post hoc test. $P < 0.05$ was considered to achieve statistical significance.

RESULTS

Respiratory Effects of MK-801 Administration During Eucapnia

Administration of MK-801 in intact conscious rats resulted in changes in respiratory pattern and timing during eucapnia. V_T was reduced by ~30% ($P < 0.05$), and f increased modestly ($P = 0.05$; Figs. 1 and 2), with the overall result of a slight, but significant, reduction in $\dot{V}_E$. Mild alveolar hypoventilation (Table 1) was

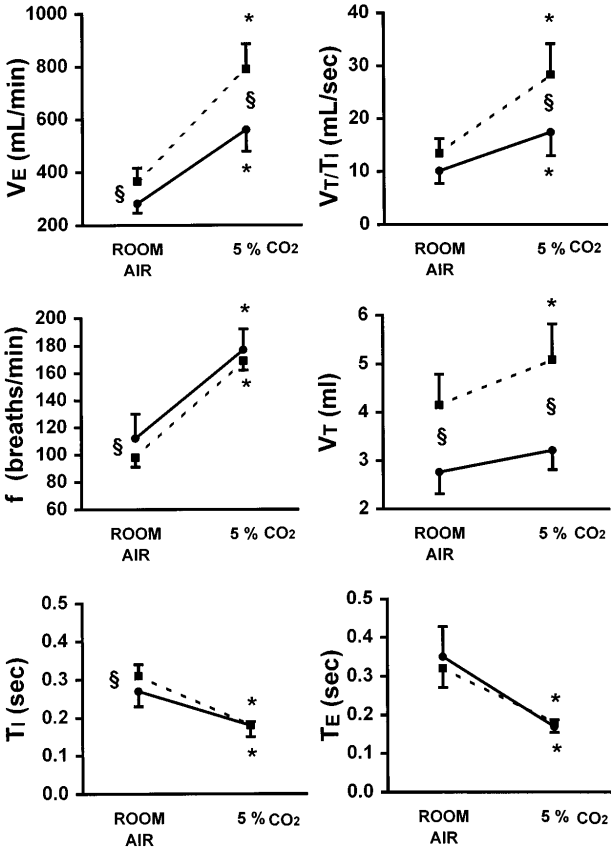

Fig. 1. Ventilation ($\dot{V}_E$), respiratory drive (V_T/T_I), respiratory frequency (f), tidal volume (V_T), and inspiratory (T_I) and expiratory (T_E) time in response to 5% CO_2 before (■) and after (●) MK-801 administration. *Significantly different from room air; §significant differences between conditions.

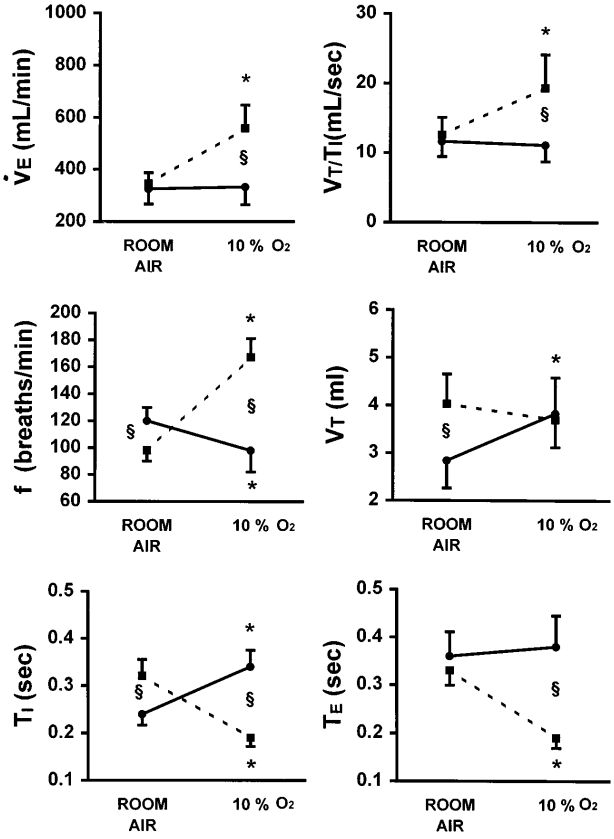

Fig. 2. $\dot{V}_E$, V_T/T_I, f, V_T, T_I, and T_E in response to 10% O_2 before (■) and after (●) MK-801 administration. *Significantly different from room air; §significant differences between conditions.

associated with administration of MK-801 to intact animals, as demonstrated by an average increase in arterial P_{CO_2} (Pa_{CO_2}) of ~3 Torr ($P < 0.02$). Effects on respiratory timing elicited by MK-801 administration were limited to significant reductions in T_I, whereas T_E was unaffected.

