

Glutamatergic neurotransmission modulates hypoxia-induced hyperventilation but not anapyrexia

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Abstract

The interaction between pulmonary ventilation (V_E) and body temperature (T_b) is essential for O_2 delivery to match metabolic rate under varying states of metabolic demand. Hypoxia causes hyperventilation and anapyrexia (a regulated drop in T_b), but the neurotransmitters responsible for this interaction are not well known. Since L-glutamate is released centrally in response to peripheral chemoreceptor stimulation and glutamatergic receptors are spread in the central nervous system we tested the hypothesis that central L-glutamate mediates the ventilatory and thermal responses to hypoxia. We measured V_E and T_b in 40 adult male Wistar rats (270 to 300 g) before and after intracerebroventricular injection of kynurenic acid (KYN, an ionotropic glutamatergic receptor antagonist), α -methyl-4-carboxyphenylglycine (MCPG, a metabotropic glutamatergic receptor antagonist) or vehicle (saline), followed by a 1-h period of hypoxia (7% inspired O_2) or normoxia (humidified room air). Under normoxia, KYN (N = 5) or MCPG (N = 8) treatment did not affect V_E or T_b compared to saline (N = 6). KYN and MCPG injection caused a decrease in hypoxia-induced hyperventilation (595 ± 49 for KYN, N = 7 and 525 ± 84 ml $kg^{-1} min^{-1}$ for MCPG, N = 6; $P < 0.05$) but did not affect anapyrexia (35.3 ± 0.2 for KYN and $34.7 \pm 0.4^\circ C$ for MCPG) compared to saline (912 ± 110 ml $kg^{-1} min^{-1}$ and $34.8 \pm 0.2^\circ C$, N = 8). We conclude that glutamatergic receptors are involved in hypoxic hyperventilation but do not affect anapyrexia, indicating that L-glutamate is not a common mediator of this interaction.

Key words

- Kynurenic acid
- α -Methyl-4-carboxyphenylglycine (MCPG)
- Hypoxia
- Ventilation
- Body temperature

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Introduction

It is well known that oxygen is crucial for ATP synthesis, cell function and consequently survival of all aerobic organisms. Respiratory, thermal and metabolic adjustments have been reported to attenuate the reduction in

O_2 supply during exposure to hypoxia (for a review, see Ref. 1). At first sight, these responses appear to occur in opposite directions, i.e., increases in ventilatory drive and decreases in body temperature (T_b) and metabolism (2). However, this apparent contradiction may reflect the complexity of the still

unknown mechanisms and neuromodulators involved in these responses.

Stimulation of peripheral chemoreceptors during hypoxia is associated with release of the excitatory neurotransmitter L-glutamate in several brain areas associated with pulmonary ventilation (V_E). Accordingly, chemodenervation or inhibition of L-glutamate receptors nearly abolishes the hyperventilatory response to hypoxia (3). However, no reports are available about the role of L-glutamate in the mediation of hypoxic anapnoea even though several studies have shown that L-glutamate may be involved in thermoregulation (4-6). Therefore, it is reasonable that central L-glutamate may play a role in the ventilatory and thermoregulatory responses to hypoxia, mediating this interaction.

In view of these considerations, we administered intracerebroventricularly (*icv*) an ionotropic (kynurenic acid) or metabotropic (α -methyl-4-carboxyphenylglycine, MCPG) non-selective glutamatergic antagonist and measured V_E and Tb under normoxic and hypoxic conditions.

Material and Methods

Animals

Experiments were performed on awake adult male Wistar rats weighing 270 to 300 g, housed at controlled temperature ($25 \pm 1^\circ\text{C}$) and exposed to a daily 12-h light-dark cycle, with free access to water and food. Animal care was provided in compliance with the guidelines set by the American Physiological Society (7). The rats used in this study were divided into six major groups: 1) *icv* kynurenic acid injection (N = 5), 2) *icv* MCPG injection (N = 8), and 3) *icv* vehicle (saline) injection (N = 6) in rats exposed to normoxia; 4) *icv* kynurenic acid injection (N = 7), 5) *icv* MCPG injection (N = 6), and 6) *icv* vehicle injection (N = 8) in rats exposed to hypoxia.

