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mPGES-1 and prostaglandin E_2: vital role in inflammation, hypoxic response, and survival

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BACKGROUND: Apnea associated with infection and inflammation is a major medical concern in preterm infants. Prostaglandin E_2 (PGE_2) serves as a critical mediator between infection and apnea. We hypothesize that alteration of the microsomal PGE synthase-1 (mPGES-1) PGE_2 pathway influences respiratory control and response to hypoxia.

METHODS: Nine-d-old wild-type (WT) mice, mPGES-1 heterozygote (mPGES-1$^{+/-}$), and mPGES-1 knockout (mPGES-1$^{-/-}$) mice were used. Respiration was investigated in mice using flow plethysmography after the mice received either interleukin-1β (IL-1β) (10 μg/kg) or saline. Mice were subjected to a period of normoxia, subsequent exposure to hyperoxia, and finally either moderate (5 min) or severe hypoxia (until 1 min after last gasp).

RESULTS: IL-1β worsened survival in WT mice but not in mice with reduced or no mPGES-1. Reduced expression of mPGES-1 prolonged gasping duration and increased the number of gasps during hypoxia. Response to intracerebroventricular PGE_2 was not dependent on mPGES-1 expression.

CONCLUSION: Activation of mPGES-1 is involved in the rapid and vital response to severe hypoxia as well as inflammation. Attenuation of mPGES-1 appears to have no detrimental effects, yet prolongs autoresuscitation efforts and improves survival. Consequently, inhibition of the mPGES-1 pathway may serve as a potential therapeutic target for the treatment of apnea and respiratory disorders.

Infection during the neonatal period commonly induces potentially life-threatening apnea episodes (1). Respiratory syncytial virus infection and subsequent inflammation may induce autonomic dysfunction in preterm as well as young term infants (2). Moreover, infection often precedes episodes of sudden infant death syndrome (3).

Interleukin 1β (IL-1β) is produced during the acute phase immune response to infection (4). It evokes a variety of sickness behaviors including respiratory depression (5,6). A major and rapid pathway for IL-1β to act across the blood–brain barrier is by binding to IL-1 receptors on vascular endothelial cells of the blood–brain barrier, which then induces cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase-1 (mPGES-1) activity (7,8).

Following induction of COX-2 and mPGES-1 activity by a proinflammatory stimulus such as IL-1β, prostaglandin E_2 (PGE_2) is released into the brain parenchyma (7) and mediates several central effects including fever (9), pain (10), anorexia (11), and respiratory depression (12). PGE_2 exerts its actions via E prostanoid-receptors (EPRs), including EP3R, in respiratory-related regions of the brainstem (e.g., the nucleus of tractus solitarius (NTS) and rostral ventrolateral medulla) (13). PGE_2 has been shown to depress breathing in fetal and newborn sheep, mice, and humans *in vivo* (8,12,14) and inhibit respiratory-related neurons *in vitro* (5,6,8). In addition to inflammatory stimuli, anoxia itself induces PGE_2 synthesis (15) and might lead to deleterious brain damage in postnatal rats (16). We and others have suggested that PGE_2 may serve as a critical mediator between infection and apnea (1,2,8,17).

In this study, we further examined the role of mPGES-1 activity in the respiratory response to IL-1β and acute anoxia. This study suggests that attenuation of the mPGES-1-pathway can decrease inflammation-related respiratory depression in neonates. Therefore, a reduction of mPGES-1 activity, achieved via specific mPGES-1 inhibiting drugs, might be considered in the treatment of neonatal apnea. Furthermore, we show that mPGES-1 activation and an immediate release of PGE_2 are involved in the acute response to severe hypoxia.