Effects of MK-801 Administration on the Hypercapnic Ventilatory Response

The ventilatory response to hypercapnia was determined by 30 min of exposure to 5% CO_2. In intact animals, hypercapnia increased Pa_{CO_2} by ~10 Torr (Table 1) and resulted in a $\dot{V}_E$ increase of ~100% (Fig. 1). This increase was due primarily to increased f, with lesser contributions of V_T (Fig. 1). During hypercapnia, T_I and T_E were affected equally, both decreasing by ~60% ($P < 0.05$).

After administration of MK-801, the ventilatory response to CO_2 remained intact, as indicated by the overall increase in $\dot{V}_E$. The change in $\dot{V}_E$ (Fig. 1) and Pa_{CO_2} (Table 1) after 30 min at 5% CO_2 was not different between the control and MK-801 conditions, although the absolute level of $\dot{V}_E$ was lower in the MK-801-treated condition ($P < 0.05$). The f increased similarly in blocked and unblocked conditions, whereas V_T did not increase during CO_2 challenges, remaining at a level lower than in the unblocked condition. Respiratory drive (V_T/T_I) increased significantly in response to

Table 1. *Arterial blood-gas measurements during hypoxia and hypercapnia before and after MK-801 administration in intact and CBD rats*

	Room Air			5% CO_2		
	pH	Pa_{O_2}, Torr	Pa_{CO_2}, Torr	pH	Pa_{O_2}, Torr	Pa_{CO_2}, Torr
			Intact			
Control	7.401 ± 0.019	100 ± 4	36.5 ± 1.8	7.321 ± 0.015†	110 ± 11†	46.3 ± 2.0†
MK-801	7.342 ± 0.013*	99 ± 5	39.3 ± 2.9*	7.320 ± 0.017†	110 ± 14	47.4 ± 3.8†
	Room Air			10% O_2		
	pH	Pa_{O_2}, Torr	Pa_{CO_2}, Torr	pH	Pa_{O_2}, Torr	Pa_{CO_2}, Torr
			Intact			
Control	7.377 ± 0.029	100 ± 6	36.2 ± 1.7	7.545 ± 0.021†	35 ± 1†	23.7 ± 1.9†
MK-801	7.359 ± 0.025	96 ± 5*	39.5 ± 2.7*	7.332 ± 0.022*†	35 ± 1†	37.5 ± 1.8*†
			CBD			
Control	7.367 ± 0.027	69 ± 15	45.3 ± 6.2	7.399 ± 0.061	32 ± 3†	39.0 ± 5.0†
MK-801	7.341 ± 0.090	75 ± 17	47.9 ± 5.2	7.356 ± 0.054	31 ± 4†	39.1 ± 3.3†

Values are means ± SD. Pa_{O_2} and Pa_{CO_2}, arterial P_{O_2} and P_{CO_2}; CBD, carotid body denervated. *Significantly different from control; †significantly different from room air.

hypercapnia before and after MK-801 administration, although the increase was smaller after NMDA receptor blockade (Fig. 1). Changes in T_I and T_E during hypercapnia after MK-801 were similar to control responses (Fig. 1).

Effects of MK-801 Administration on the Hypoxic Ventilatory Response

Intact animals. Ventilatory responses to 10% O_2 are illustrated in Fig. 2. In intact animals in the unblocked condition, $\dot{V}_E$ increased ~100% because of increases in f ($P < 0.05$), with V_T remaining unchanged. Hypoxia induced changes in respiratory timing, with T_I and T_E being decreased ~40%. Significant increases in V_T/T_I were observed. Arterial P_{O_2} (Pa_{O_2}) decreased to ~35 Torr during hypoxia and was associated with a mean decrease in Pa_{CO_2} of 12.5 Torr, indicating significant alveolar hyperventilation (Table 1).