Surgery

Animals were submitted to general anesthesia by intraperitoneal administration of 250 mg/kg 2,2,2-tribromoethanol (Aldrich, Milwaukee, WI, USA). Rats of the kynurenic acid, MCPG and vehicle groups were fixed in a stereotaxic frame and implanted with a stainless steel guide cannula (0.7 mm, OD) into the third ventricle (coordinates: anterior -0.4 mm, lateral 0 mm, dorsal 7.8-8.5 mm) for *icv* administration (8). The displacement of the meniscus in a water manometer assured correct positioning of the cannula in the third ventricle. The cannula was attached to the bone with stainless steel screws and acrylic cement. A tight-fitting stylet was kept inside the guide cannula to prevent occlusion. All animals were submitted to paramedian laparotomy for the insertion of a biotelemetry capsule (model: ER 4000; Mini-Mitter Co. Inc., Sunriver, OR, USA) into the peritoneal cavity. The wound was then closed and the implanted capsule was used for Tb measurements. At the end of the surgical procedures, rats were injected intramuscularly with an antibiotic cocktail (benzylpenicillin-dihydrostreptomycin-streptomycin; 80,000 IU/kg and 33 mg/kg and 33 mg/kg, respectively). The surgical procedures were performed over a period of about 40 min. Experiments were initiated 1 week after surgery in the *icv* kynurenic acid, *icv* MCPG or vehicle groups.

Determination of ventilation

Measurements of V_E were performed by the body plethysmograph method (9,10). Each animal was individually placed in a Plexiglas chamber (5 liters) connected to a reference chamber of identical size and construction. Use of the reference chamber made the system independent of minor pressure interference from the laboratory, such as those caused by opening and closing doors. During V_E measurements, the flow was in-

errupted and the chamber sealed for short periods of time (~2 min). The principle is the following: the animal is placed in an air-tight chamber in which the temperature differs from T_b . The cyclic variations of temperature and water vapor partial pressure of the tidal volume (V_T) produce variations of the pressure in the chamber proportional to this volume (11). This procedure was performed once during each time of V_E measurement. Signals from a differential air-pressure transducer (model MP45-14-871; Validyne, Northridge, CA, USA) were connected to a differential pressure signal conditioner (Gould Instrument Systems, Inc., Valley View, OH, USA), passed through an analog-to-digital converter, digitized in a micro-computer equipped with a data acquisition software (Acquire 6600; Data Acquisition System, Gould Instrument Systems, Inc.), and then analyzed with a data-analysis software (Windaq Software for Windows by Dataq Instruments, Inc., Akron, OH, USA). Calibration for volume was obtained during each experiment by injecting the animal chamber with a known amount of air (1 ml) using a graduated syringe. Respiratory variables such as respiratory frequency (fr) and V_T were measured, the latter being calculated by the Malan formula (11):

$$V_T = \frac{PT}{PK} \times VK \times \frac{TC}{TR} \times \frac{(PB - PC)}{(PB - PC) - \frac{TC}{T_b} \times (PB - PR)}$$

where PT is the pressure deflection associated with each V_T , PK is the pressure deflection associated with injection of the calibration volume (VK), TC is the air temperature in the animal chamber, PB is the barometric pressure, PR is the vapor pressure of water at T_b , PC is the vapor pressure of water vapor in the animal chamber, T_b is the body temperature, and TR is the room temperature. V_E was calculated by multiplying fr by V_T . V_E and V_T are presented at the ambient barometric pressure, at T_b , and saturated with water vapor at this temperature.

Determination of body temperature

T_b was measured by biotelemetry. The animal chamber was placed on the ER 4000 telemetry receiver (Mini-Mitter Co. Inc.). The output of the receiver displayed the pulse frequency of the transmitter capsule and the corresponding T_b on the screen of a microcomputer containing appropriate software (Vital View, version 3.1; Mini-Mitter Co. Inc.).

