Title: Role of glutamate and serotonin on the hypoxic ventilatory response in high-altitude-adapted plateau Pika

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ABSTRACT

The highland 'Plateau Pika' is considered to be adapted to chronic hypoxia. We hypothesized that glutamate N-methyl-D-aspartate (NMDA) and non-NMDA receptors, nitric oxide (NO) synthase, and serotonin are involved in hypoxic ventilatory response (HVR) in pikas. We tested the effects of NMDA (memantine) and non-NMDA receptors (DNQX) antagonists, NO synthase inhibitor (L-NAME), and selective serotonin reuptake inhibitors (fluoxetine) on ventilation and HVR in Pikas. Ventilatory parameters were measured before and after drug (or vehicle) injections in conscious Pikas at their natural living altitude (P_IO_2 86mmHg) and after a hypoxic challenge (P_IO_2 57 mmHg, 3 min) to assess the influence of peripheral chemoreceptor on HVR. Minute ventilation (V_I) and tidal volume (Vt), increased during hypoxic challenge after vehicle injection whereas the Ti/Ttot ratio remained unchanged. The increase in V_I and Vt observed with vehicle at P_IO_2-57, when compared to P_IO_2-86, was inhibited after memantine and fluoxetine injection whereas DNQX injection increased HVR. At P_IO_2-57, L-NAME induced an increase in the Ti/Ttot ratio when compared to vehicle. Therefore, the glutamate through NMDA-R/AMPA receptors bindings and serotonin pathway are implicated at the peripheral chemoreceptor level in HVR in Pikas. However, NO influences the ventilatory pattern of Pikas at their habitual living altitude.

Keywords: Hypoxia – Adaptation - Control of breathing – Serotonin – Glutamate pathway –

1. INTRODUCTION

Long-term high altitude exposure induces cardiopulmonary adaptations to optimize tissue oxygenation under hypoxic conditions. These improvements appear at the level of ventilation ($\dot{V}_I$), pulmonary diffusion, circulation and tissue diffusion (Scott and Milsom, 2007). The acute hypoxic ventilatory response (HVR) is characterized by transient hyperventilation followed by a relative ventilatory decline (Teppema and Dahan, 2010), whereas the time-dependent increase in resting baseline ventilation occurring after several hours to months is called ventilatory acclimatization to chronic hypoxia (VAH) (Powell et al., 1998). VAH is linked to an increased sensitivity of the respiratory control system (Bisgard, 2000; Bisgard and Neubauer, 1995; Gozal et al., 2000; Olson and Dempsey, 1978; Powell et al., 2000; Prabhakar et al., 1996) due to both enhanced O_2 peripheral chemosensitivity (Bisgard, 2000; Powell, 2007) and central responsiveness to peripheral chemoreceptors input (Powell et al., 2000). However, neurotransmitters involved in adaptations of ventilatory control in highland animals have not been fully studied.

The plateau Pika (*Ochotona curzoniae*) is a small lagomorph (100-150g) living in high altitude Qinghai-Tibetan plateau between 3200 to 5300 m above sea level. Pika fossils found in Tibetan plateaus are up to 20/30 millions years old (Mason, 2003; Wang et al., 2008; Wang et al., 2012; Zhu et al., 2005) and Pikas could be therefore considered as adapted to high altitude hypoxia. Previous results showed that plateau Pikas have improved their basal ventilation in hypoxia through changes in ventilatory pattern as compared to lowland animals exposed to high altitude (Pichon et al., 2009). These adaptations are mainly due to an increase in tidal volume and inspiratory time in Pikas leading to a better ventilatory efficiency (Pichon et al., 2009).

The involvement of excitatory amino acids as major neurotransmitters in the mammalian nervous system is well established. The α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors contribute to the fast components of excitatory postsynaptic current whereas the slow components are mediated by NMDA receptors channel (Stern et al., 1992). It has been proposed, in

non-adapted animals, that the N-methyl-D-aspartate (NMDA) / nitric oxide (NO) pathway (also called NO central pathway) could be involved in HVR. Indeed, some authors have shown that activation of NMDA receptors (NMDA-R) could participate in HVR in mice (Gozal et al., 2000) or in hibernator mammals (Harris and Milsom, 2001, 2003). When glutamate binds to NMDA-R, the intracellular calcium concentration increases, which may be activated by nNOS and induce a rise in NO production, then acting as an excitatory neurotransmitter in HVR (Ogawa et al., 1995). Therefore, chronic hypoxia was shown to stimulate a NO central pathway including NMDA-R and NO synthase (NOS) and could act on ventilatory response in highland species. Indeed, previous results suggest that nNOS had no effect on baseline ventilation but could inhibit ventilatory drive and reduce HVR in Pikas (Pichon et al., 2009) when they are exposed to acute severe hypoxia in their burrows (Kuhnen, 1986; Lechner, 1976).