RESULTS

Animal Characteristics

All animals exhibited a similar skin temperature before experimentation (34.7 ± 0.1 °C) and at 70 min after injection of NaCl or IL-1β (34.8 ± 0.1 °C). In response to anoxia, there was a concomitant decrease in skin temperature (–2.6 ± 0.1 °C). Animal weight was similar between groups (4.5 ± 0.1 g) (**Table 1**). Weight did not correlate with gasping duration, numbers of gasps, or survival. Finally, there was no difference in the distribution of males and females between groups, and sex did not affect any of the measured respiratory variables or survival.

### Effects of IL-1β on Respiration During Normoxia and Hyperoxia

During normoxia, mice with IL-1β or NaCl treatment had similar mean respiratory frequency (f_R), minute ventilation (V_E), and tidal volume (V_T). Furthermore, no differences in

Table 1. Characteristics of mPGES-1^{+/+}, mPGES-1^{+/−}, mPGES-1^{−/−} mice, and effects of IL-1β or NaCl

	mPGES^{+/+}		mPGES^{+/−}		mPGES^{−/−}	
	NaCl	IL-1β	NaCl	IL-1β	NaCl	IL-1β
Animals (n)	33	33	25	20	15	19
Weight (g)	4.3 ± 0.1	4.5 ± 0.1	4.7 ± 0.1	4.6 ± 0.2	5.2 ± 0.2	4.7 ± 0.2
f_R normoxia (min−1)	249 ± 5	236 ± 6*	241 ± 6	243 ± 7	243 ± 8	254 ± 7
f_R hyperoxia (min−1)	181 ± 8	155 ± 7**	168 ± 9	173 ± 9	195 ± 14	206 ± 9

