IL-1 β Depresses Respiration and Anoxic Survival via a Prostaglandin-Dependent Pathway in Neonatal Rats

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ABSTRACT

IL-1 β has been proposed to be an important mediator linking infection, apnea, and sudden infant death syndrome. We hypothesized that IL-1 β acts in this capacity by depressing brainstem respiratory neurons *via* a prostaglandin-dependent pathway. For studying the effects of IL-1 β on respiration as well as the mechanism underlying its actions, 7-d-old rats received an initial injection (i.p.) of NaCl or a cyclooxygenase inhibitor (indomethacin, 10 mg/kg) followed by a second injection (i.p.) at 30 min of NaCl, recombinant rat IL-1 β (10 μ g/kg), or lipopolysaccharide (LPS; 100 μ g/kg). Respiration during normoxia and in response to anoxia (100% N₂) was examined at 60 min after the second injection using flow and barometric plethysmography. Animals given IL-1 β breathed more slowly and died more often after anoxia. LPS also reduced the rats' ability to autoresuscitate and survive an anoxic challenge. Indomethacin prevented the depressive effects during normoxia and the adverse effects on survival. For investigating drug-induced changes in central respiratory activity, IL-1 β (1.0 or 1.25 ng/mL) and prostaglandin E₂ (5 or 20

μ g/L) was applied to the brainstem–spinal cord preparation of 0- to 4-d-old rats. Whereas IL-1 β exerted no effect on respiration measured at the C4 ventral root during a 60-min period, prostaglandin E₂ reversibly inhibited respiratory activity. These findings suggest that IL-1 β does not inhibit respiratory neurons directly but may depress breathing and hypoxic defense *via* a prostaglandin-mediated mechanism. (*Pediatr Res* 54: 326–331, 2003)

Abbreviations

BBB, blood-brain barrier
COX-2, cyclooxygenase-2
f_R, respiratory frequency
LPS, lipopolysaccharide
NTS, nucleus of the solitary tract
PGE₂, prostaglandin E₂
RVLM, rostral ventrolateral medulla

Infection, hypoxia, and apnea each have been implicated in the pathogenesis of sudden infant death syndrome, and it has been postulated that the proinflammatory cytokine IL-1 β may serve as the critical link between them (1, 2). IL-1 β is released during an acute-phase immune response and subsequently induces multiple effects within the CNS, including the initiation of fever, sleep, and neuropeptide release [for review, see (3)]. Previous studies indicated that this immunomodulator may also alter respiration and autoresuscitation, yet the mechanism underlying such effects must be further elucidated (4–7). Systemic administration of IL-1 β has been shown to induce

time- and dose-dependent expression of early immediate genes in the nucleus of the solitary tract (NTS) and rostral ventrolateral medulla (RVLM), both of which contain neurons important for respiratory control and the latter houses the central pattern generator for breathing (8, 9). Because of its size and lipophilic properties, IL-1 β does not diffuse readily across the blood-brain barrier (BBB) (10). However, IL-1 β may still communicate with the brain across the BBB *via* active transport passage, through circumventricular organs, vagal afferent stimulation, and *via* second messenger induction at the BBB [for review, see (11)].

We propose that IL-1 β may affect brainstem respiratory control *via* a prostaglandin-dependent mechanism. This hypothesis is supported by evidence that circulating IL-1 β induces the local release of prostaglandin E₂ (PGE₂) into the brain parenchyma and that PGE₂ receptor mRNA expression and a high density of PGE₂ binding occur near respiratory-related regions in the brainstem, such as the NTS, RVLM, nucleus ambiguus, and nucleus parabrachialis (12, 13). Prostaglandin has been shown to suppress fetal breathing move-

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ments as well as cause hypoventilation by decreasing respiratory frequency, induce apnea, and alter central respiratory rhythm in newborn animals (14–17). It has also been correlated with an increased number of central apneas in human neonates (18). Conversely, indomethacin, a drug that inhibits cyclooxygenase (COX) and thus prostaglandin synthesis, stimulates ventilation in both fetal and newborn animals (19, 20).