Moreover, the central respiratory drive is modulated by excitatory amino acids acting on non-NMDA receptors in mice (Greer et al., 1991). Glutamate could also bind on non-NMDA receptors (AMPA/kainate receptors) and could act on the ventilatory pattern in mammals (Bonham, 1995) and especially in lagomorphs (Mutolo et al., 2005). Serotonin or 5-hydroxytryptamine (5-HT) is also known to play a crucial role in the control of breathing under hypoxic conditions (Hilaire et al., 2010). However, little is known about the neurotransmitters involved in the adaptation of control of ventilation induced by long-term exposure to hypoxia in mammals. Therefore, the plateau Pika is a good model to study this phenomenon.

Then, we used a pharmacological approach to test the hypothesis that NMDA/non-NMDA receptors, nitric oxide synthase, and serotonin are involved in the HVR and baseline ventilation in pikas. Therefore, we tested the effects of NO synthase inhibitor (L-NAME), NMDA (memantine) and non-NMDA receptors (DNQX) antagonists and selective serotonin reuptake inhibitors (fluoxetine) on ventilation in Pikas at their living altitude and during an hypoxic challenge.

2. MATERIALS AND METHODS

2.1. Ethical statements

Qinghai University ethics committee gave its agreement for all procedures conducted in animals (agreement number QHU-RCHAMD-2013-04-441 for Research Center for High Altitude Medicine). All the experiments were carried out following the Guiding Principles in the Care and Use of Animals (Institute of Laboratory Animal Research 1996). Animals were sedated or anesthetized appropriately to reduce suffering during procedures required.

2.2. Experimental animals

Wild adult males (n=6) and females (n=26) Pikas, weighing 135 ± 14 g, were captured at an altitude between 4050m and 4150m by traps in the Tianjun private area of Haixi Mongol and Tibetan Autonomous Prefecture on the Qinghai-Tibetan Plateau. The owners of the capture zone had given permission to capture 32 Pikas for scientific purposes. The experiments were conducted during the 5 days following the capture. The animals were transferred from the Tianjun area to Xining by car, and they were put into the hypobaric chamber reproducing their living altitude (4100m) half a day later. During the travel, the animals had food and water available *ad libitum* with one animal by cage.

2.3. Study and experimental design (Figure 1)

Experiments were carried out in Xining (P_IO_2=111 mmHg, 2262m Qinghai Province, People's Republic of China) but were performed at Pikas' usual living altitude by simulating P_IO_2 of 86 mmHg (4100m simulated altitude) in a hypobaric chamber ($P_{CHAMBER}$=454 mmHg). Minute ventilation ($\dot{V}_I$) and HVR were first measured through the whole body plethysmograph placed in the hypobaric chamber at this simulated altitude (4100m). Hypoxia was induced by injection of nitrogen from a tank placed in the hypobaric chamber. The plethysmograph outflow was released inside the hypobaric chamber. The Pikas were then exposed through the plethysmograph to a gas

mixture with a F_IO_2 of 13.9% to induce the hypoxic challenge at a P_IO_2 of 57 mmHg during 3 min corresponding to a simulated altitude of 6800m. As ventilatory parameters measurements were done between 2min 30 sec and 3min we studied the first part of the biphasic hypoxic response mainly linked to the carotid bodies (Maxova and Vizek, 2001; Teppema and Dahan, 2010). These experiments were first completed after intra-peritoneal injections of NaCl (vehicle) in 32 Pikas. The following days, and after an additional 30 min within the plethysmograph for a maximal drug action and animal habituation, Pikas were re-exposed to PIO_2=86 mmHg (PIO_2-86) and PIO_2=57 mmHg (PIO_2-57) during 3 min (Figure 1) to reassess HVR. The control group was divided and Pikas had received memantine (NMDA receptor antagonist, 10 mg/kg, n=8), DNQX (AMPA and Kainate receptor antagonist, 10 mg/kg, n=7), L-NAME (NO synthase inhibitor, 10 mg/kg, n=7) or fluoxetine (selective serotonin reuptake inhibitor, 10 mg/kg, n=10). Doses were chosen on the basis of previous experiments in which the effects of drugs on ventilation were examined on rats or mice (Ryan et al., 1996; Schwarzacher et al., 1992; Smeraski et al., 1999).

2.4. Whole body Plethysmography

As already described (Pichon et al., 2009), ventilation was measured in unanaesthetised and unrestrained animals *via* a whole body plethysmograph placed in the hypobaric chamber ($P_{CHAMBER}$=454 mmHg). The system consisted of two 1L high-density polyethylene rigid and transparent experimental chambers. The plethysmographic chamber was connected to a differential pressure transducer (model TSD 160A, Biopac) that measured pressure fluctuations within the closed chamber relative to a reference chamber. During each recording session, the chamber was hermetically sealed and temperature was continuously measured. The pressure signal was sent to a demodulator (model DA100c, Biopac) and data were recorded by a Biopac system (MP150, BIOPAC System Inc., Santa Barbara, California, USA). The pressure transducer was calibrated before each experiment with a manual manometer. Moreover, calibration pulses (0.2 mL) were generated by a gas-tight syringe and injection of air pulses into the plethysmograph at a rate similar

to the animal's inspiratory rhythm to assess volume/pressure changes relationship. Barometric pressure was measured routinely before experiments and temperature inside the chamber was kept stable and continuously monitored with a digital thermometer (Thermo Frigo OTAX). Relative humidity was monitored with a digital hygrometer. Rectal temperature was assessed before and after ventilation measurement using an electronic thermometer. It is known that on inspiration, the air entering the lungs is warmed and humidified, leading to an increase in volume, which, in turn, causes a rise in pressure. Therefore, breathing was measured from the change in pressure associated with the change in volume resulting from the warming and humidification of air during inspiration (Bartlett and Tenney, 1970; Drorbaugh and Fenn, 1955).