Effects of IL-1β or NaCl on respiratory frequency (f_R) during normoxia and hyperoxia were quantified 75 min after i.p. injection. Comparing all groups wild-type mice treated with IL-1β and exposed to hyperoxia exhibited a lower f_R. When comparing treatment effects within each genotype, IL-1β did reduce basal f_R in mPGES-1+/+ mice during normoxia (t-test; *P = 0.038). The f_R effect of IL-1β on wild-type mice is further evident under physiological deafferentation of the carotid bodies during hyperoxia. Data are presented as mean + SEM. IL-1β, interleukin-1β; mPGES-1, microsomal prostaglandin E synthase-1.
*P < 0.05, **P < 0.01.
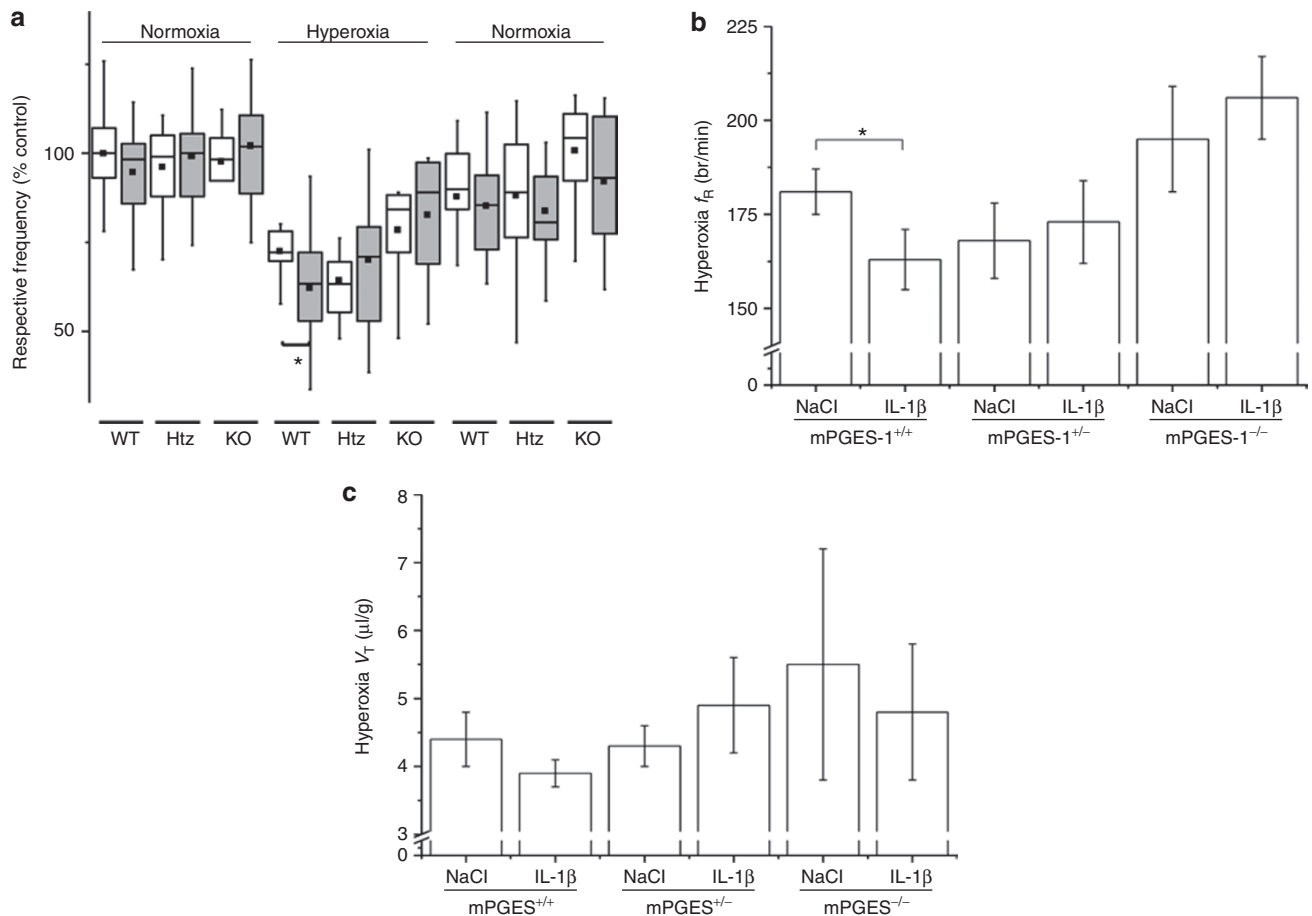
Figure 1. Interleukin-1β (IL-1β) depresses respiration through microsomal prostaglandin E synthase-1 (mPGES-1) activation, evident during hyperoxia. Response to 1 min hyperoxia varied by genotype and treatment. (**a**, **b**) All mice responded to hyperoxia with a reduction in f_R (breaths/min). During hyperoxia, lower f_R was present in IL-1β- as compared with NaCl-treated mPGES-1^{+/+} mice, whereas IL-1β did not alter respiration in normoxia or hyperoxia in heterozygote (mPGES-1^{+/−}) or knockout (mPGES-1^{−/−}) mice. (**c**) No changes in V_T (tidal volume) were found between groups during hyperoxia. Note the lower depression as well as post-hyperoxic f_R recovery in mPGES-1^{−/−} mice independent of treatment. Data are presented as mean ± SEM. *P < 0.05. In the boxplot: mean = black boxes, median = vertical lines, NaCl = white boxes, and IL-1β = gray boxes.

breathing were seen because of genotype. When comparing treatment effects within each genotype, IL-1β did reduce basal f_R in mPGES-1^{+/+} mice during normoxia (t-test, * P = 0.038). Data are summarized in Table 1.

All mice, irrespective of treatment, responded to hyperoxic challenge with a reduction in f_R. IL-1β decreased respiratory frequency in wild-type (WT) mice, but not in mPGES-1^{+/−} or mPGES-1^{−/−} mice (ANOVA, *P = 0.013) (Figure 1a,b and

Supplementary Figures S1 and S2 online). No difference in V_e or V_T during hyperoxia was found (Figure 1c).

