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Interleukin-1 β suppresses the ventilatory hypoxic response in rats via prostaglandin-dependent pathways

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Abstract: We investigated the effect of the major inflammatory cytokine interleukin-1 β (IL-1 β) on the ventilatory response to hypoxia. The goal was to test the hypothesis that IL-1 β impairs the hypoxic ventilatory response *in vivo* by indirectly inhibiting respiratory neurons in the brainstem via prostaglandins. Thus, IL-1 β was delivered by cerebroventricular injection, and the ventilatory hypoxic response was assessed in anesthetized, spontaneously breathing rats pretreated with or without diclofenac, a nonspecific inhibitor of prostaglandin synthesis. We found that the slope of the ventilatory response to hypoxia decreased almost twofold from 10.4 ± 3.02 to 4.06 ± 0.86 mL min⁻¹ mmHg⁻¹ (-61 %) 90 min after administration of IL-1 β ($p < 0.05$). The slope of tidal volume and mean inspiratory flow also decreased from 0.074 ± 0.02 to 0.039 ± 0.01 mL mmHg⁻¹ (-45 %, $p < 0.05$), and from 0.36 ± 0.07 to 0.2 ± 0.04 mL s⁻¹ mmHg⁻¹ (-46 %, $p < 0.05$), respectively. Pretreatment with diclofenac blocked these effects. Thus, the data indicate that IL-1 β degrades the ventilatory hypoxic response by stimulating production of prostaglandin. The increase of cerebral levels of IL-1 β , which is induced by the activation of immune cells in the brain, may impair respiratory chemoreflexes.

Key words: cytokines, interleukin-1 β , breathing, ventilation, hypoxia, respiratory chemoreflex, prostaglandin.

Introduction

Accumulating evidence now suggest that systemic inflammation modulates ventilatory control, the key elements of which include the hypoxic and hypercapnic respiratory chemoreflexes. These elements maintain the homeostasis of gases in arterial blood. In previous studies, we demonstrated that elevated levels of the major inflammatory cytokine interleukin-1 β (IL-1 β) in the cerebrospinal fluid reduces the sensitivity of the central chemoreflex to carbon dioxide, and that prostaglandins mediate the effects of IL-1 β on the hypercapnic response (Aleksandrova and Danilova 2010; Aleksandrova et al. 2015). Intravenous injection of lipopolysaccharide, and the subsequent release of inflammatory cytokines, were also reported to repress the ventilatory response to hypoxia (Fernandez et al. 2008; Zapata et al. 2011). However, the mechanisms by which inflammation impairs respiratory chemoreflexes are still not clear, and may involve multiple inflammatory molecules that activate central and peripheral chemoreceptors or other respiratory neurons (reviewed in Huxtable et al. 2011).

The hypoxic ventilatory response arises predominantly from peripheral arterial chemoreceptors in the carotid body. Notably, Lam et al. (2008) found that systemic inflammation causes morphological changes in the carotid body. Such changes are associated with changes in the levels inflammatory cytokines, and reduce the chemosensitivity to hypoxia (Gauda et al. 2013). Moreover, glomus cells, which are the primary O₂-sensing elements in the carotid body (Zhang et al. 2007), express IL-1 receptor type I and IL-6 receptor α even under normoxic conditions (Wang et al. 2002, 2006). These cells can depolarize in response to hypoxia and release neurotransmitters that activate sensory nerve fibers terminating in the brainstem respiratory center. Taken together, these observations indicate that the carotid body has immunosensory activity, and may modulate the hypoxic ventilatory response during inflammation.

However, a central component in the brainstem complements the peripheral component of the hypoxic chemoreflex. Thus, inflammation-related functional changes in brainstem

respiratory neurons may also evoke changes in the hypoxic ventilatory response. Indeed, IL-1 β has been shown to induce expression of c-fos, a marker of functionally activated neurons, in respiration-related regions in the brainstem, including the nucleus tractus solitarius (Ericsson et al. 1997). However, the nucleus tractus solitarius has been reported to not express IL-1 receptors, and brainstem respiration-related neurons were found to be insensitive to IL-1 β *in vitro* (Olsson et al. 2003). We now present evidence that IL-1 β may impair the central hypoxic ventilatory response *in vivo* through indirect suppression of respiratory neurons in the brainstem via prostaglandins. This phenomenon is demonstrated in anesthetized, spontaneously breathing rats injected with IL-1 β , and which were pretreated with or without diclofenac, a nonspecific inhibitor of prostaglandin synthesis.