After NMDA receptor blockade with MK-801, no changes in $\dot{V}_E$ occurred in response to hypoxia (Fig. 2). Despite the absence of ventilatory augmentation in 10% O_2, changes in respiratory pattern occurred that differed from those seen in control conditions. In contrast to the increase in f observed in response to hypoxia before MK-801 administration, after MK-801 f decreased ~20% from eucapnic levels and V_T increased >30%. In contrast to the reduction in T_I and T_E in response to hypoxia in untreated animals, after NMDA receptor blockade T_I increased ~40% and T_E remained unchanged during hypoxia (Fig. 2). No increases in V_T/T_I were seen in response to hypoxia in the blocked condition. The markedly attenuated ventilatory response to hypoxia was further confirmed by arterial blood-gas measurements. Indeed, although Pa_{O_2} decreased to the same level in control and MK-801 conditions, alveolar hyperventilation was not observed, inasmuch as Pa_{CO_2} was only modestly decreased in the blocked condition.

CBD animals. To assess the role of NMDA receptors in mediating the hypoxic ventilatory response originating from peripheral chemoreceptors other than carotid bodies, selective CBD was performed in seven animals. Compared with intact animals at rest, CBD resulted in decreases in resting $\dot{V}_E$ and alveolar ventilation

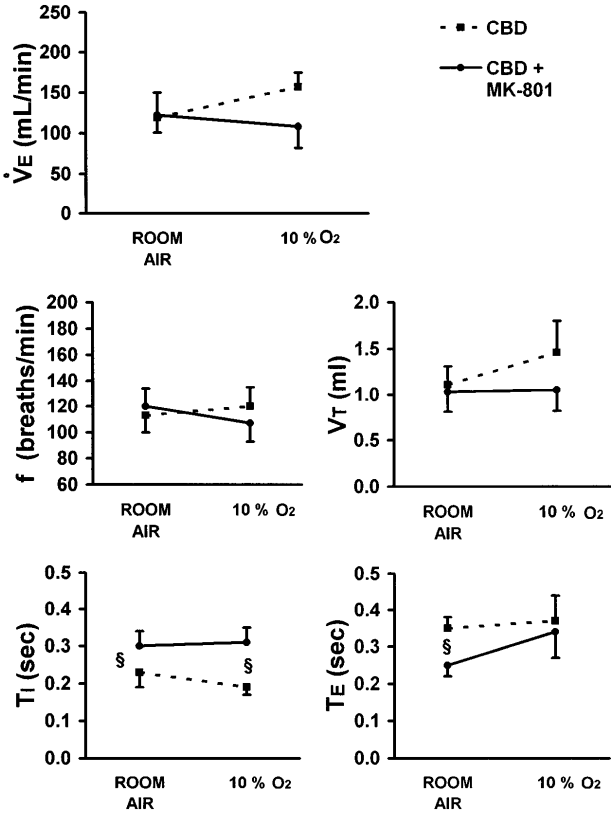

Fig. 3. $\dot{V}_E$, f, V_T, T_I, and T_E in response to 10% O_2 before and after MK-801 administration in animals with selective carotid body denervation (CBD). § Significant differences between conditions.

(Fig. 3). T_I and T_E were unaffected. Administration of MK-801 was without effect on ventilation or arterial blood-gas measurements during room air breathing (Fig. 3, Table 1). In contrast to the intact animals, however, NMDA receptor blockade was associated with an increase in T_I and a decrease in T_E.

Ventilatory responses to hypoxia were examined after CBD before and after MK-801 administration (Fig. 3). In untreated CBD animals, a 30% increase in $\dot{V}_E$ was observed after 30 min of hypoxia compared with the 100% increase in intact animals. Thus, after CBD, a residual peripheral chemoreceptor afferent reflex remained operative. Nonsignificant increases in f and V_T contributed to the significant, albeit mild, ventilatory response to hypoxia in CBD rats. Hypoxic exposure was without effect on T_I and T_E.