In the current study, we investigated whether an inflammatory response may affect respiration and the ability of rats to survive anoxic challenge through a cytokine-mediated, prostaglandin-dependent pathway. The effects of IL-1 β , lipopolysaccharide (LPS), and indomethacin on respiratory activity during normoxia and anoxia were studied in 7-d-old rats. Brainstem respiratory pattern generation after application of IL-1 β and PGE₂ was also examined in 0- to 4-d-old rats.

METHODS

Animals. Male and female Bkl:SD rats (B&K Universal, Sollentuna, Sweden), postnatal ages 0–4 d (*in vitro*) and 7 d (*in vivo*), were used. The animals were born and reared by their mothers under standardized conditions with a 12-h light:12-h dark cycle. Food and water were provided *ad libitum*. Animals studied using flow plethysmography were handled regularly during the 3–4 d before experimentation. The study was performed in accordance with European Community guidelines and approved by the regional animal research ethics committee.

Drugs. Recombinant mouse IL-1 β (Boehringer Mannheim Biochemica, Hamburg, Germany), recombinant rat IL-1 β (Nordic BioSite AB, Täby, Sweden), LPS (from *Escherichia coli* serotype 0128:B12), PGE₂, and indomethacin crystalline (the last three from Sigma Chemical Co.-Aldrich, Stockholm, Sweden) were used. For the *in vivo* study, recombinant rat IL-1 β was mixed with NaCl to produce a 1 μ g/mL of working solution. An LPS stock solution (10 $\times$) was prepared in NaCl, and an indomethacin stock solution (50 $\times$) was prepared in ethanol and Trisphosphate buffer. All *in vivo* drugs were stored at -20°C and thawed to room temperature on the day of experimentation. LPS and indomethacin were then diluted in NaCl to working solutions of 10 μ g/mL and 1 mg/mL, respectively. For the *in vitro* study, a PGE₂ stock solution (20 $\times$) was prepared in ethanol and sterile H₂O and stored at -20°C . Concentrated solutions (100,000 IU/mL) of recombinant mouse and rat IL-1 β were stored at -80°C . On the day of experimentation, the PGE₂ and IL-1 β solutions were diluted in artificial cerebrospinal fluid (aCSF) to 5 or 20 μ g/L and 1.0 or 1.25 ng/mL (50–62.5 IU/mL), respectively.

Flow plethysmography. A Plexiglas chamber (110 mL) was connected to a highly sensitive direct airflow sensor (0–200 mL/min; RSP 1040 and TRN3100 respectively, Kent Scientific Corporation, Litchfield, CT, U.S.A.). Gas flow through the chamber was maintained at approximately 110 mL/min. Gases were exchanged using a computer-controlled valve switch. Control experiments verified that 95% of gas exchange occurred within 30 s. The flow signal was amplified by a four-channel amplifier (P/N 770 S/N 5; SENSElab, Somedic Sales, Hörby, Sweden), converted to digital signal, and recorded at 100 Hz by an online computer using DasyLab software (Data-

log GmbH & Co. KG, Mönchengladbach, Germany). Chamber temperature was measured continuously using a digital thermometer and recorded in parallel with the flow signal. It was maintained at 30°C in accordance with the documented thermoneutral zone for rats of similar age (21) by using a heating blanket attached to the chamber roof and connected to a power supply (ES 030-5; Delta Electronika, Zierikzee, The Netherlands).

Barometric plethysmography. A Plexiglas chamber (175 mL) was connected along with an identical reference chamber to a highly sensitive differential pressure transducer (DP103; Validyne, Northridge, CA, U.S.A.). Pressurized gas was administered continuously into the chamber and evacuated with a vacuum pump. Inlet and outlet resistance was adjusted using needle valves to maintain atmospheric pressure in the chamber. Gas flow was maintained at 1.0 L/min, and 95% of gas exchange occurred within 30 s of gas administration. The pressure signal was amplified and recorded by an online computer for off-line analysis. Chamber temperature was measured using a digital thermometer and maintained at 30°C using a thermoradiator and heating pad.