Experimental protocols

Measurements of V_E and T_b were made simultaneously. Animals were exposed first to humidified room air and then to a humidified hypoxic poikilocapnic gas mixture containing 7% O_2 (AGA, Sertãozinho, SP, Brazil). Each animal was exposed to a 1-h period of hypoxia and received a single *icv* injection of kynurenic acid, MCPG or vehicle. Experiments were carried out randomly between 8:00 am and 1:00 pm.

Experiment 1. Effect of kynurenic acid or MCPG on the ventilatory and thermoregulatory response to normoxia. Each animal was placed in an individual Plexiglas chamber (5 liters) and allowed to move about freely while the chamber was flushed with humidified air. After the animals remained calm (~30 min), control V_E was measured and T_b started to be measured continuously at 5-min intervals. Subsequently, experimental rats were treated with kynurenic acid (100 nmol/0.2 μ l) or MCPG (200 nmol/0.2 μ l; Sigma, St. Louis, MO, USA) injected into the third ventricle, while the vehicle groups received the same volume of pyrogen-free sterile saline (150 mM 0.9% NaCl), followed by V_E measurement at 10, 15, 30, 45, 60, 70, 85, 100, 115, and 130 min during normoxia (the chamber was ventilated with humidified room air).

Experiment 2. Effect of kynurenic acid or MCPG on hypoxia-induced hyperventilation and anapyrexia. After the same procedures as performed in Experiment 1, experimental rats

were treated with kynurenic acid (100 nmol/0.2 μ l) or MCPG (200 nmol/0.2 μ l) injected into the third ventricle, while the vehicle groups received the same volume of pyrogen-free sterile saline (150 mM 0.9% NaCl), followed by V_E measurement 10 min later. Subsequently, a hypoxic gas mixture (7% inspired O_2) was

flushed through the chamber for 60 min and V_E was measured at 5, 20, 35, 50, and 60 min during hypoxia. Finally, rats were returned to a 60-min period of normoxia exposure (the chamber was ventilated for 60 min with room air), and V_E was measured again after 15, 30, 45, and 60 min.