After MK-801 administration, the residual ventilatory response to hypoxia was abolished. No changes were observed in $\dot{V}_E$, f, V_T, and T_I after MK-801.

Effects of MK-801 Administration on the Ventilatory Response to NaCN

Intravenous administration of NaCN was employed to assess the role of NMDA receptors in the mediation of peripheral chemoreceptor afferent input without the confounding effects of systemic hypoxia. Ventilatory responses to NaCN were investigated in intact animals before and after MK-801 administration (Fig. 4). Ventilatory increases were dose dependent in intact animals, the response becoming significant at an NaCN dose of 5 μg/kg. After MK-801 administration the ventilatory response to NaCN was markedly attenuated, with the response curve being shifted to the right by 1 log dose; i.e., significant increases in $\dot{V}_E$ occurred at an NaCN dose of 50 μg/kg.

Effects of MK-801 Administration on the Cardiovascular Response to Hypoxia

Intact animals. MAP and HR responses to hypoxia were measured in animals before and after administration of MK-801 in intact and CBD animals (Fig. 5). Transient increases in MAP and HR occurred immediately after MK-801 injection and abated within 5–10 min. Before MK-801 administration, no changes were observed in MAP and HR in response to hypoxia, although a trend toward lower MAP was present near the end of the 30-min hypoxic challenge. After NMDA receptor blockade in intact animals, MAP and HR during room air breathing or in response to 10% O_2 were not different from those measured in control conditions.

CBD animals. In animals after CBD, MAP and HR responses to hypoxia were also investigated before and after MK-801 administration (Fig. 5). In control CBD animals, hypoxia was associated with a 60% reduction in MAP, whereas HR remained unchanged. After NMDA receptor blockade, MAP and HR were not different from the unblocked condition in room air and hypoxic conditions.

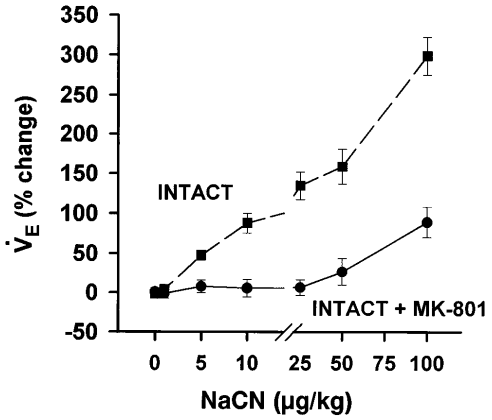

Fig. 4. $\dot{V}_E$ response to intravenous administration of increasing doses of NaCN before (■) and after (●) MK-801 in intact rats.

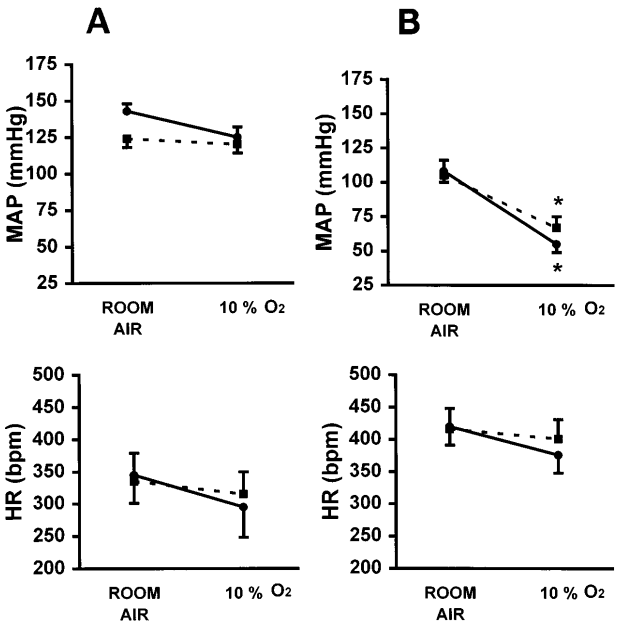

Fig. 5. Mean arterial blood pressure (MAP) and heart rate (HR) in response to 10% O_2 before (■) and after (●) MK-801 administration in intact animals (*A*) and animals with selective CBD (*B*). bpm, Beats/min. * Significantly different from room air.

