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MK-801 alters diaphragmatic activities in unanesthetized rats differently between normoxia and hypoxia

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Abstract

This study was designed to examine how systemic administration of an *N*-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, altered respiratory timing in unanesthetized rats under normoxia and hypoxia. To detect fine changes in inspiratory time (TI) and expiratory time (TE), and cycle duration (TTOT), we prepared a diaphragmatic electromyogram (EMGdia).

Diaphragm electrodes and arterial and venous catheters were inserted into Wistar rats ($n=8$) under pentobarbital anesthesia. The next day, EMGdia was recorded before and after intravenous administration of MK-801 (3 mg/kg) under normoxia and hypoxia (12% O_2) without anesthesia, and the respiratory timing (TI, TE, TTOT), respiratory frequency (fR), and amplitude of the integrated EMGdia were measured. Arterial blood gases (ABGs), mean arterial pressure (MAP), and heart rate (fH) were also measured with the EMGdia.

Under normoxia, MK-801 increased fR owing to a significant decrease in TE, and elevated both MAP and fH. Under hypoxia, MK-801 suppressed an increase in fR owing to a significant increase in TI, and did not accelerate fH. In both gaseous conditions, on ABGs, MK-801 did not alter partial pressure of O_2 (PaO_2) or CO_2 ($PaCO_2$), and slightly decreased pH (but not less than 7.4). MK-801 significantly decreased hypoxic response (%change from normoxia) in fR, and increased that in EMGdia amplitude, and did not alter a total ventilatory index (fR × EMGdia amplitude).

The results suggest that an NMDA receptor-mediated mechanism partially determines fR through significant alterations in respiratory timing, particularly in which the hypoxic ventilatory response was obtained in unanesthetized rats.
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Keywords: Respiratory timing; Conscious rats; Diaphragm electromyogram; Hypoxic response; Glutamate receptor blockade; *N*-methyl-D-aspartate; Respiratory system; Cardiovascular system

Introduction

The *N*-methyl-D-aspartate (NMDA) receptor channel blocker MK-801 crosses the blood-brain barrier, and systemic administration of MK-801 can regulate peripheral and central NMDA receptor-mediated regulatory processes in the cardiopulmonary system in anesthetized (Foutz et al., 1989; Monteau et al., 1990; Connelly et al., 1992; Cassus-Soulanis et al., 1995; Harris and Milsom, 2001; Tarakanov et al., 2004) and unanesthetized (Lewis et al., 1989; Foutz et al., 1994; Ohtake et al., 1998; Harris and Milsom, 2001; Reid and Powell, 2005) animals.

It has been suggested that hypoxia has a greater effect on respiratory timing than peak phrenic nerve activity in anesthetized dogs (Ledlie et al., 1981), and that excitatory amino acid receptors located in the pons, medullary lateral tegmental field, as well as those in the pre-Bötzinger complex, are important for respiratory timing (Fung et al., 1994; Orer et al., 2006). Thus, the NMDA receptor-mediated mechanism may play a significant role in determining respiratory timing (and fR) not only under normoxia but also under hypoxia. Recent studies done on the effects of MK-801 on the hypoxic ventilatory response in conscious rats (Ohtake et al., 1998; Reid and Powell, 2005) suggest that MK-801 attenuates the response, and the effects of MK-801 become more significant during postnatal development (e.g. 5-d vs. 15-d-old rats) (Ohtake et al., 2000). However, these studies used whole-body plethysmography to evaluate respiratory indices, and the effects of

Abbreviations: ABGs, arterial blood gases; EMGdia, diaphragm electromyogram; fH, heart rate; fR, respiratory frequency; MAP, mean arterial pressure; NMDA, *N*-methyl-D-aspartate; $PaCO_2$, partial pressure of CO_2 in arterial blood; pH, arterial blood pH; PaO_2, partial pressure of O_2 in arterial blood; SAB(s), spontaneous augmented breath(s); TE, expiratory time; TI, inspiratory time; TTOT, cycle duration.