Materials and methods

Experiments

Data were collected from 36 tracheostomized rats with mean weight 270 ± 10 g, and which were anesthetized intraperitoneally with 1000 mg kg^{-1} urethane, a level sufficient to eliminate pain reflexes. Anesthetic depth was monitored by corneal reflex and response to tactile stimuli. The anaesthetized rats were placed in a stereotactic frame in the supine position with a fixed head. The skin and connective tissues were removed from the skull, a hole was drilled, and a microinjection cannula was placed into the right ventricle of the brain. The stereotaxic coordinates were as follows: 0.8 mm posterior to bregma, 1.5 mm lateral from the midline, and 3.7 mm below the surface of the skull (Paxinos and Watson 1982). All animal procedures were conducted in accordance with the ethical guidelines of the European Community Council Directives 86/609/EEC, and were reviewed and approved by the animal care committee at Pavlov Institute of Physiology RAS. The animals were also cared for in accordance with the Guide to the Care and Use of Experimental Animals by the Canadian Council on Animal Care.

Ventilation and alveolar gases

A respiratory flow head connected to a pneumotachometer (AD Instruments ML141 Spirometer, Dunedin, New Zealand) was used to measure peak airflow and respiratory rate. Airflow was integrated to obtain respiratory volume. Minute ventilation was calculated from mean tidal volume and respiratory frequency of ten respiratory cycles, while mean inspiratory flow was calculated as tidal volume divided by inspiratory time. End-tidal partial pressure of oxygen ($P_{ET}O_2$) was analyzed by a rapid-response quadruple mass spectrometer (Model MC-100, Institute for Analytical Instrumentation, Saint Petersburg, Russia), which was calibrated immediately before and after use with onboard gas mixtures of known composition.

Ventilatory response to hypoxia

Hypoxic ventilatory response was measured using isocapnic progressive hypoxia rebreathing, following Read (1967), with some modification. Briefly, animals were made to breathe from a bag with 15 % O_2 , 5 % CO_2 , and 80 % N_2 . A gradual decrease in end-tidal O_2 was recorded for 4 min, and was monitored on a breath-by-breath basis. CO_2 concentration in the bag was kept constant by passing expirations through a CO_2 -absorbing filter. The response of tidal volume (V_T), mean inspiratory flow (V_{in}), and minute ventilation (V_E) to decreased $P_{ET}O_2$ was assessed by the linear regression slopes of $\Delta V_T/\Delta P_{ET}O_2$, $\Delta V_{in}/\Delta P_{ET}O_2$, and $\Delta V_E/\Delta P_{ET}O_2$ obtained at $P_{ET}O_2$ between 80 and 40 mm Hg. Rectal temperature was measured throughout the experiment, and kept at about 37 °C.

Drug administration

The animals were divided into four groups. The animals of the first group ($n=9$) were administered IL-1 β (350 ng of human recombinant IL-1 β in 10 μ l of saline, Sigma) into the lateral ventricle of the brain. Animals of the second group ($n=9$) were first injected intraperitoneally with diclofenac (0.5 μ g, Belmedpreparaty RUE, Russia) and then IL-1 β .

Diclofenac, a non-specific cyclooxygenase inhibitor, was used to investigate the role of the cyclooxygenase pathway in the effects of IL-1 β . The third group of animals ($n=9$) was the control, these animals received 10 μ l of saline instead of IL-1 β . Fourth group ($n=9$) was used in order to find possible action of diclofenac on the carotid body. Animals included this group were administered diclofenac intraperitoneally.