IL-1β Reduces the Ability to Autoresuscitate Following 5-min Anoxic Challenge via mPGES-1

IL-1β decreased survival after the 5-min anoxic challenge in WT mice (χ^2, *P = 0.013) but not in mPGES-1^{+/−} mice or mPGES-1^{−/−} mice (Figure 2).
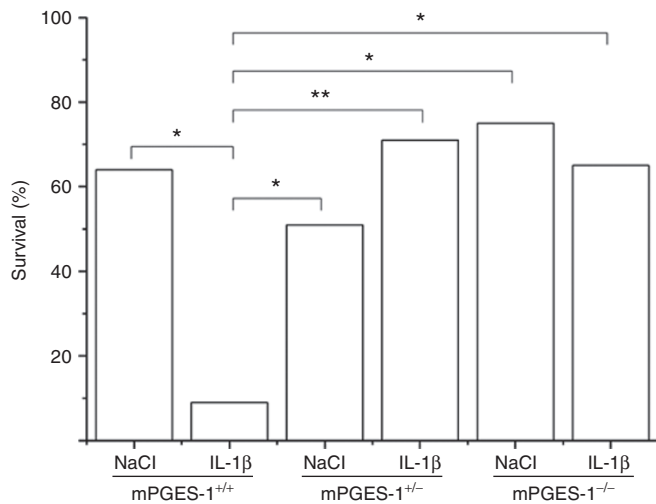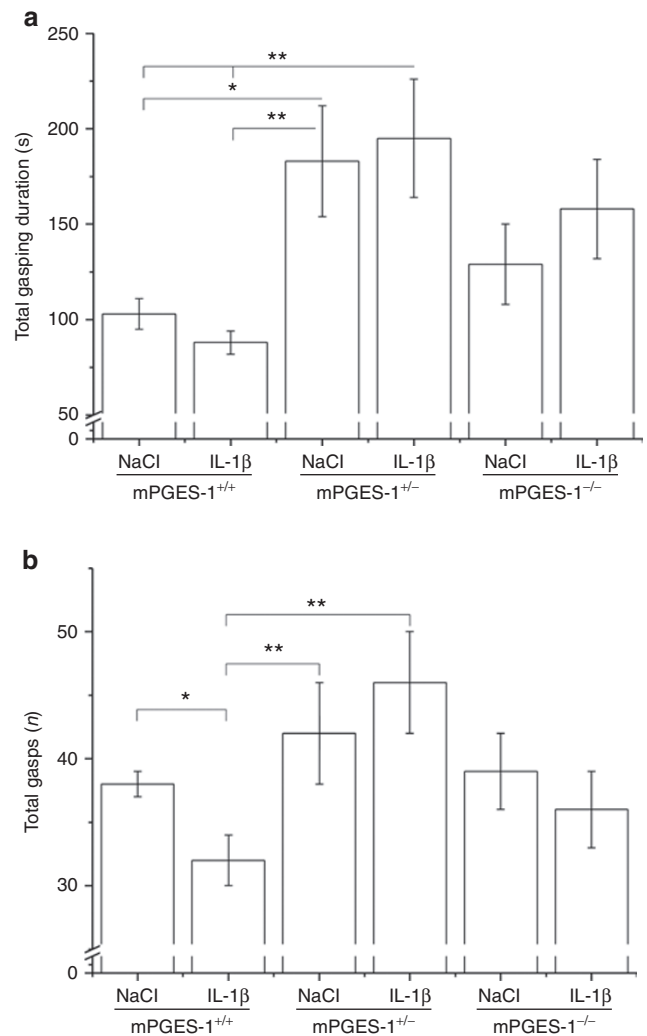
Figure 2. Interleukin-1β (IL-1β) reduces anoxic survival via microsomal prostaglandin E synthase-1 (mPGES-1). Nine-d-old mPGES-1^{+/+} mice (n = 25), mPGES-1^{+/-} (n = 14), and mPGES-1^{-/-} (n = 12) were exposed to 5 min of anoxia (100% N$_2$) at 80 min after peripheral administration of IL-1β (n = 26) or vehicle (n = 25). IL-1β reduced survival in wild-type mice. This effect was not observed in mice with reduced or absent expression of mPGES-1. Data are presented as mean ± SEM. *P < 0.05, **P < 0.01.