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MK-801 on fR, tidal volume (VT), and minute ventilation (VE) seem more consistent than those on respiratory timing. In fact, the effects of MK-801 on respiratory timing (e.g., inspiratory time) in these studies (Ohtake et al., 1998; Reid and Powell, 2005) are opposite to those obtained in anesthetized or decerebrated animals (Foutz et al., 1989; Monteau et al., 1990; Connelly et al., 1992; Cassus-Soulanis et al., 1995). Although the differences may be related to anesthesia or decerebration, recordings of diaphragmatic or phrenic nerve activities, which have been used in anesthetized or decerebrated animals (Foutz et al., 1989; Connelly et al., 1992; Monteau et al., 1990), may have an advantage compared to whole-body plethysmography in determining respiratory timing. Whole-body plethysmography (or the barometric method) records pressure oscillations generated while an animal breathes in a housing chamber and derives VT from changes in the chamber pressure. Therefore, whole-body plethysmography allows us to measure pulmonary ventilation in unrestrained animals without the use of anesthesia or any invasive preparations. However, there are some methodological problems like baseline drift because of warming, humidification, and pressure generated by airflow during inspiration and expiration. Although these problems may be partially solved by providing a slow leak with an adequately long time constant compared to the animal's inspiratory time, expiratory time, and cycle duration (Mortola and Frappell, 1998), it seems difficult to detect fine changes in respiratory timing during unstable respiratory movements such as those seen after MK-801 administration (Monteau et al., 1990; Connelly et al., 1992; Cassus-Soulanis et al., 1995). In contrast, measurement of diaphragmatic or phrenic nerve activities is not influenced by temperature, humidification or pressure changes in the chamber, and may be advantageous to detect inspiratory activity during unstable breathing.

In this study, we implanted diaphragm electrodes to record EMGdia activity in conscious rats, and examined whether the effects of MK-801 on respiratory indices, in particular those related to respiratory timing, were different from those obtained in earlier studies using anesthetized rats, or those using the barometric method with unanesthetized rats.

Materials and methods

Animals

The study was approved by the Animal Research Committee of Nippon Dental University, School of Life Dentistry at Tokyo. Experiments were performed on 8 adult male Wistar rats weighing 236–284 g. The animals were cared for in accordance with Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences (The Physiological Society of Japan), and the American Physiological Society's Guiding Principles for Research Involving Animals and Human Beings.

EMGdia electrodes, and arterial and venous catheter preparations

The day before the experiment, after premedication with atropine sulfate (0.5 mg/kg, s.c.), rats were anesthetized with sodium pentobarbital (35 mg/kg, i.p.). Two bipolar electrodes for a diaphragmatic electromyogram (EMGdia) made of twisted 80-μm copper wires were implanted in the costal portion of the diaphragm, just beneath the xiphoid process, via a small midline incision (about 2 cm). The wires were of sufficient length (about 30 cm) to allow them to loop in the subcutaneous space to dampen tension exerted on the wires during movement, and to emerge from the exit point in the rump through a subdermal tunnel.

Following EMGdia electrode implantation, chronically indwelling catheters (PE-10 and PE-50, Intramedic®; Becton, Dickinson and Co., Franklin Lakes, NJ) with a total volume of 0.1 ml were implanted in the caudal artery and vein. For each catheter implantation, a 1-cm incision of the skin was performed at the level of the ventral (for the artery) or lateral (for the vein) proximal portion of the tail. The arterial catheter was implanted to sample blood for analysis of arterial blood gases (ABGs); i.e. partial pressure of O2 (PaO_2), partial pressure of CO2 ($PaCO_2$), pH, and to measure the mean arterial pressure (MAP) and heart rate (fH). The venous catheter was prepared for drug infusion. The catheters were filled with a saline-heparin solution (100 U/ml).

These catheters and the emerging EMGdia electrode wires were made into a bundle, which was wound around the tail (for about 2 cm), and protected with a soft plastic tube (1.5 mm thickness, 5 cm long). After intravenous administration of antibiotic and analgesic, the tube was covered with a fine stainless steel net (6 cm × 6 cm) until the recording. During the surgical preparations, incisions were performed and wounds were sutured under local anesthesia (lidocaine hydrochloride, 5–10 mg, subcutaneously), while the animals were still under the influence of sodium pentobarbital. After these surgical preparations, rats were given atropine sulfate (0.5 mg/kg, s.c.) again and antibiotics (aspoxicillin 2.5 mg, i.p.), and returned to the home cage. The animals appeared to recover from anesthesia 4 to 5 h after the operation. They were then given aspoxicillin (5 mg, i.v.) again and analgesic flurbiprofen axetil (0.25 mg, i.v.) to prevent postoperative complications, and were allowed free access to water and laboratory chow. Measurements were started the next morning.

EMGdia measurements

We measured EMGdia activity under unanesthetized conditions. A fine Chromel-Alumel thermocouple (OMEGA® Model 871A; OMEGA Engineering, Stamford, CT) was inserted 6 cm into the colon for continuous monitoring of colonic temperature, taken as representative of body temperature (Tb, °C). A rat was placed in a cylindrical restrainer made of loose stainless steel netting (diameter 6 cm, about 27 cm long), which was then placed in a Plexiglas chamber, and the rat was allowed at least 30 min to become accustomed to the environment. The EMGdia and Tb electrodes and the arterial and venous catheters emerged from the chamber through a hole (diameter 2 cm) in the back of the restrainer. This did not obstruct forward motion but almost entirely precluded any back-to-front rotation, thus facilitating blood sampling and avoiding twisting of the electrodes during the experiment (Saiki et al., 1994). Air was continuously delivered

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