Plethysmography protocol. Each animal ($n = 112$) received an initial injection (i.p.) of NaCl or indomethacin (10 mg/kg), followed by a second injection (i.p.) at 30 min of NaCl, IL-1 β (10 μ g/kg), or LPS (100 μ g/kg; the last examined using flow plethysmography only). The unrestrained animal was placed in the plethysmograph chamber 60 min after the second injection and exposed to room air (21% O₂; approximately 3 min and 7 min for barometric and flow plethysmography, respectively). The inlet gas was then switched to 100% N₂, and respiratory activity was measured during anoxia (Fig. 1). One minute after initiation of secondary apnea, 100% O₂ was administered into the chamber (22). O₂ flow continued for approximately 15 min or until the animal autoresuscitated, whichever occurred first. In a sample population ($n = 35$), skin temperature was measured using a thermistor probe attached to the animal's back with adhesive tape. Experiments were performed during the light cycle.

Figure 1. Basal respiration and response to anoxia. Flow plethysmograph recording depicts the respiratory activity of a control animal during normoxia (21% O₂; initial 5 min not shown), anoxia (100% N₂), and hyperoxia (100% O₂). Note the biphasic anoxic response, including hyperpnea, primary apnea, gasping, and secondary apnea, followed by autoresuscitation during hyperoxia.

Brainstem–spinal cord preparation. The brainstem and spinal cord of 0- to 4-d-old rats ($n = 20$) was dissected under deep ether anesthesia and isolated as described previously (23, 24). The preparation was perfused continuously in a 2-mL chamber at a rate of 3.0–3.5 mL/min with the following aCSF: 124 mM of NaCl, 5.0 mM of KCl, 1.2 mM of KH_2PO_4 , 2.4 mM of CaCl_2 , 1.3 mM of MgSO_4 , 26 mM of NaHCO_3 , and 30 of mM glucose. The aCSF was equilibrated with 95% O_2 and 5% CO_2 to pH 7.4 at 28.5°C.

Respiratory activity was recorded using suction electrodes applied to the proximal end of the cut C4 ventral root containing respiratory motoneuron axons. C4 activity, corresponding to phrenic nerve discharge (24), was amplified and band-pass filtered (10 Hz to 5 kHz; differential AC amplifier model 1700; A-M Systems, Everett, WA, U.S.A.), rectified, and then integrated by a third-order R-C filter (time constant 100 ms). C4 activity was monitored continuously using Axotape and Axoscope programs (Axon Instruments, Foster City, CA, U.S.A.). It was digitized (1–5 kHz) and stored on a computer hard drive for off-line analysis. After perfusion with control aCSF for 30–50 min, the drug-containing aCSF solution was applied for 30 min. IL-1 β perfusion was continued for 60 min in five preparations. Drug application was followed by washout with the control aCSF.

Measurements and analysis. From the plethysmograph recordings during normoxia, at least three periods of 10–60 s with minimal movement artifacts were selected. Mean respiratory frequency (f_R ; breaths/min) was determined for each animal. Inspiratory time (s), expiratory time (s), tidal volume (mL/breath), and minute ventilation (mL/min) were also calculated from the flow plethysmograph data. The duration of hyperpnea, primary apnea, and gasping as well as the gasping frequency were determined manually. The gasping period of animals examined using flow plethysmography was further divided into three phases as described previously (25). The duration and gasping frequency of each phase were calculated manually. Survival was recorded for all animals, and the duration of secondary apnea and time required to autoresuscitate after N_2 administration were determined in survivors. *In vitro* recordings were analyzed using Axoscope and JMP (SAS Institute, Cary, NC, U.S.A.). Respiratory frequency (f_R ; burst/min) was calculated from the mean C4 burst interval during 2- to 5-min intervals both before and after IL-1 β and PGE_2 administration. All data were normalized to facilitate statistical comparisons.