Anoxia Reduces Gasping Efforts in WT as Compared With mPGES-1^{+/-} and mPGES-1^{-/-} Mice

All mice exhibited a biphasic response to anoxia with an initial increase in ventilation (i.e., hyperpnea) followed by hypoxic respiratory depression (i.e., primary apnea and gasping). A difference between genotypes in gasping effort was found, both in duration and total number of gasps. mPGES-1^{+/-} mice produced more gasps as compared with WT mice (ANOVA, **P = 0.001) and also had a longer gasping duration than WT (Tukey-Kramer, *P = 0.04). IL-1β depressed total number of gasps in WT, but not in mPGES-1^{+/-} mice (ANOVA, P = 0.014) (Figure 3a,b). The number of gasps and gasping duration was positively correlated to survival (logistic regression, P = 0.03 and P = 0.04, respectively). mPGES-1^{-/-} and mPGES-1^{+/-} mice responded similarly to anoxia; IL-1β did not affect the gasping behavior in these mice.

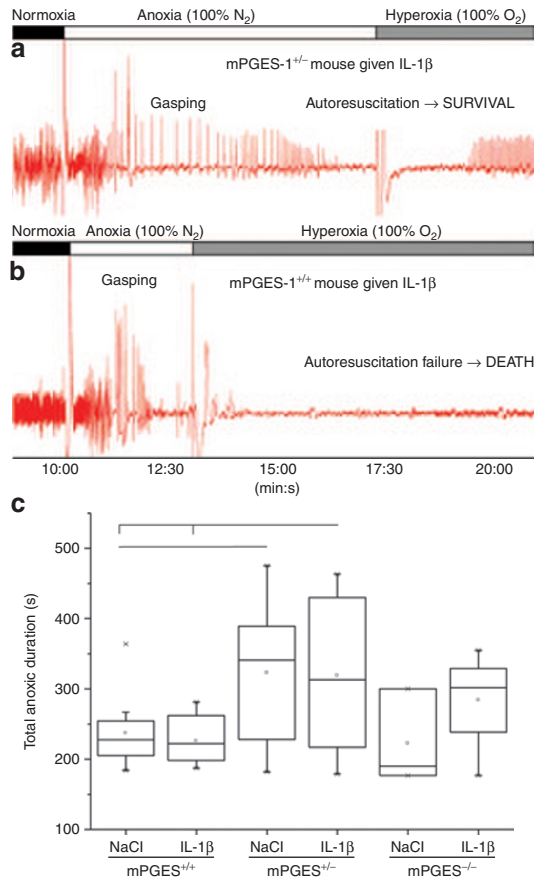## Reduced mPGES-1 Expression Prolongs Respiratory Efforts During Severe Anoxic Challenge

Survival was correlated to duration of gasping, and ability to gasp for a prolonged period improved survival. When mice were exposed to the severe anoxic challenge protocol (i.e., anoxic exposure until 1 min after the last gasp of the mouse pup), the mPGES-1^{+/-} mice were subjected to a longer period of anoxia before autoresuscitation than WT mice because of their prolonged respiratory efforts (Figure 4). This was independent of treatment. Despite being exposed to anoxia for a significantly longer duration than WT mice (321 + 16 s and 231 + 16 s, respectively), there was no difference in survival after anoxic exposure between genotypes or treatment groups using this severe anoxic challenge. The mPGES-1^{-/-} mice were exposed to anoxia for 201 ± 18 s and 284 ± 38 s with NaCl and IL-1β treatment, respectively.