The experiment lasted 120 minutes, IL-1 β or saline was administered at the thirtieth minute. Diclofenac was injected on the tenth minute of the experiment, that is, 20 minutes before the administration of IL-1 β , since according to official information the manufacturer the maximum content of diclofenac in blood is reached after 10-20 minutes even after injection. Hypoxic ventilatory response was measured before microinjection of IL-1 β or saline and on 20, 40, 60, and 90 min after administration.

Statistics

Data are reported as mean $\pm$ SE. Values before and after cerebroventricular injections were compared using Wilcoxon signed rank test and one-way analysis of variance for repeated measures. Differences were considered significant at $p < 0.05$. For statistical processing of experimental data STATISTICA 7.0 software package was used.

Results

Resting breathing

Cerebroventricular injections of interleukin IL-1 β , but not saline injections, increased the tidal volume and mean inspiratory flow during resting minute ventilation. Respiratory frequency also tended to increase, but $P_{ET}CO_2$ did not change significantly. Breathing indices at baseline and after cerebroventricular injections of saline and IL-1 β are listed in Table 1.

Ventilatory response to hypoxia

During hypoxia, significant correlation was observed between minute ventilation, tidal volume, mean inspiratory flow, and decrease in $P_{ET}O_2$, both before and after cerebroventricular injections of IL-1 β . However, IL-1 β decreased the ventilatory response to hypoxia (Fig. 1), and flattened the hypoxic response slopes (Fig. 2A-C), with maximal changes observed 90 min after injection. The slope of the ventilatory response to hypoxia, a measure of the rate at which ventilation rises with decreasing $P_{ET}O_2$, decreased more than twofold from $10.4 \pm 3.02 \text{ mL min}^{-1} \text{ mmHg}^{-1}$ before injection to $4.06 \pm 0.86 \text{ mL min}^{-1} \text{ mmHg}^{-1}$ (-61 %) 90 min after injection ($p < 0.05$). The slopes of tidal volume and mean inspiratory flow also decreased from 0.074 ± 0.02 to $0.039 \pm 0.01 \text{ mL mmHg}^{-1}$ (-45 %, $p < 0.05$), and from 0.36 ± 0.07 to $0.2 \pm 0.04 \text{ mL s}^{-1} \text{ mmHg}^{-1}$ (-46 %, $p < 0.05$), respectively (Fig. 3). In contrast, cerebroventricular injection of saline did not affect response slopes. Collectively, the data indicate that elevated IL-1 β in the brain decreases the ventilatory response to hypoxia, *i.e.* the inflammatory cytokine depresses the central component of the hypoxic chemoreceptor control of breathing.

Ventilatory response to hypoxia after diclofenac pretreatment

The effect of IL-1 β on the ventilatory hypoxic response was blocked in rats pretreated with diclofenac, a nonspecific cyclooxygenase inhibitor (Fig. 1). In particular, IL-1 β did not significantly change either the position or the slope of the ventilation- $P_{ET}O_2$ line when administered after an intraperitoneal injection of diclofenac (Fig. 2D-F). Similarly, the relationship between $P_{ET}O_2$ and tidal volume or mean inspiratory flow did not significantly change from baseline (Fig. 3).

Discussion

The data demonstrate a marked attenuation in the hypoxic ventilatory response after cerebral IL-1 β is artificially elevated in anesthetized rats. Thus, IL-1 β appears to modulate not only peripheral chemoreceptors (Fernandez et al. 2008; Wang et al. 2002; Zhang et al. 2007;