One-way ANOVA was used to compare parameters with normal distribution and equal variance. Multiple comparisons were performed using Tukey-Kramer's HSD *post hoc* test. The

Wilcoxon test (two groups) and Kruskal-Wallis test (>two groups) were used for nonparametric measurements and data with non-Gaussian distributions or unequal variance. Change in variables over time was examined using MANOVA repeated measures design. Three-way ANOVA and logistic regression were used to evaluate differences between independent variables. The Spearman rank correlation test was used to determine correlations between variables. Data are presented as mean $\pm$ SEM. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Respiration during normoxia. Animals given IL-1 β exhibited a significantly lower f_R than control animals ($p < 0.01$) and animals treated only with indomethacin ($p < 0.05$; Table 1, Fig. 2). LPS also tended to reduce f_R compared with the control ($p = 0.079$) and indomethacin only ($p = 0.064$). A lower f_R during normoxia was correlated to a longer gasping duration ($p < 0.0001$), a depressed gasping frequency ($p < 0.0001$), and reduced survival ($p < 0.01$). Indomethacin reversed the depressive effects of IL-1 β and LPS on f_R but did not stimulate respiration above basal levels in control animals. Respiratory frequency was higher in animals studied using barometric plethysmography than in those examined using flow plethysmography ($p < 0.001$). Treatment had no effect on inspiratory time, expiratory time, tidal volume, and minute ventilation (Fig. 2). Skin temperature during normoxia ($34.6 \pm 0.1^\circ\text{C}$) did not differ between groups.

Response to anoxia. All animals displayed a biphasic response to anoxia with the following characteristic stages: 1) hyperpnea, 2) primary apnea, 3) gasping, and 4) secondary or terminal apnea (Table 2, Fig. 1) (26). IL-1 β significantly lowered the total gasping frequency compared with control ($p < 0.01$). Indomethacin did not prevent this depression of gasping. IL-1 β , LPS, and indomethacin had no effect on the duration of hyperpnea, primary apnea, or gasping and did not alter the duration or frequency of the three gasping phases. The duration of secondary apnea and time of autoresuscitation in survivors as well as the duration of anoxic exposure (685 ± 12 s) also did not differ between treatment groups.

Both IL-1 β and LPS worsened recovery after anoxia. Survival was significantly lower in animals given IL-1 β than in control animals and animals treated with indomethacin ($p < 0.05$; Fig. 3). Similarly, animals given LPS died more readily than control animals ($p < 0.05$) and animals that received only indomethacin ($p < 0.01$). Pretreatment with indomethacin

Table 1. Respiratory effects of IL-1 β and indomethacin using barometric plethysmography

	NaCl pretreatment		Indomethacin pretreatment	
	NaCl	IL-1 β	NaCl	IL-1 β
Animals (n)	12	12	6	13
Normoxia f_R (breaths/min)	241 $\pm$ 12	185 $\pm$ 5	214 $\pm$ 14	209 $\pm$ 11
Hyperpnea (s)	52 $\pm$ 7	65 $\pm$ 4	53 $\pm$ 6	54 $\pm$ 3
Primary apnea (s)	22 $\pm$ 4	24 $\pm$ 4	22 $\pm$ 8	15 $\pm$ 3
Gasping (s)	472 $\pm$ 44	640 $\pm$ 59	425 $\pm$ 22	525 $\pm$ 25
Gasping f_R (gasps/min)	6.4 $\pm$ 0.4	5.4 $\pm$ 0.3	5.2 $\pm$ 0.2	5.2 $\pm$ 0.2
Secondary apnea (s)	502 $\pm$ 54	501 $\pm$ 50	398 $\pm$ 21	449 $\pm$ 22
Autoresuscitation (s)	1102 $\pm$ 93	1154 $\pm$ 95	878 $\pm$ 96	1094 $\pm$ 46