Each animal was habituated in the chamber during at least 30 min for familiarization before the assessment of ventilation. Measurements were made when the animals were absolutely quiet but awake. Each data file was analysed breath-by-breath throughout all baseline and experimental periods, and was stored for offline analysis to determine the respiratory frequency (fR in breath.min^{-1}), the tidal volume (Vt, mL) normalised as the ratio Vt divided by the body weight (Vt, mL.kg^{-1}), the inspiratory time (Ti), and the total time of the respiratory cycle (Ttot). Vt calculations were based on the equation described by Drorbaugh and Fenn (1955). Minute ventilation ($\dot{V}_I$, ml.min^{-1}.Kg^{-1}) was calculated as the product of fR and Vt. Expiratory time (Te) was calculated by subtracting Ti from Ttot. The Ti/Ttot ratio, a measure of respiratory timing, was calculated. An index of inspiratory drive was also determined by calculating the Vt/Ti ratio (Milic-Emili and Grunstein, 1976). The HVR was assessed (figure 1) in each animal by the changes in $\dot{V}_I$ from 86 mmHg to 57 mmHg of P_IO_2 ($\Delta\dot{V}_I$).

2.5. Statistical analyses

Data are expressed as means ± SD. The normality of distribution was assessed by the Kolmogorov-Smirnov test. The effects of vehicle, memantine, DNQX, L-NAME, and fluoxetine and of P_IO_2 on each ventilatory parameter were assessed by two-way ANOVAs for each drug.

Newman-Keuls test was used for *post-hoc* test. All statistical analyses were done using the Statistica software (StatSoft, Inc, Tulsa, USA). A P value <0.05 was considered as a significant difference.

3. RESULTS

3.1. Ventilatory response to hypoxia (Table 1)

Whatever group of Pikas tested, animals showed a significant increase in $\dot{V}_I$, Vt and Vt/Ti when exposed from P_IO_2 of 86 mmHg to P_IO_2 of 57 mmHg after vehicle (NaCl) (Table 1, ANOVA main effect). Pikas body temperature did not changed after memantine as compared to vehicle (38.6±0.25°C vs 38.7±0.9°C), DNQX (38.9±0.5°C vs 39.2±0.4°C), L-NAME (38.6±0.4°C vs 38.1±0.7°C) or fluoxetine injections (38.9±0.2°C vs 39.0±0.3°C).

3.2. Effects of NMDA receptors antagonist

Baseline $\dot{V}_I$ at P_IO_2 of 86 mmHg was not influenced by injection of NMDA receptors antagonist (Figure 2A). Contrary to what was observed after vehicle injection, there was no more significant change of $\dot{V}_I$, Vt and Vt/Ti after memantine injection during the hypoxic challenge (Table 1). Thus, HVR decreased after memantine as compared to vehicle, suggesting that NMDA receptors have a significant effect on HVR in Pikas (Figure 3A).

3.3 Effects of AMPA receptors antagonist

Baseline $\dot{V}_I$ at P_IO_2 of 86 mmHg was not modified by injection of AMPA receptors antagonist (Figure 2B). However, DNQX injection induced a significantly higher $\dot{V}_I$ when compared to vehicle at P_IO_2 of 57 mmHg, leading to an increase in HVR after AMPA receptors antagonist injection (Table 1, Figure 3B). This result suggests that AMPA receptors stimulation normally reduces HVR in Pikas. DNQX injection also increased the Vt/Ti ratio between P_IO_2 of 86 mmHg and 57 mmHg.

3.4. Effects of NOS inhibitor

NOS inhibitor had no effect on baseline $\dot{V}_I$ at P_IO_2 of 86 mmHg (Figure 2C). At P_IO_2 of 57 mmHg as compared to 86 mmHg, L-NAME injection increased Vt but without difference as compared to the vehicle group (Table 1), suggesting an effect of NOS on peripheral ventilatory control. Moreover, L-NAME induced a significant increase in the Ti/Tt ratio at P_IO_2 of 57 mmHg. However, NOS inhibitor had no significant effect on HVR (Figure 3C).

3.5 Effects of a selective 5-HT reuptake inhibitor

The injection of selective 5-HT reuptake inhibitor had no effect on Baseline $\dot{V}_I$ at P_IO_2 of 86 mmHg (Figure 2D). After fluoxetine injection, $\dot{V}_I$ and Vt/Ti were non-significantly increased during hypoxic challenge (at P_IO_2 of 57 mmHg) conversely to what was observed after vehicle. Moreover, $\dot{V}_I$ and Vt/Ti values at P_IO_2 of 57 mmHg were significantly lower under fluoxetine when compared to vehicle. Taken together, these results suggest that serotonin accumulation decreased HVR (Figure 3D).