Zapata et al. 2011), but also central component of respiratory chemoreflexes. The main direction of the flow of cerebrospinal fluid is: lateral ventricle – openings monrovi – III ventricle – Silvius aqueduct – fourth ventricle – hole Luski and Magendie – a big cistern and outer subarachnoid space of the brain, the central canal and subarachnoid space of the spinal cord – the ultimate cistern. The NTS is the most accessible target for drugs contained in the cerebrospinal fluid because it is located at the bottom of the fourth ventricle near its surface. Therefore, we can assume that IL-1 β which was injected in the lateral ventricle was reached the bottom of the fourth ventricle and could exert their effects on neurons of the NTS. Because the primary afferents arising from the peripheral chemoreceptors terminate within the NTS it could be expected that the cerebroventricular injection of IL-1 β would lead to altered sensitivity to hypoxia. We believe that cerebral IL-1 β indirectly depresses the hypoxic ventilatory response by inhibiting respiration-related neurons in the brainstem, such as those in the nucleus tractus solitarius and the rostral ventrolateral medulla. Consequently, the same hypoxic stimulus might elicit less response from these neurons in conditions of elevated IL-1 β than in normal conditions. In turn, desensitization of the central inspiratory activity would decrease the efferent activation of spinal respiratory motoneurons, and thereby suppress the generation of force from inspiratory muscle contractions in response to hypoxia, as well as lower the inspiratory driving pressure. Tidal volume and ventilatory hypoxic response would also decrease as a result. Similarly, we have previously demonstrated that cerebral IL-1 β also inhibits the hypercapnic ventilatory response (Aleksandrova and Danilova 2010; Aleksandrova et al. 2015). Taken together, our studies suggest that elevated cerebral IL-1 β due to inflammation may result in hypoxemia and hypercapnia in the absence of natural compensatory reactions that increase ventilation. Indeed, IL-1 β may impair respiration during infection (Hofstetter 2007; Olsson 2003).

We also now demonstrate that cerebral IL-1 β depresses the hypoxic chemoreflex via cyclooxygenase, indicating that endogenous prostaglandins may modulate brainstem respiratory neurons during hypoxia, in line with previous studies. For example, IL-1 β is already known to

elicit cyclooxygenase activity and release of multiple secondary mediators, including prostaglandins. Further, Graff and Gozal (1999) reported that IL-1 β induces ventilatory effects via eicosanoid-dependent mechanisms. Similarly, we previously demonstrated that inhibiting prostaglandin synthesis with diclofenac clearly blocks the effects of IL-1 β on breathing patterns and on the hypercapnic ventilatory response in adult rats (Aleksandrova et al. 2015). PGE₂ has also been shown to mediate the anoxic ventilatory effects of IL-1 β , while indomethacin has been shown to mitigate the adverse effects of IL-1 β on hypoxic gasping and anoxic survival in neonatal rats (Hofstetter et al. 2007; Olsson et al. 2003). In addition, it was shown that intravenous administration of endotoxin not only elicited release of inflammatory cytokines, but also altered the respiratory motor output and the cyclooxygenase pathway (Preas et al. 2001).

Stimulation of prostaglandin synthesis appears to be a fundamental and specific mechanism by which IL-1 β modulates neuronal function, including in respiration. For instance, IL-1 β induces release of cyclooxygenase-2 and PGE₂ in glial and neuronal ganglion cells cultured from rat trigeminal ganglia (Neeb et al. 2011). Our own experiments with diclofenac suggest that IL-1 β depends on a similar mechanism to elicit respiratory effects in the brainstem, presumably via glial and neural cells in respiration-related units and that express receptors for IL-1. Upon stimulation, these cells would release prostaglandins into the extracellular space, and thereby stimulate neurons with PGE₂ receptors such as EP3R. Indeed, EP3R is strongly expressed in respiration-related tissues of the brainstem such as the NTS and RVLM (Ek et al. 2000; Nakamura et al. 2000). Accordingly, IL-1 β was shown to inhibit autoresuscitation after hypoxic apnea in wild-type mice, but not in mice lacking EP3R (Hofstetter et al. 2007).

At present, prostaglandins are believed to inhibit respiration. For instance, PGE₂ has been shown to depress breathing *in vivo* in fetal and newborn sheep, and to inhibit respiration-related neurons *in vitro* (Guerra et al. 1988; Olsson et al. 2003). It was also shown that PGE₂ injection into the rat right lateral ventricle induces expression of c-fos in the nucleus of the solitary tract and in the ambiguous nucleus (Lacroix et al. 1996). These are anatomic evidence that central

injection of PGE₂ evokes selective and specific expression of c-fos in brain structures that regulate respiration. In our own experiments, the hypoxic response also degraded within 30-40 minutes after intraventricular administration of IL-1 β .