DISCUSSION

Figure 2. Effects of IL-1 β , LPS, and indomethacin on basal respiration. *A*, Flow plethysmograph recording depicts respiration during normoxia for a control animal and an IL-1 β -treated animal. IL-1 β depressed f_R but did not alter inspiratory time, expiratory time, tidal volume, or minute ventilation. *B*, f_R during normoxia was compared between all animals according to group ($n = 112$). NaCl or indomethacin was given before a second injection of NaCl, IL-1 β , or LPS (□, NaCl; ▨, IL-1 β ; ■, LPS). IL-1 β reduced f_R compared with control ($p < 0.01$) and indomethacin only ($p < 0.05$). Animals given LPS also tended to breathe more slowly than control animals ($p = 0.079$) and animals treated only with indomethacin ($p = 0.064$). Indomethacin attenuated the depressive effects of IL-1 β and LPS but did not alter f_R in control animals. Data are presented as mean $\pm$ SEM. ** $p < 0.01$ compared with control.

significantly improved the outcome of animals subsequently given IL-1 β and LPS ($p < 0.05$).

Skin temperature decreased similarly during anoxia in all groups to $32.5 \pm 0.2^\circ\text{C}$. No difference in chamber temperature ($29.7 \pm 0.1^\circ\text{C}$), animal weight (15.5 ± 0.2 g), or sex distribution was observed between groups. Skin and chamber temperature as well as animal sex were not correlated with any respiratory variables or survival. The type of plethysmograph system also had no effect on anoxic response.

Brainstem respiratory activity. IL-1 β applied to the brainstem–spinal cord preparation for 30 min did not alter burst distance (7.0 ± 1.7 s) compared with control (7.0 ± 0.7 s; Fig. 4). IL-1 β perfusion for 60 min also had no significant effect on respiratory activity (f_R ; $-4.5 \pm 1.8\%$ of control). PGE₂ application increased burst distance (20.8 ± 3.5 s) compared with control (9.3 ± 1.3 s), thereby decreasing f_R ($p < 0.01$). This effect was dose dependent and reversible within 20 min of reperfusion with control aCSF.

The principal finding of this study is that peripheral IL-1 β depresses f_R and worsens recovery from anoxic challenge in neonatal rats and that these effects are attenuated by pretreatment with indomethacin. In addition, this study reveals that IL-1 β has no direct effect on respiratory rhythm generation in the isolated brainstem, whereas PGE₂ significantly inhibits brainstem respiratory neurons. Thus, we propose that an activated immune system may alter central respiration and autoresuscitation *via* a cytokine-induced, prostaglandin-mediated pathway.

Two different plethysmograph systems were used to evaluate the effects of IL-1 β on respiration and anoxic survival. Both recording methods revealed essentially similar treatment results. However, f_R during normoxia was higher in animals examined using the barometric method, possibly because of an increased stress response resulting from a shorter acclimation period during normoxia and the lack of handling before experimentation. The more pronounced effects of IL-1 β and indomethacin on basal respiration but not anoxic response in these potentially more stressed animals are consistent with previous work showing that stress elevates f_R during normoxia but does not alter the respiratory response to hypoxia (27).

Animals given IL-1 β exhibited a sustained respiratory depression throughout normoxia and anoxia, which may have contributed to their markedly reduced survival rate. These findings are in accordance with other studies demonstrating that IL-1 β depresses respiration and adversely affects recovery from apnea induced by laryngeal stimulation (6, 7). However, the specific pathway by which IL-1 β acts cannot be determined with our methods. Direct transport mechanisms have been demonstrated for IL-1 β (28), although they have low capacity and are rapidly saturated. Furthermore, indomethacin does not affect this active transport and receptors for IL-1 are predominantly present on barrier-related cells (29). Because IL-1 β applied directly to the brainstem preparation does not alter respiratory activity, it is unlikely that direct activation of brainstem areas by IL-1 β through this transport mechanism or *via* distant circumventricular organs plays a major role in the induction of the respiratory effects seen in the present study.