DISCUSSION

In this study of conscious, freely behaving rats, blockade of NMDA receptors virtually abolished the ventilatory response to hypoxia while preserving the ventilatory response to hypercapnia. NaCN ventilatory responses were also markedly attenuated after NMDA receptor channel blockade, further suggesting that peripheral chemoreceptor-mediated ventilatory increases are critically dependent on NMDA receptor-mediated mechanisms in the conscious adult rat. In addition, tonic activity of NMDA receptors appears to prolong T_I in intact animals, whereas in the absence of carotid body chemoafferent input, NMDA-mediated pathways may facilitate termination of the inspiratory phase. Regulation of blood pressure and HR during baseline conditions and during peripheral and central

chemoreflex stimulation was not altered by the administration of MK-801 in conscious rats.

Role of NMDA Receptors in Control of Respiratory Timing and Pattern Generation

Maintenance of resting ventilation after systemic administration of NMDA receptor channel blocker suggests that NMDA receptor activity is not essential for respiratory rhythm generation in the adult conscious rat. However, after NMDA receptor antagonist administration, some degree of respiratory output inhibition may have occurred, as indicated by the ~3-Torr increase in Pa_{CO_2}. These findings are consistent with similar studies in conscious rats (27), spontaneously breathing anesthetized rats (8), and intact awake cats (10).

NMDA receptors, but not non-NMDA receptors, are critically involved in phase switching from inspiration to expiration in cats (10, 33), rats (8), and nonhuman primates (34). Intravenous administration of NMDA receptor antagonists significantly prolonged T_I (8, 10), and vagotomy further accentuated this effect, leading to apneusis (8, 11). In the present study of conscious intact rats, MK-801 administration decreased T_I, suggesting that, if indeed NMDA receptors mediate inspiratory termination, other compensatory mechanisms are recruited during wakefulness. This observation emphasizes the importance of state, i.e., wakefulness vs. anesthesia, in the control of respiratory timing. Indeed, administration of even low doses of anesthetic agents was associated with significant alterations in inspiratory off-switch mechanisms (5), suggesting that, when particular respiratory drives are abolished or inhibited by an anesthetic, residual mechanisms responsible for termination of inspiration or ventilatory output are NMDA dependent.

In contrast to the intact animal, NMDA receptor antagonism after CBD resulted in a prolongation of T_I similar to that observed in anesthetized intact rats with peripheral chemoreceptors debuffered by breathing 100% O_2 (8). Such additional information suggests that peripheral afferent input is essential for recruitment of compensatory mechanisms that are not NMDA dependent. Thus, if afferent input is reduced (anesthesia or CBD) or abolished (vagotomy), NMDA dependency of the inspiratory off-switch will emerge, ranging from inspiratory prolongation to apneusis.

NMDA receptor antagonism was without effect on T_E in intact conscious rats; however, after CBD, MK-801 administration was associated with shortened T_E. This is consistent with observations that activation of NMDA receptors in the NTS increases T_E in carotid-denervated anesthetized cats (4). Therefore, it appears that removal of peripheral afferent activity unmasks several NMDA receptor mechanisms involved in respiratory timing regulation.

MK-801 Effects on Ventilatory Response to Hypercapnia in Intact Conscious Animals

Results of the present study indicate that the ventilatory response to hypercapnia in awake intact rats is only mildly affected by NMDA receptor antagonism. Indeed, although the eucapnic level of ventilation was reduced after MK-801 administration in some animals, in others this response was not observed. In addition, animals were capable of mounting a significant ventilatory response to the 5% CO_2 challenge after NMDA receptor antagonist administration. Preservation of the ventilatory response to CO_2 after NMDA receptor antagonism has been observed in conscious rats (27) and anesthetized cats (33). Indeed, ventilatory responses during hypercapnic stimulation were mediated predominantly by non-NMDA receptors within the retrotrapezoid nucleus (29) and on the ventrolateral medullary surface in the region of the hypoglossal rootlets (24). Alternatively, other studies suggest that the hypercapnic ventilatory response may not involve glutamate receptors. Systemic blockade of NMDA and non-NMDA receptors was unable to suppress the ventilatory response to CO_2 in anesthetized cats (33). However, these apparently contradictory results may reflect the route of administration of antagonists, inasmuch as systemic drug administration elicited, in general, different results from studies in which central drug administration was employed.