4. DISCUSSION

The main results of this study are that L-NAME modifies the ventilatory pattern in plateau Pikas, whereas HVR is controlled by mechanisms involving NMDA and AMPA/kainate receptors and serotonin pathways. Baseline ventilation in Pikas at their usual living altitude is insensitive to the alteration of NMDA, AMPA/kainate, and serotonin pathways, suggesting that adapted mechanisms of baseline ventilation are less sensitive (invariant) than HVR. Furthermore, systemic inhibition of NMDA receptors and the administration of the reuptake inhibitor, that would indicate an accumulation of serotonin, seem to limit HVR in this high-altitude indigenous animal by acting mainly on peripheral chemoreceptors. On the contrary, the inhibition of AMPA/kainate receptors, another target of glutamate, leads to an increase in HVR.

4.1. Methodological consideration

Given that drugs were administered systemically, the effects could be acting anywhere in the reflex pathway from O_2 sensing to respiratory motor output, depending on the permeability of the blood brain barrier (BBB) to the drug. Permeability of the BBB to L-NAME is controversial (Kaufmann et al., 2004; Wagner et al., 1997) and DNQX is blocked by the BBB (Tokarev and Jezova, 1997). However, memantine (Mehta et al., 2013) and fluoxetine (Warren, 2012) cross the BBB and act on the central nervous system. In the future, an injection into the fourth ventricle should be interesting to more easily distinguish central from peripheral effects of the drugs on the ventilatory control. Moreover, since ventilatory recordings were performed during the 3 first minutes of hypoxic stimulation, the changes observed after drug injection were mainly due to peripheral chemoreceptors (Teppema and Dahan, 2010). Moreover, some of the drugs used in this study could act on the cardiovascular system. For example L-NAME could induce hypertension or hypotension depending on the doses and NO bioavailability (Kopincova et al., 2012) whereas memantine could activate the cardiovascular system (Collins et al., 2006).

4.2. Role of NMDA receptors in HVR

It is well known that glutamate and its binding on NMDA-R receptors are involved in the ventilatory control in mammals (Burton and Kazemi, 2000; Waters and Machaalani, 2005). Indeed, there is a lot of neurohistological evidence for NMDA expression in multiple brainstem regions that are known to be involved in ventilatory control, even if some discrepancies could be observed by a pharmacological approach (agonist/antagonist), probably due to differences in experimental design or species concerned (Waters and Machaalani, 2005). In conscious rats, carotid bodies and other peripheral chemoreceptor-mediated hypoxic ventilatory responses are critically dependent on NMDA receptor activation (Ohtake et al., 1998). Moreover, it has been suggested that arterial chemosensory input during hypoxia may induce the release of glutamate in the *nucleus tractus solitarii* (Mizusawa et al., 1994). In the central respiratory centers, NMDA receptors have been identified in the ventral (Zheng et al., 1998) and dorsal part of the pons (Dutschmann and Herbert, 1998). In this experiment, memantine had no significant effect on basal ventilation in Pikas at their usual living altitude (P_IO_2=86 mmHg), suggesting that NMDA is not involved in ventilatory adaptation to hypoxia in this species. Moreover, whereas ventilation is known to increase under hypoxic condition, we observed only a slight increase in ventilation during the hypoxic challenge after injection of NMDA receptors antagonist, suggesting an effect of the NMDA pathway on HVR in Pikas.

4.3. Role of AMPA receptors in HVR

From the neonatal development (Whitney et al., 2000) to the adulthood, it has been shown that AMPA and kainate receptors could be involved in the respiratory pattern under hypoxic environment (Mutolo et al., 2005) but not in long term facilitation (McGuire et al., 2008). In mice, at sea level, AMPA application over the pre-Bötzinger complex, the C4 ventral motoneurons and the hypoglossal motor nucleus produced an increase in inspiratory frequency, tonic discharge on C4 ventral nerve roots and inward currents in inspiratory hypoglossal motoneurons (Funk et al., 1997).

In our model of adaptation to hypoxia, we have shown that AMPA and kainate receptors antagonist increased ventilation of Pikas during an acute severe hypoxic challenge (P_IO_2=57 mmHg) but not in their usual hypoxic environment (P_IO_2=86 mmHg). These results suggest that non-NMDA (AMPA/kainate) receptors are not involved in the normal ventilatory pattern of Pikas adapted to hypoxia, at their habitual living altitude, but could decrease the hypoxic ventilatory drive, as shown by the increase in Vt/Ti after DNQX injection, and limit the increase in ventilation during acute severe hypoxia. Since DNQX does not cross the BBB (Tokarev and Jezova, 1997) and we measured ventilation 2min 30 after hypoxia initiation, the non-NMDA receptors could be involved in the peripheral chemoreception to adjust the ventilatory drive at the central level.