Instead, our study implicates a mediatory role of prostaglandins in these IL-1 β -induced respiratory effects. This is consistent with reports that prostaglandin mediates other IL-1 β actions beyond the BBB [for review, see (11)] and can in itself depress both fetal and neonatal breathing (14–17). Furthermore, prostaglandin has been shown to inhibit the rhythm-generating neurons of the pre-Bötzinger complex involved in both eupnea and gasping (30, 31), which may reduce the animal's ability to autoresuscitate. It was recently shown that circulating IL-1 β binds to its receptor located on vascular endothelial cells at the BBB in the brainstem and induces COX-2 and microsomal PGE synthase mRNA expression within an hour of i.v. administration (32). This results in a local release of PGE₂ and the subsequent activation of neurons in the VLM and NTS with highly expressed PGE₂ receptors. This is corroborated by the findings of a similar c-fos and PGE₂ receptor mRNA expression in the VLM and NTS, as that seen 1 h after i.v. IL-1 β is detected 45 min after injection of PGE₂.

Table 2. Respiratory effects of IL-1 β , LPS, and indomethacin using flow plethysmography

	NaCl pretreatment			Indomethacin pretreatment		
	NaCl	IL-1 β	LPS	NaCl	IL-1 β	LPS
Animals (n)	10	14	11	12	11	11
Normoxia f_R (breaths/min)	192 $\pm$ 7	174 $\pm$ 8	173 $\pm$ 8	193 $\pm$ 7	184 $\pm$ 6	186 $\pm$ 5
Hyperpnea (s)	64 $\pm$ 4	63 $\pm$ 4	61 $\pm$ 4	60 $\pm$ 3	64 $\pm$ 4	62 $\pm$ 4
Primary apnea (s)	13 $\pm$ 2	23 $\pm$ 5	16 $\pm$ 5	23 $\pm$ 5	18 $\pm$ 1	17 $\pm$ 2
Gasping (s)	442 $\pm$ 21	481 $\pm$ 28	515 $\pm$ 34	518 $\pm$ 26	571 $\pm$ 41	472 $\pm$ 38
Gasping f_R (gasps/min)	5.8 $\pm$ 0.1	5.3 $\pm$ 0.2	5.6 $\pm$ 0.3	5.2 $\pm$ 0.2	5.3 $\pm$ 0.3	5.5 $\pm$ 0.3
Secondary apnea (s)	462 $\pm$ 22	472 $\pm$ 20	495 $\pm$ 9	470 $\pm$ 28	489 $\pm$ 34	476 $\pm$ 26
Autoresuscitation (s)	1024 $\pm$ 39	1107 $\pm$ 39	1088 $\pm$ 20	1113 $\pm$ 46	1138 $\pm$ 67	1069 $\pm$ 49

Figure 3. Effects of IL-1 β , LPS, and indomethacin on the ability to autoresuscitate after hypoxic apnea. Animals given IL-1 β and LPS died more readily than control animals ($p < 0.05$) and animals treated only with indomethacin ($p < 0.05$ and $p < 0.01$, respectively; $\square$, NaCl; $\blacksquare$, IL-1 β ; $\blacksquare$, LPS). Indomethacin improved outcome of these animals ($p < 0.05$). Data are presented as mean $\pm$ SEM. * $p < 0.05$ compared with control. **Figure 4.** Effects of IL-1 β and PGE₂ on respiratory rhythm generation. IL-1 β (Δ) and PGE₂ ($\bullet$) were applied to the brainstem–spinal cord preparation of neonatal rats. IL-1 β did not change f_R , whereas PGE₂ reversibly inhibited f_R ($p < 0.01$). Data are presented as mean $\pm$ SEM.

in the lateral ventricles (33). It is possible that the effects of peripheral IL-1 β and LPS are mediated by COX-2 mRNA production, as indicated by several previous studies and subsequent release of PGE₂, although this was not investigated here. The proposed cascade of events involving prostaglandin synthesis and action may explain why direct application of IL-1 β to respiratory neurons in the brainstem–spinal cord preparation—where the BBB endothelium is absent and thus IL-1 β could not induce prostaglandin release—had no effect on respiratory neuronal activity.