Figure 3. Interleukin-1β (IL-1β) as well as severe anoxia reduces ability to gasp via microsomal prostaglandin E synthase-1 (mPGES-1). Nine-d-old mPGES-1^{+/+} mice (n = 33), mPGES-1^{+/-} (n = 21), and mPGES-1^{-/-} (n = 15) were exposed to anoxia (100% N$_2$) at 80 min after peripheral administration of IL-1β (n = 35) or vehicle (n = 34). (**a**) IL-1β reduced gasping duration in mPGES-1^{+/+} mice as compared with NaCl-treated mPGES-1^{+/+} mice as well as compared with mPGES-1^{+/-} mice, independent of treatment. (**b**) IL-1β reduced the number of gasps in the mPGES-1^{+/+} mice, whereas this effect was not observed in mice with reduced or absent expression of mPGES-1. Moreover, mPGES-1^{+/-} mice that were pretreated with IL-1β were able to produce more gasps than IL-1β-treated mPGES-1^{+/+} mice. Data are presented as mean ± SEM. *P < 0.05, **P < 0.01.

PGE$_2$ Induces Apneic Breathing Independent of mPGES-1 Expression

PGE$_2$ (n = 8) induced irregular breathing and apnea after intracerebroventricular administration, whereas intracerebroventricular administration of NaCl (n = 6) did not (Figure 5). Skin temperature increased 20 min after anesthesia and intracerebroventricular injection in mice that received PGE$_2$ as compared with vehicle (PGE$_2$: 35.2 + 0.3 °C; NaCl: 33.4 + 0.4 °C, **P < 0.05). The effects of PGE$_2$ were independent of mPGES-1 expression (WT: n = 4; mPGES-1^{+/-}: n = 6; mPGES-1^{-/-}: n = 5).

Figure 4. A reduced microsomal prostaglandin E synthase-1 (mPGES-1) expression prolongs autoresuscitation. (**a**) Plethysmograph recording of an mPGES-1$^{+/-}$ mouse given interleukin-1β (IL-1β) 80 min before anoxic exposure (100% N$_2$) until 1 min after last breath when 100% O$_2$ (hyperoxia) was administered. The picture depicts the initial hyperpnea period, followed by a long anoxic period due to the prolonged ability to produce gasps. This is followed by autoresuscitation during the hyperoxia period administered 1 min after the last gasp. (**b**) Plethysmograph recording of mPGES-1$^{+/+}$ (wild-type (WT)) mouse given IL-1β showing initial hyperpnea, the following gasping period during anoxia, and then the inability to autoresuscitate during hyperoxia, leading to death. (**c**) Mice with a reduced expression of mPGES-1 (*n* = 22) are able to sustain gasps during 90 s additional anoxia as compared with mPGES-1$^{+/+}$ mice (*n* = 23) using the severe anoxic protocol. This occurred irrespective of treatment with NaCl (*n* = 25) or IL-1β (*n* = 20), suggesting that anoxia itself induces mPGES-1. mPGES-1$^{-/-}$ mice (*n* = 7) exhibited a similar increased duration of gasping and anoxic duration although not significant as compared with WT mice. Illustrated as a boxplot, the box depicts the 25th and 75th percentiles, with whiskers enclosing maximum and minimum and *x*s representing outliers. Time is shown in minutes (min) and seconds (s). Data are presented as mean ± SEM. *P < 0.05, **P < 0.01.