4.4. Role of NOS in HVR

It is well known that NOS, through NMDA receptors activation, could be involved in the control of ventilation during acute (Prabhakar, 2006) or chronic (Reid and Powell, 2005) exposure to hypoxia and modulate ventilatory response at the peripheral (chemoreceptors) (Bisgard, 2000) or at the central levels (Schwenke et al., 2006). However, these possible effects of nNOS on HVR and VAH were recently contradicted in rats (Pamenter et al., 2015). In our previous work, we have shown that nNOS is probably involved in the limitation of ventilatory response to severe hypoxia in Pikas (Pichon et al., 2009). In the present experiment and in contrast to SMTC (Pichon et al., 2009) we observed no effect on HVR of a global NOS inhibitor (L-NAME). In fact, SMTC and L-NAME could have different time course of response in rats, possibly because of the peripheral and central action of NOS inhibitors (Gozal et al., 1996). The different NOS isoenzymes could act with different delays and different amounts of NO synthesis at the peripheral and the central levels and lead to dichotomous responses of the peripheral desinhibition of the carotid bodies (Lahiri et al., 2006; Wang et al., 1994) and of the immediate activation and delayed inhibition of central respiratory centres (Gozal et al., 1996). However, L-NAME induced significant changes in Vt of Pikas (Table 1), suggesting an effect of NOS on the control of ventilatory pattern, and more precisely on the Vt/fR regulation (El Hasnaoui-Saadani et al., 2007; Patel et al., 1998; Voituron et

al., 2014). Moreover, L-NAME increased significantly Ti/Ttot ratio, which seems to be an invariant in Pikas during acute hypoxic challenge, probably because of the restriction of fR under NOS inhibition.

4.5. Role of serotonin in HVR

In control conditions (vehicle) we observed the expected increase in ventilation during acute hypoxia in Pikas. However, after fluoxetine injection, we observed a decrease in $\dot{V}_I$ and in the inspiratory drive (Vt/Ti) in hypoxia (P_IO_2=57 mmHg), suggesting that 5-HT accumulation led to the reduction in HVR. 5-HT accumulation at the central level after few minutes of hypoxic challenge in cats has been shown to induce a depression of the respiratory neurones excitability by activation of potassium channels, leading to a subsequent decrease in phrenic nerves activity (Richter et al., 1999). This effect of 5-HT was also observed in MAOA-KO mice, in which the excess of 5-HT decreased the ventilatory responses to hypoxia (Burnet et al., 2001). However, 5-HT could also play a role at the peripheral level by increasing long-term facilitation and carotid bodies excitation (Peng et al., 2006). In our adapted animals, systemic fluoxetine injection decreased the ventilatory drive in hypoxia, suggesting that the central effect of 5-HT accumulation seems to be more potent than the peripheral effect in the acute ventilatory response to hypoxia. Again, 5-HT accumulation after fluoxetine injection had no effect on the normal ventilatory pattern in Pikas at their living simulated altitude (4100m) suggesting that long-term ventilatory adaptation to high altitude is highly stable.

4.6. Conclusions

Basal ventilatory pattern of Pikas at their usual living altitude did not change after NMDA or non-NMDA receptors antagonists, or after NOS inhibitors or selective serotonin reuptake inhibitor, suggesting that the mechanisms of ventilatory adaptation are strongly invariant. However, whereas the inhibition of NMDA and the accumulation of serotonin seemed to limit HVR, NOS had no effect on HVR in Pikas. Inhibition of AMPA/kainate receptors, another target of glutamate, led to an increase in HVR. Therefore, glutamate, through NMDA-R/AMPA receptors bindings, and serotonin pathway are involved in HVR in our model of mammals adapted to high altitude.

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TABLES

Table 1 –Tidal volume (Vt), breathing frequency (fR), inspiratory time (Ti), total time of one breath (Tt), the inspiratory drive (Vt/Ti) and the respiratory timing (Ti/Tt) for P_IO_2 of 57 and 86 mmHg in Pikas after injection of memantine (n=7), DNQX (n=7), L-NAME (n=7) and fluoxetine (n=10).

Drugs	P_IO_2 (mmHg)	Vt ($\mu l.g^{-1}$)	fR (breath.min^{-1})	Ti (s)	Tt (s)	Vt/Ti ($mL.Kg^{-1}.s^{-1}$)	Ti/Tt
NaCl	**86**	13.07±2.19	178±37	0.20±0.06	0.35±0.09	69.3±22.9	0.56±0.03
	57	20.52±3.89 *	193±41*	0.18±0.03	0.32±0.05	113.9±34.7 *	0.58±0.03
Memantine	**86**	15.47±1.28	150±21	0.23±0.04 °	0.41±0.05	66.7±9.6	0.58±0.03
	57	19.27±3.51	166±15	0.21±0.02	0.36±0.03	94.2±24.5	0.58±0.03
NaCl	**86**	14.54±2.54	169±27	0.21±0.04	0.36±0.06	69.7±10.8	0.58±0.04
	57	17.25±2.09 *	175±25	0.20±0.04	0.35±0.05	89.2±13.8	0.56±0.04
DNQX	**86**	14.67±1.34	177±37	0.21±0.05	0.35±0.07	72.3±18.6	0.60±0.04
	57	19.49±4.55 *	196±48	0.18±0.04	0.32±0.06	116.9±54.1 *	0.57±0.03
NaCl	**86**	18.01±3.17	140±11	0.25±0.02	0.42±0.03	72.6±8.2	0.58±0.04
	57	21.43±4.50	178±37 *	0.19±0.04 *	0.34±0.07	116.2±46.0	0.56±0.03
L-NAME	**86**	15.57±2.35	148±23	0.23±0.03	0.41±0.06	67.5±12.2	0.57±0.04