The prostaglandin-dependent action on central respiratory neurons may be mediated by both central and vagal pathways,

and the predominant signaling pathway could be affected by factors such as drug administration route and dose (34, 35). It is possible that peripheral IL-1 β communicates with the NTS by acting at vagal afferents in a prostaglandin-dependent manner (36), subsequently affecting respiratory centers. It is worth noting that vagotomy does not prevent IL-1 β 's induction of fos-ir expression in the NTS and RVLM (36, 37). It also does not affect indomethacin's reduction of expression in these respiratory regions or indomethacin's stimulation of respiration (20, 36, 37). This supports the proposal of a central rather than vagal prostaglandin-dependent pathway for IL-1 β action upon brainstem respiratory-related neurons in the newborn rat.

LPS induced similar effects on respiration, although less pronounced, as those seen with IL-1 β . Previous studies have demonstrated that LPS stimulates macrophages to release IL-1 β , resulting in detectable levels of IL-1 β in the plasma at 2 h and the brain at 6 h after intraperitoneal injection (38). LPS (i.p.) also activates prostaglandin-synthesizing enzymes; however, it does so following a more delayed time course than with direct application of IL-1 β (i.v.) (39, 40). Although a longer duration between the inflammatory challenge and respiratory recordings in our study may have produced greater alterations in respiration and the ability to autoresuscitate, a shorter time period was chosen to limit confounding systemic changes. The effects of IL-1 β and LPS on respiration and survival in the present study do not seem to result from other drug-induced centrally mediated responses such as fever or sleep. Temperature measurements during normoxia correspond to previously reported values for rats of similar age (41), and no difference was observed between treatment groups. Furthermore, all animals exhibited a similar characteristic hypothermic response to reduced O₂ concentrations (42). These findings are in agreement with studies showing that IL-1 β and LPS do not elicit a significant temperature increase in rats until at least 90 min after intraperitoneal administration (43, 44). In addition, our recordings were performed in the light cycle within 90 min of drug injection, thus making the sleep-inducing effects of IL-1 β and LPS shown previously to begin at 2 h after drug administration during the light cycle unlikely (45), and no difference in gross motor activity was observed between treatment groups.

Although prostaglandin may relay IL-1 β and LPS signals to the central respiratory network during an immune challenge, it does not seem to influence eupnea in healthy 7-d-old rats. The inability of indomethacin to stimulate breathing beyond basal levels in such animals may be explained by the critical transition in the respiratory effects of PGE₂ during early postnatal

development. Although PGE₂ inhibits breathing movements in the fetus and directly after birth (14, 15), it later induces little or no change in central respiration during normal conditions (17). Consequently, the effects of indomethacin on eupneic breathing will vary depending on the animal's immune state and the specific stage of perinatal development at which it is given.

Prostaglandin seems to play a critical role in the ability of rats to autoresuscitate and survive an anoxic challenge, yet no alterations in the anoxic respiratory pattern by indomethacin were detected. Prostaglandin involvement during anoxia remains likely as indomethacin effects were seen both before and after N₂ exposure. In addition, prostaglandin is considered to be one of the inhibitory neuromodulators contributing to respiratory depression during anoxia (46). In addition to its effects on neuronal activity, prostaglandin may diminish the responsiveness to anoxia by reducing central chemosensitivity to CO₂ (12, 17, 30, 31). Prostaglandin may do so by acting on PGE₂ receptors highly expressed in the NTS, nucleus ambiguus, and parabrachial nucleus or by hyperpolarizing pre-Bötzinger neurons recently shown to possess intrinsic hypoxic chemosensitivity and respond to changes in P_{CO2}. In exerting these effects, prostaglandin may contribute to the decreased ability to autoresuscitate and thereby worsen recovery from anoxic stress.

The present investigation explored one possible mediator of recurrent apnea and infection: IL-1 β and a mechanism by which it may exert modulating effects on vital autonomic function within the CNS. This study shows that an inflammatory response can inhibit respiration as well as the ability to autoresuscitate and survive anoxic challenge. We propose that these effects are mediated by IL-1 β and its induction of prostaglandin release in the vicinity of respiratory neurons in the brainstem. Our findings support the hypothesis that infection may induce apnea and possibly also sudden infant death syndrome *via* a prostaglandin-dependent, cytokine-mediated pathway.

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