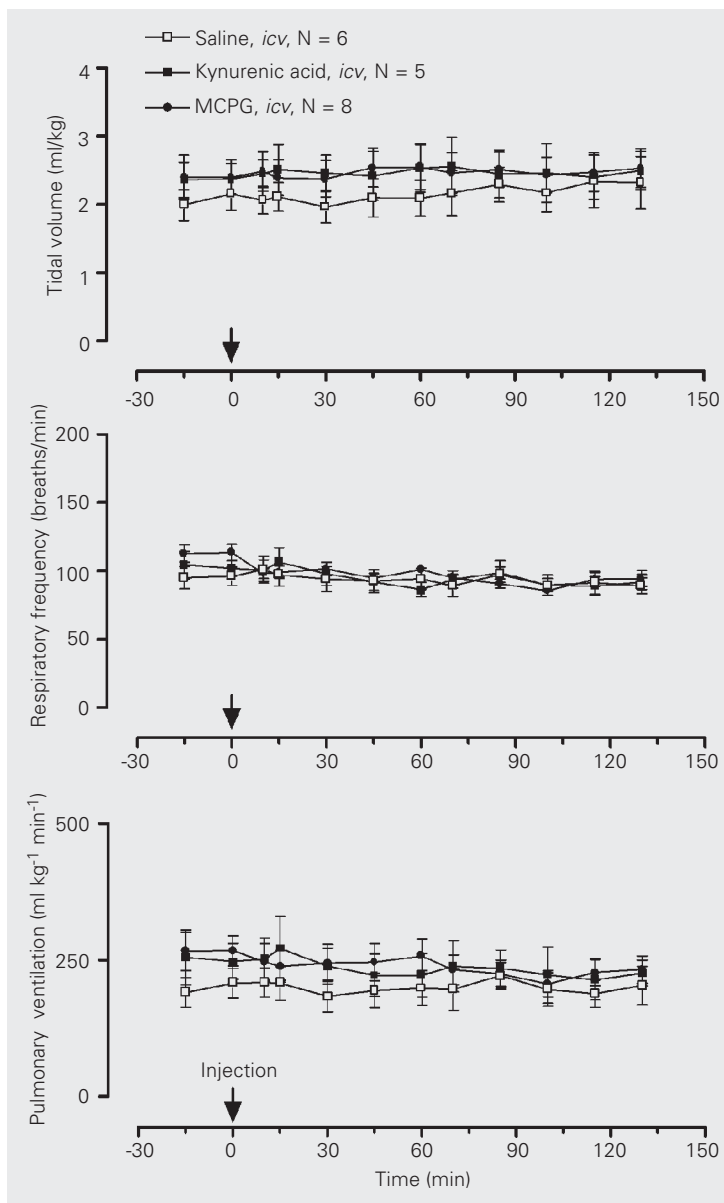


Figure 1. Effects of *icv* injection of kynurenic acid (100 nmol/0.2 μ l), α -methyl-4-carboxyphenylglycine (MCPG, 200 nmol/0.2 μ l) or vehicle (saline) on tidal volume, respiratory frequency and pulmonary ventilation of Wistar rats exposed to normoxic conditions (21% O_2). The arrows indicate the time of injection. Data are reported as means \pm SEM.

Statistical analysis

Data are reported as means \pm SEM and were analyzed statistically by repeated measures multivariate analysis of variance (MANOVA), with factors being treatment (kynurenic acid or MCPG), time and stimulus (hypoxia). In the case of significant interactions, one-way ANOVA was performed each time. The Duncan test was used for multiple comparisons. The statistical analysis was performed using the SPSS software (SPSS Inc. for Windows 6.0, Chicago, IL, USA). The level of significance was set at $P < 0.05$.

Results

During the experiments the mean (\pm SEM) chamber temperature was $24.0 \pm 0.5^\circ\text{C}$ and mean room temperature was $23.0 \pm 0.7^\circ\text{C}$. There was no statistically significant difference in T_b between normoxic ($37.1 \pm 0.1^\circ\text{C}$) and hypoxic ($36.8 \pm 0.1^\circ\text{C}$) animals.

Experiment 1. Effects of *icv* injection of kynurenic acid or MCPG on resting ventilation and body temperature

Under normoxic conditions, *icv* injection of kynurenic acid or MCPG caused no significant changes in V_E (Figure 1) or T_b (Figure 2).

Experiment 2. Effects of *icv* injection of kynurenic acid and MCPG on hypoxia-induced hyperventilation and anapnoea

Figure 3 shows the effect of *icv* injection of kynurenic acid and MCPG on V_T , f_R and

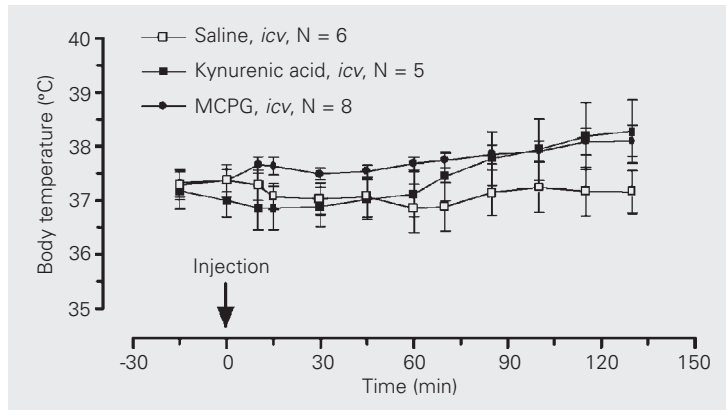


Figure 2. Effects of *icv* injection of kynurenic acid (100 nmol/0.2 μ l), α -methyl-4-carboxyphenylglycine (MCPG, 200 nmol/0.2 μ l) or vehicle (saline) on the body temperature of rats exposed to normoxic conditions (21% O_2). The arrow indicates the time of injection. Data are reported as means \pm SEM.