	57	22.55±5.43 *	160±27	0.22±0.03	0.38±0.06	102.2±32.4	0.59±0.03 *°
NaCl	**86**	12.23±1.66	161±21	0.21±0.03	0.38±0.05	57.65±11.79	0.56±0.04
	57	17.36±5.93 *	174±29	0.20±0.05	0.35±0.06	89.29±26.72 *	0.56±0.06
Fluoxetine	**86**	13.00±3.37	165±31	0.22±0.04	0.37±0.07	60.01±17.66	0.60±0.08
	57	15.32±2.22 *	159±21	0.22±0.03	0.38±0.05	71.45±13.74 °	0.57±0.04

Grey tint, $P<0.05$ when compared to P_IO_2 86 mmHg, main effect; *, $P<0.05$ when compared to P_IO_2 86 for the same group; °, $P<0.05$ when compared to vehicle (NaCl) for the same P_IO_2 and the same group of drug injection.

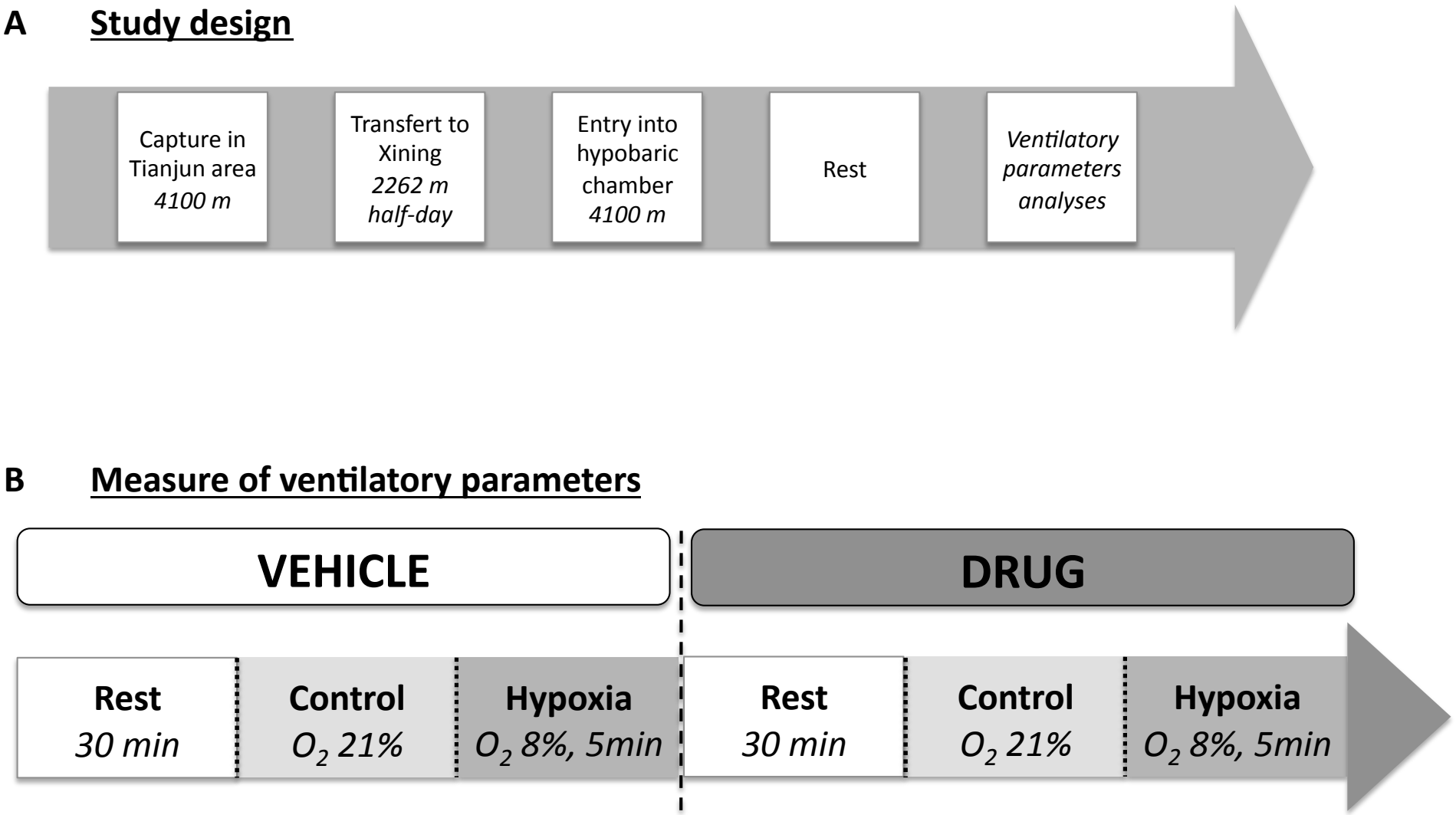
FIGURES LEGENDS

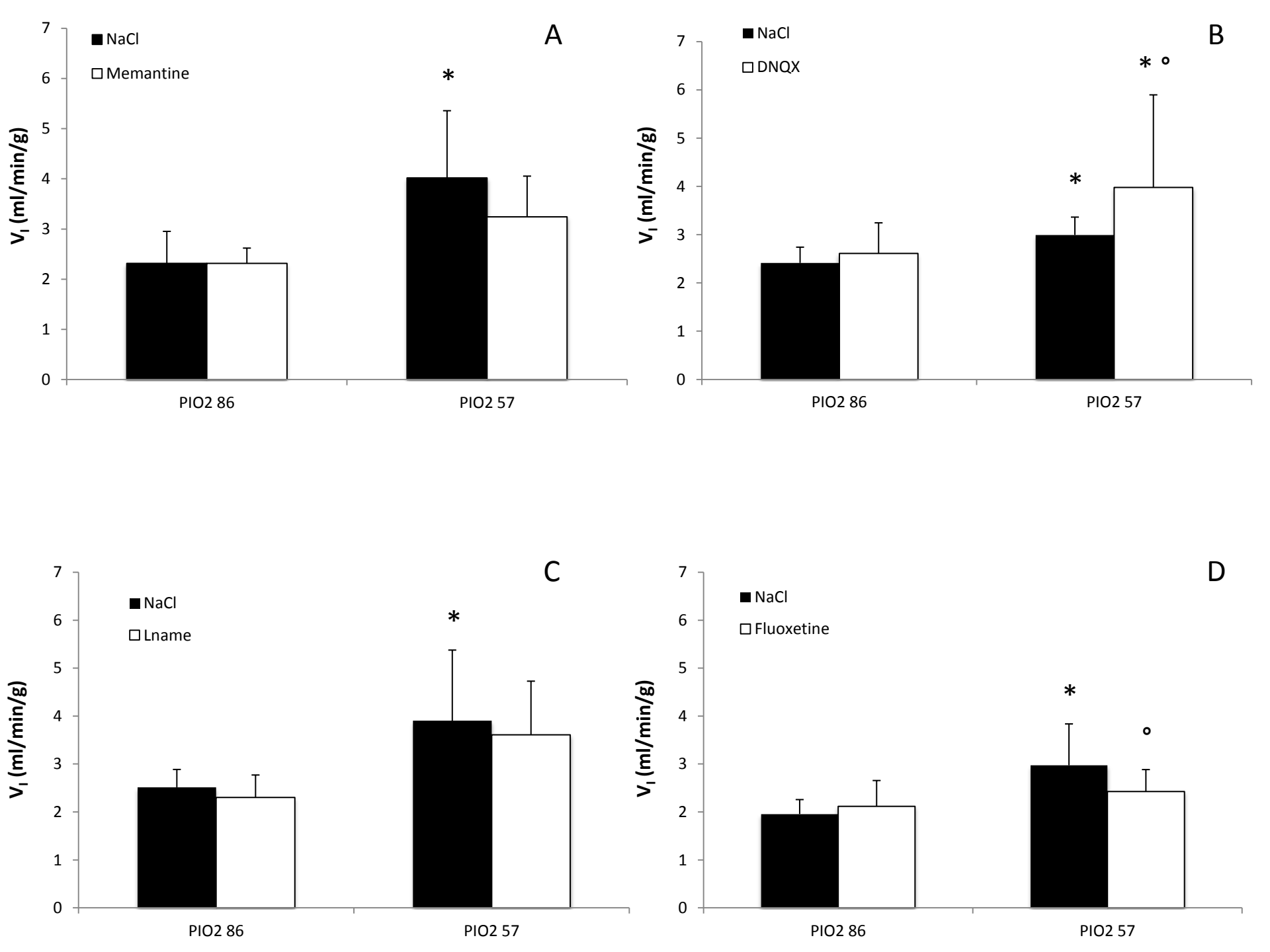
Figure 1 – Study design. The ventilatory parameters were evaluated by plethysmographic approach placed inside a hypobaric chamber (simulated altitude 4100m). The acute ventilatory response to hypoxia was checked by replacing air (P_IO_2 of 86 mmHg) by hypoxic gas mixture (P_IO_2 of 57 mmHg) before (vehicle) and after drug injection.