Although NMDA receptor mechanisms do not appear critical for the ventilatory response to hypercapnia, V_T/T_I responses to hypercapnia were reduced after MK-801 administration, suggesting that NMDA receptors may underlie some component of the hypercapnic response (Fig. 1). NMDA receptor antagonism could eliminate a component of tonic facilitation to eucapnic and hypercapnic ventilation. Indeed, neurons within the retrotrapezoid nucleus region have endogenous glutaminergic input, which is involved in the maintenance of phrenic amplitude and timing during eucapnic and hypercapnic conditions (29). NMDA receptor antagonism would reduce this source of tonic drive and may account for an alteration in threshold of the CO_2 ventilatory response.

MK-801 Effects on Ventilatory Response to Hypoxia in Intact Conscious Animals

After NMDA receptor blockade, ventilation in intact conscious rats did not increase in response to inhalational hypoxia, suggesting that intact NMDA receptor mechanisms are critical for the expression of the hypoxic ventilatory response. Although identification of the neural sites affected by MK-801 administration in the present study is impossible by virtue of systemic administration of the antagonist, MK-801 is known to readily cross the blood-brain barrier and act within the pontomedullary structures (41). A high density of NMDA receptors has been demonstrated within the NTS (28), and evidence exists to support involvement of NMDA receptor mechanisms within the NTS in the ventilatory response to hypoxia. Glutamate is released in the NTS during exposure to 10% O_2, and this release is accompanied by an increase in ventilation (27). Furthermore, hypoxic exposure in conscious rats is associated with activation of the c-*fos* gene within the NTS and subsequent expression of *fos*-like immunoreactive protein

(18), whereas hypoxia in the presence of NMDA receptor antagonism was associated with attenuation of *fos* expression. Small lesions to the commissural nucleus of the NTS, induced by microinjection of kainic acid, attenuated the ventilatory response to hypoxia (19), whereas lesions rostral to the obex were ineffective. Similar results were obtained by local microdialysate infusion of MK-801 into the NTS in conscious rats (30). The present study in intact conscious rats is in close agreement with the above findings.

Attenuation of the ventilatory response to hypoxia after MK-801 administration may be related to an interruption of peripheral chemoreflex transmission of bulbospinal respiratory drive to spinal motoneurons, which has been shown to involve NMDA and non-NMDA receptors (9). However, because the ventilatory response to hypercapnia remained intact in the present study, it is unlikely that the attenuation of the hypoxic ventilatory response was at this point in the peripheral chemoreflex loop.

NMDA receptor blockade may impact the ventilatory response to hypoxia through actions involving nitric oxide (NO) synthase (NOS). NMDA receptor activation contributes to the maintenance of hypoxia-induced increases in respiratory output through activation of NOS within the NTS, which leads to the release of NO (16). Activation of NMDA receptors elevates intracellular calcium by voltage-dependent calcium channel opening or by release of intracellular calcium stores. Calcium will bind with calmodulin and activate NOS to produce NO, which in turn will modulate neurotransmitter release via activation of guanosine 3′,5′-cyclic monophosphate-dependent protein phosphorylation cascades. Alternatively, NO will rapidly diffuse into the presynaptic neuron, where it enhances glutamate release (13, 36). Thus, within respiratory control regions, NO is thought to play a pivotal role in sustaining ventilatory output during hypoxia (14, 15). Therefore, NMDA receptor blockade would decrease NO release and, thus, decrease the ventilatory response to hypoxia.