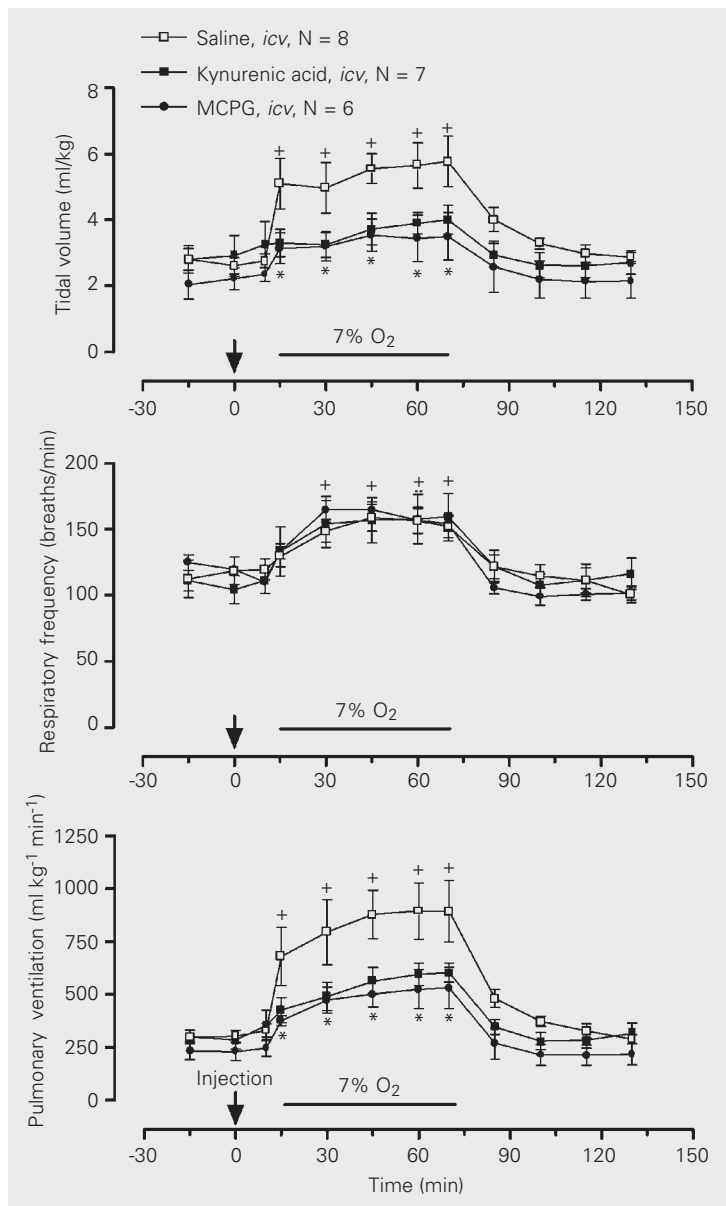


Figure 3. Effects of *icv* injection of kynurenic acid (100 nmol/0.2 μ l), α -methyl-4-carboxyphenylglycine (MCPG, 200 nmol/0.2 μ l) or vehicle (saline) on tidal volume (V_T), respiratory frequency (f_R) and pulmonary ventilation (V_E) of rats exposed to hypoxia (7% O_2). *Significant increase ($P < 0.05$) in V_T , f_R and V_E during hypoxia compared to control values (time zero) (MANOVA followed by the Duncan test). *Significant difference ($P < 0.05$) between vehicle (saline) and kynurenic acid and MCPG treatments during hypoxia (MANOVA followed by the Duncan test). The arrows indicate the time of injection. Data are reported as means \pm SEM.