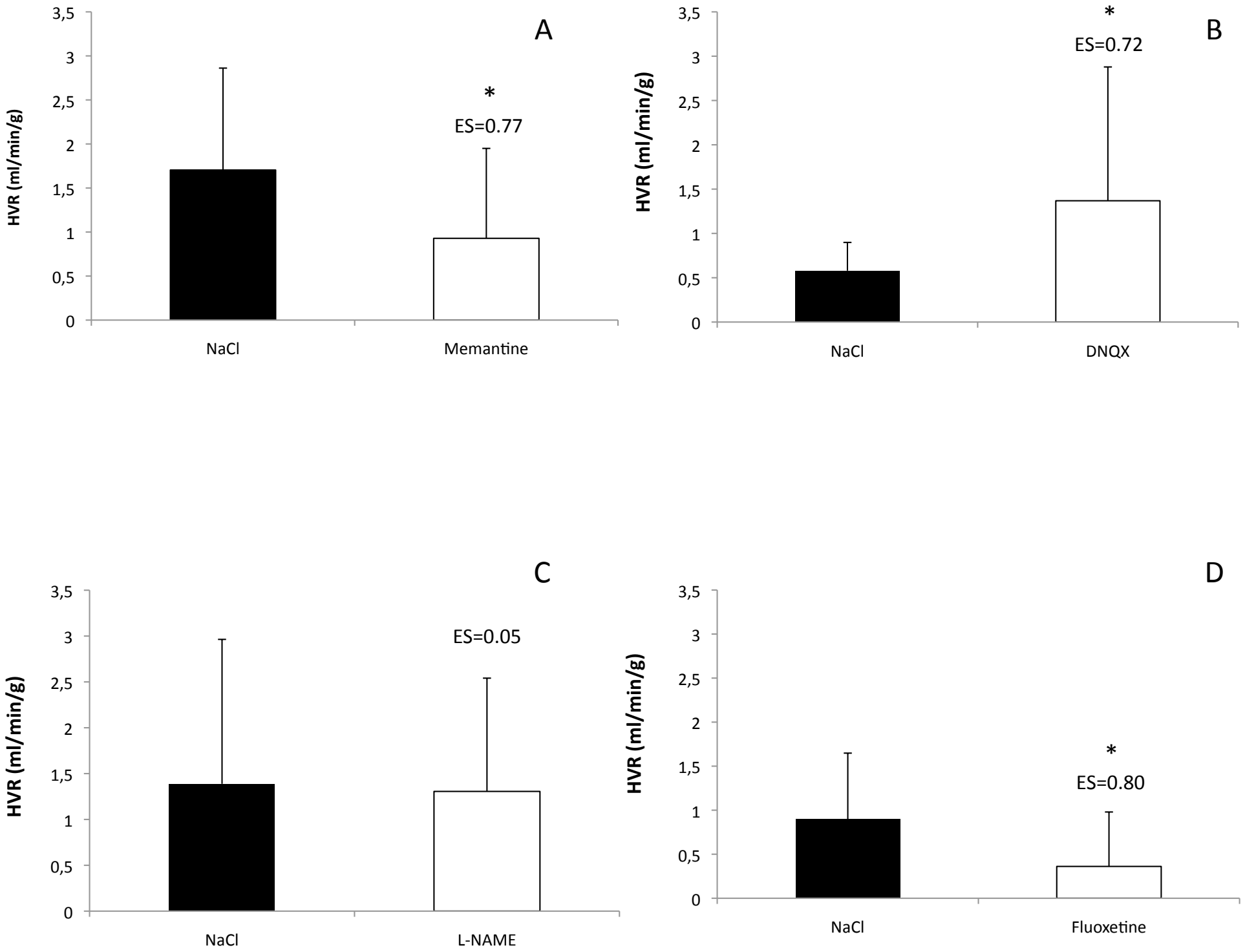
Figure 2 - Inspired ventilation ($\dot{V}_I$) in plateau Pikas for simulated P_IO_2 of 57 and 86 mmHg after memantine (Panel A), DNQX (Panel B), L-NAME (Panel C) and fluoxetine (Panel D) injections as compared to vehicle (NaCl).*, $P<0.05$ when compared to P_IO_2 87 for the same group; °, $P<0.05$ when compared to vehicle (NaCl) for the same P_IO_2 and the same group of drug injection.

Figure 3 – Hypoxic ventilatory response (HVR) in plateau Pikas for simulated P_IO_2 of 57 and 86 mmHg after memantine (Panel A), DNQX (Panel B), L-NAME (Panel C) and fluoxetine (Panel D) injections as compared to vehicle (NaCl). Effect size (ES) from the Cohen's d values was given on the chart suggesting a moderate to large effect of drug for memantine, DNQX and fluoxetine on HVR.

Figure 1


A Study design
Capture in Tianjun area 4100 m
Transfert to Xining 2262 m half-day
Entry into hypobaric chamber 4100 m
Rest
Ventilatory parameters analyses
B Measure of ventilatory parameters
VEHICLE
DRUG
Rest 30 min
Control O2 21%
Hypoxia O2 8%, 5min
Rest 30 min
Control O2 21%
Hypoxia O2 8%, 5min
HVR = V̇E at O2 8% - V̇E at O2 21%


A
NaCl
Memantine
V_I (ml/min/g)
PIO2 86
PIO2 57
*
B
NaCl
DNQX
V_I (ml/min/g)
PIO2 86
PIO2 57
* °
C
NaCl
Lname
V_I (ml/min/g)
PIO2 86
PIO2 57
*
D
NaCl
Fluoxetine
V_I (ml/min/g)
PIO2 86
PIO2 57
*
°


A
HVR (ml/min/g)
3,5
3
2,5
2
1,5
1
0,5
0
*
ES=0.77
NaCl
Memantine
B
HVR (ml/min/g)
*
ES=0.72
NaCl
DNQX
C
HVR (ml/min/g)
ES=0.05
NaCl
L-NAME
D
HVR (ml/min/g)
*
ES=0.80
NaCl
Fluoxetine