MK-801 Effects on Ventilatory Response to Hypoxia in CBD Animals

After selective CBD in this study, a residual ventilatory response to hypoxia was still present and amounted to ~30% of the response observed in intact animals. Such residual hypoxic ventilatory response is attributable to hypoxic stimulation of noncarotid chemoreceptor glomus tissue situated in the cervical region in rats and other ancillary glomus tissue served by glossopharyngeal nerves and abdominal vagi (25), since aortic bodies do not significantly contribute to hypoxic respiration in the rat (25). Alternatively, the increase in ventilation may be a result of the arterial hypotension that occurred, inasmuch as even small reductions in MAP are known to stimulate ventilation in conscious animals (32). However, because MAP changed similarly in the blocked and unblocked conditions, the changes in ventilation are unlikely to be related to the alteration in blood pressure. Our findings that MK-801 administration in CBD animals abolished the residual ventilatory response to hypoxia indicate that all peripheral chemoreflexes (carotid and noncarotid) require NMDA receptor-mediated synaptic neurotransmission for expression of the hypoxic ventilatory response.

MK-801 Effects on Ventilatory Response to NaCN in Intact Animals

Repeated NaCN injections in normoxic rats allow delivery of a strong carotid chemoreceptor stimulus in the absence of the potential confounding effects that accompany systemic hypoxia. In conscious rats, respiratory responses occur within 1–2 s, whereas blood pressure changes lag by ~3–4 s, thus allowing for accurate analysis of the ventilatory component of the response (12). In this study the ventilatory response to increasing doses of NaCN was markedly attenuated after NMDA receptor antagonist administration. The modest increase in ventilation observed during NMDA receptor antagonism after administration of very high doses of NaCN may reflect central effects of this compound (17).

Within the carotid body, the major organ associated with peripheral chemoreception, high concentrations of glutamate have been demonstrated within the O_2-sensitive glomus cells (38). However, glutamate does not appear to be the neurotransmitter released by glomus cells (38). Thus it is unlikely that the marked attenuation of the hypoxic ventilatory response induced by MK-801 was due to its action on carotid body function. Our findings of attenuation of the ventilatory response to NaCN after NMDA receptor antagonism provide novel evidence for a mandatory role of NMDA receptors in the full expression of the ventilatory response to peripheral chemoreceptor stimulation.

MK-801 Effects on Resting Arterial Blood Pressure During Normoxia and Hypoxia

Glutamate receptors within the NTS (31) and the rostral ventrolateral medulla (39), as well as at the spinal level (3), are important in determining resting levels of arterial blood pressure and HR. Therefore, the absence of significant changes in MAP or HR after MK-801 administration in the present study is surprising. One possible explanation lies in the consideration of state. The aforementioned studies were performed during anesthesia, whereas the present studies were undertaken in the conscious state. Recently, it was demonstrated that antagonism of NMDA receptors in the NTS with (±)-2-amino-5-phosphonovaleric acid in conscious rats was without effect on resting arterial pressure and HR (7), similar to the findings of the present study. On the other hand, our findings are in contrast to those of Lewis et al. (21), who reported that tonically active excitatory amino acid pathways that involve NMDA receptors regulate resting blood pressure and HR in conscious rats. Reconciliation of these disparate results may lie in the 10-fold higher dose of NMDA antagonist employed by Lewis et al. However, despite such high MK-801 dosages, resting arterial pressure increased by only 10 mmHg (21). Thus NMDA

receptor mechanisms appear to be of minimal importance in the regulation of resting blood pressure in the awake state.

During hypoxia in the awake rat, arterial blood pressure remains unchanged (23) because of the balancing of an increased cardiac output with a vasodilation in most systemic tissues. In contrast, after carotid body deafferentation, hypoxia will induce a decrease in arterial pressure (23), which was seen in the present study. Arterial blood pressure responses to hypoxia were not modified by NMDA receptor blockade, further suggesting that, in the awake state, cardiovascular regulation during carotid chemoreceptor stimulation does not appear to involve NMDA receptor mechanisms.

Summary

NMDA receptor activation is critical for the full expression of the ventilatory response to hypoxia but not to hypercapnia. The afferent limb of the peripheral chemoreflex is critically dependent on NMDA receptor-mediated synaptic transmission, as indicated by the marked attenuation of the ventilatory response to peripheral chemoreceptor stimulation by NaCN. NMDA receptor pathways facilitate termination of inspiration, and this effect becomes apparent when peripheral afferent input is diminished or absent. Finally, NMDA receptors are not essential for regulation of blood pressure and HR during peripheral and central chemoreflex stimulation in conscious rats.

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