DISCUSSION

This study demonstrates that mPGES-1 and subsequent release of PGE$_2$ in the vicinity of respiration-related brainstem areas are involved in the acute response to hypoxia as well as inflammation (**Figures 3, 4,** and **6**) (8). Moreover, decreased expression of mPGES-1 protects neonatal mice during acute phases of inflammation and anoxia, enabling prolonged respiratory effort and increased survival. Of note, reduced mPGES-1 activity increases the ability
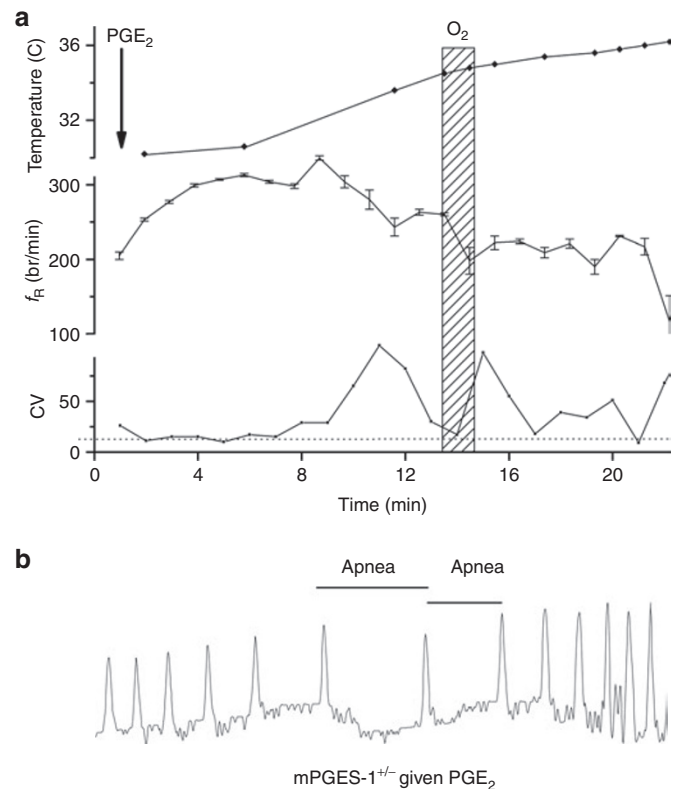

Figure 5. Prostaglandin E$_2$ (PGE$_2$) induces irregular breathing and apnea independent of microsomal prostaglandin E synthase-1 (mPGES-1) expression. Respiration during normoxia, hyperoxia, and anoxia was examined in neonatal mice with variable expression of mPGES-1 (following intracerebroventricular administration of vehicle (*n* = 7) or PGE$_2$ (*n* = 8). (**a**) Respiratory activity in a newborn mPGES-1$^{+/-}$ mouse given PGE$_2$ at time point 0. A temporary increase in respiratory frequency (f_R, breaths/min) during initial post-anesthesia period is followed by a depression of basal f_R despite simultaneous increase in animal body temperature, with measuring points represented by black diamonds. Note irregular respiratory rhythm with elevated coefficient of variation (CV) during normoxia and hyperoxia. Note adequate reduced f_R response to hyperoxic (100% O$_2$) exposure (striped box) but no subsequent recovery of f_R. (**b**) Plethysmograph recording in newborn mPGES-1$^{+/-}$ mouse demonstrates apneic episodes in response to PGE$_2$ during basal respiration.

to gasp and autoresuscitate during hypoxic exposure (**Figures 3** and **4**). These results improve our knowledge of an important pathway underlying infection and inflammation, hypoxia, and apnea in neonates and provide new venues for possible therapeutic interventions to treat apnea and respiratory dysfunction.