V_E . Typical hypoxia-induced hyperventilation (increases in V_T , f_R and V_E) was observed after *icv* saline (vehicle) injection, whereas *icv* injection of kynurenic acid and MCPG reduced the ventilatory response to hypoxia, acting essentially on V_T .

Typical hypoxia-induced anapyrexia was observed after saline treatment. Kynurenic acid and MCPG *icv* injection did not affect the drop in T_b induced by hypoxia (Figure 4).

Discussion

The present study shows that both ionotropic and metabotropic glutamatergic receptors play an excitatory role in the ventilatory response to hypoxia but do not participate in the neural pathways that mediate hypoxia-induced anapyrexia, indicating that L-glutamate is not a common mediator of these responses.

Ventilatory response to hypoxia

Hypoxia elicits a number of compensatory responses, including increased ventilation (for a review, see Ref. 1). It is known that hyperventilation induced by hypoxia results primarily from the activation of peripheral chemoreceptors located in the aortic and carotid bodies and the subsequent processing of information by the central ner-

vous system (CNS; 12). Mizusawa et al. (13) demonstrated that afferent nerve fibers carrying peripheral chemoreceptor impulses to the CNS cause release of L-glutamate during hypoxia. Accordingly, inhibition of L-glutamate receptors nearly abolishes the hyperventilatory response to hypoxia (3,13-17, present data).

Glutamate receptors are divided into two major classes, ionotropic and metabotropic glutamatergic receptors. Ionotropic glutamatergic receptors are ligand-gated ion channels and can be antagonized by kynurenic acid (18). Metabotropic glutamatergic receptors are G protein-coupled receptors that modulate second messenger systems and can be antagonized by MCPG (19).

In the present study we report that *icv* injections of kynurenic acid or MCPG (Figure 1) have no effect on ventilation under normoxia, indicating that central L-glutamate has no role in the control of pulmonary ventilation under resting conditions. However, some studies have shown that systemic administration (20), as well as microinjection into the brain stem, of both agonists and glutamatergic antagonists produce apnea (21,22). In the present study, which, to our knowledge, was the first to use *icv* injections of glutamatergic antagonists, this response was not observed. These differences may be explained by the use of different experimen-

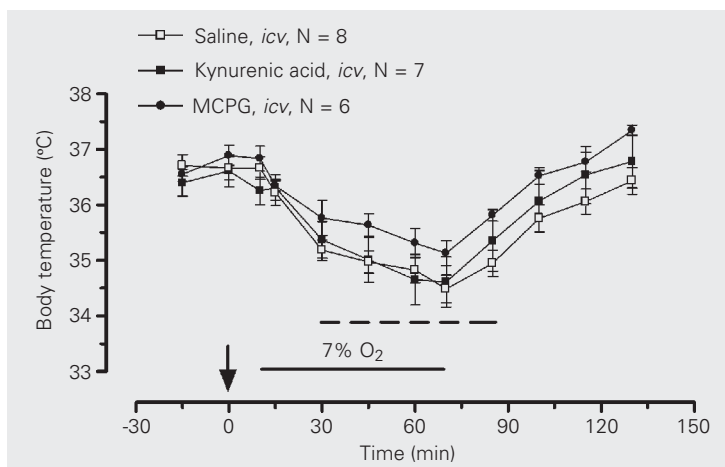


Figure 4. Effects of *icv* injection of kynurenic acid (100 nmol/0.2 μ l), α -methyl-4-carboxyphenylglycine (MCPG, 200 nmol/0.2 μ l) or vehicle (saline) on hypoxia-induced anapyrexia in rats. There was no significant difference between vehicle and kynurenic acid or MCPG treatments. The dotted bar represents the period of time during which a significant reduction in body temperature was observed in comparison to the normoxic control period (time zero; $P < 0.05$, MANOVA). The arrow indicates the time of injection. Data are reported as means \pm SEM.

tal approaches, of different species (cats) and of anesthetized animals.