In conclusion, IL-1 β appears to be an intercellular messenger molecule that links central and peripheral ventilatory control to immune activity. In particular, IL-1 β suppresses respiratory chemoreflexes by inducing production of prostaglandins. Increase cerebral levels of this cytokine, which is induced by the activation of immune cells in the brain may impair respiratory chemoreflexes at the level of the brain stem. We also conclude that the adverse influence of IL-1 β on the respiratory chemoreflexes is mediated by activation of endogenous prostaglandin production.

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Figure captions

Fig. 1. Effect of IL-1 β on the ventilatory hypoxic response. Inspiratory pneumotachogram during normoxic breathing without IL-1 β and diclofenac (A), and during hypoxic breathing (B-D). Ventilatory response to hypoxia decreased after cerebroventricular administration of IL-1 β without diclofenac pretreatment (C), but not after IL-1 β injection with diclofenac pretreatment (D). The panels are representative traces from animals of different experimental groups: placebo (B), IL-1 β (C) and IL-1 β with diclofenac (D) groups. The panel A is representative basal trace from animal without any treatments.

Fig. 2. Ventilatory response to hypoxia in a representative animal before (solid line) and 90 min after (dashed line) cerebroventricular administration of IL-1 β with (A-C) or without (D-F) diclofenac pretreatment. IL-1 β treatment without diclofenac decreased the slope of minute ventilation, mean inspiratory flow, and tidal volume response, whereas IL-1 β treatment with diclofenac did not change the ventilatory response.

Fig. 3. Time course of the ventilatory response to hypoxia, as measured 20, 40, 60, and 90 min after cerebroventricular administration of IL-1 β with (hatched bars) or without (open bars) diclofenac pretreatment. Data are mean $\pm$ SEM, with baseline set at 100 %. *, $p < 0.05$ vs. baseline.

Table 1. Effects of IL-1 β , saline and diclofenac on breathe pattern

	Interleukin-1 β			Saline			Diclofenac		
	Baseline	40 min	60 min	Baseline	40 min	60 min	Baseline	40 min	60 min
V _E (mL min ⁻¹)	104 $\pm$ 9,0	126 $\pm$ 3,7**	131 $\pm$ 5,4**	95 $\pm$ 8,2	104 $\pm$ 10,5	94 $\pm$ 10,5	110 $\pm$ 8,2	117 $\pm$ 6,5	110 $\pm$ 7,8
V _T (mL)	1,0 $\pm$ 0,05	1,13 $\pm$ 0,06*	1,17 $\pm$ 0,04*	0,9 $\pm$ 0,14	1,0 $\pm$ 0,08	1,0 $\pm$ 0,12	1,1 $\pm$ 0,05	1,12 $\pm$ 0,05	1,1 $\pm$ 0,04
f (breaths min ⁻¹)	109 $\pm$ 6,0	117 $\pm$ 6,8	118 $\pm$ 6,4	104 $\pm$ 8,0	101 $\pm$ 10,4	94 $\pm$ 2,6	105 $\pm$ 7,2	107 $\pm$ 6,1	107 $\pm$ 5,9
V _{in} (mL s ⁻¹)	3,7 $\pm$ 0,27	4,4 $\pm$ 0,12*	4,5 $\pm$ 0,19*	3,7 $\pm$ 0,31	3,8 $\pm$ 0,10	3,9 $\pm$ 0,11	3,8 $\pm$ 0,3	4,1 $\pm$ 0,28	4,0 $\pm$ 0,3
P _{ET} CO ₂ (mm Hg)	34 $\pm$ 3	30 $\pm$ 3	30 $\pm$ 3,5	34 $\pm$ 2,1	34 $\pm$ 2,8	31 $\pm$ 2,3	32 $\pm$ 3,0	30 $\pm$ 2,8	31 $\pm$ 3,5

V_E - minute ventilation, V_T - tidal volume, f - respiratory frequency, V_{in} - mean inspiratory flow, P_{ET}CO₂ - the end-tidal partial pressure of carbon dioxide. Values are means $\pm$ SEM. * p < 0,05 and ** p < 0,01 for the comparison with baseline.