We also observed that *icv* injections of kynurenic acid or MCPG (Figure 3) reduce the ventilatory response to hypoxia, indicating that central L-glutamate plays an excitatory role during exposure to hypoxia. These findings corroborate previous reports in the literature that investigated the same question. When different glutamatergic receptor antagonists are applied directly to the ventral surface of the medulla (14,17), into the nucleus tractus solitarii (13), intra-ventriculo-cisternally (3) or systemically (15,16), a reduction in the magnitude of the hyperventilatory response to hypoxia is observed.

The effect of *icv* injection of kynurenic acid and MCPG on the ventilatory response to hypoxia observed in the current study resembles the effect observed after *icv* injection of L-NAME (a nonselective nitric oxide synthase blocker) (23). In fact, activation of brain L-glutamate has been shown to lead to synthesis and release of nitric oxide (24,25) and Ogawa et al. (26) have suggested a possible interaction between L-glutamate and nitric oxide in the CNS in the control of breathing during hypoxia. In summary, our data indicate that ionotropic and metabotropic glutamatergic receptors play no role in the respiration of awake rats under resting conditions, but exert an excitatory modulation in the ventilatory response to hypoxia, acting mainly on V_T .

Thermoregulatory response to hypoxia

Hypoxia reduces T_b and metabolism in newborns and adults of many species (27,28), including humans (29). Hypoxia also leads animals to select ambient temperatures that favor T_b below those seen in normoxia, suggesting the occurrence of a drop in the thermoregulatory set point (30,31). On this basis, this T_b drop has been called anapyrexia, i.e., a regulated decrease in T_b (1,32), instead of hypothermia.

No reports are available about the role of L-glutamate in hypoxia-induced anapyrexia despite the fact that several studies have shown that L-glutamate may be involved in thermoregulation. L-glutamate receptors have been shown to participate in pyrogenic fever (5) as well as in hypothermia induced by dithiothreitol (4). Furthermore, Pechnick et al. (33) observed an increase in T_b after acute inhibition of L-glutamate receptors by (\pm)-dizocilpine maleate (MK 801), an L-glutamate antagonist. Moreover, injections of L-glutamate into the dorsomedial hypothalamus reduce the thermogenic activity, while injections into the medial preoptic area lead to a biphasic response with a decrease followed by an increase in heat production (34).

In the present study, we investigated if L-glutamate influences the thermoregulatory responses to hypoxia within the CNS. Our data indicate that L-glutamate does not participate in this response (Figures 2 and 4). Interestingly, a recent study from our laboratory (6) demonstrated that *icv* pretreatment with kynurenic acid abolished vasopressin-induced hypothermia. The reason for such differences may reside in the type of stimulus, which may trigger different mechanisms resulting in a drop in T_b .

In addition to L-glutamate, other mediators have been recently reported to participate in the hypoxia-induced anapyrexia and/or hyperventilation. Central dopamine affects thermal and metabolic responses to hypoxia without affecting hyperventilation (35), which is the opposite of the present results, i.e., L-glutamate affecting ventilation but not T_b . Therefore, dopamine and L-glutamate may control each system independently. L-glutamate may act mainly in the brain stem areas associated with respiration (such as nucleus tractus solitarii and ventrolateral medulla) and dopamine may mainly act by stimulating central areas (such as the hypothalamus) involved in thermoregulation, leading to respiratory, thermal and

metabolic adjustments during hypoxia exposure. Yet, some other modulators released during hypoxia exposure in the CNS may have effects on both respiratory and thermoregulatory systems, such as adenosine (36) and nitric oxide (23), which attenuate hypoxic hyperventilation and anapyrexia.

The present results indicate that central glutamatergic receptors do not participate in the control of V_E during normoxia in awake rats, but contribute to the development and maintenance of hypoxia-induced hyperven-

tilation. On the other hand, these receptors do not play a role in the mechanism responsible for hypoxic anapyrexia. Therefore, we conclude that the effect of L-glutamate within the CNS on hyperventilation is independent from anapyrexia induced by hypoxia.

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