Our results suggest that mPGES-1 activation is necessary for IL-1β to depress central respiratory drive. We have shown that not only the absence of mPGES-1 expression (8), but also the attenuation of mPGES-1 expression, prevents respiratory depression in IL-1β-treated mice during hyperoxia. Therefore, reduced mPGES-1 expression weakens IL-1β-mediated mPGES-1 activity on the central brainstem respiratory drive at the examined time points. In addition, mice responded similarly to intracerebroventricular PGE$_2$, irrespective of their mPGES-1 expression (**Figure 5**), indicating that it is the reduced release of PGE$_2$ that underlies the effects observed in mice with reduced or absent mPGES-1 expression.
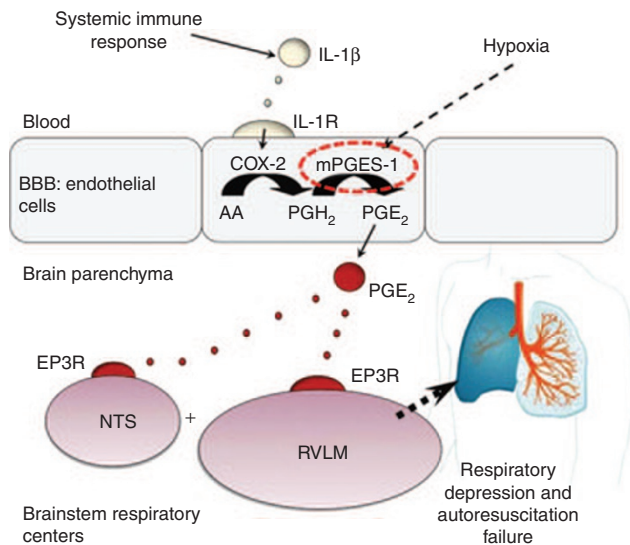
Figure 6. Model for an inflammatory and hypoxic microsomal prostaglandin E synthase-1 (mPGES-1)-mediated pathway to respiratory depression and autoresuscitation failure. During a systemic immune response, the proinflammatory cytokine interleukin-1β (IL-1β) is released into the peripheral bloodstream. It binds to its receptor (IL-1R) located on endothelial cells of the blood–brain barrier (BBB). Activation of IL-1R induces the synthesis of PGH_2 from arachidonic acid (AA) via COX-2 and the synthesis of prostaglandin E_2 (PGE_2) from PGH_2 via the rate-limiting enzyme mPGES-1. Moreover, hypoxia in itself activates mPGES-1 and the activation is additive to that of IL-1R. PGE_2 is released into the brain parenchyma and binds to its EP3R located in the respiratory control regions of the brainstem, e.g., nucleus of tractus solitarius (NTS) and the rostral ventrolateral medulla (RVLM). This results in depression of central respiration-related neurons and breathing, which may fatally decrease the ability to gasp and autoresuscitate during hypoxic events.

The NTS of the brainstem is an important nucleus for immune-to-brain as well as brain-to-immune signaling (18). The NTS is also the main site of termination of vagal afferents and contains EP3R (13). Intratracheal endotoxin-exposure in rat pups induces an attenuated respiratory response to hypoxia. This effect of local inflammation originating in the lung seems to be vagally mediated and associated with an upregulation of IL-1β in the NTS of the medulla oblongata (19).

However, it seems as if IL-1β does not directly modulate the neuronal network activity in the NTS, but instead that PGE_2, produced downstream of IL-1β, modulates synaptic transmission in the NTS (20). PGE_2 decreases vagal synaptic transmission via presynaptic EP3 receptors, which reduce glutamate release via G-proteins of the $G_{i/o}$ class coupled to adenylyl cyclase, cyclic adenosine monophosphate generation and protein kinase A activity (20).

Recently, inflammatory processes have been shown to increase O_2 sensitivity of the carotid body during chronic sustained hypoxia (21). Adaptation to chronic hypoxia seems to involve immune cell invasion and increased expression of inflammatory cytokines in rat carotid body (21). This change of hypoxic response is blocked by ibuprofen, a nonspecific COX inhibitor (22). Therefore, COX-mediated chronic inflammatory processes seem to have an excitatory effect on the carotid body

and subsequently help to stimulate the respiratory drive. In this study, the hyperoxic "Dejours test," a physiological denervation of the carotid body afferent input to the brainstem respiratory centers (23), revealed an IL-1β-induced mPGES-1 dependent depression of the central respiratory activity (**Figure 1**). It is possible that inflammation and prostaglandins also act at the carotid body, enhancing its activity. Therefore, during normoxic conditions, peripheral excitation might partly counteract the central depression induced by the inflammatory pathway.

This further emphasizes the link between inflammation and hypoxic signaling. Although this study focuses on the acute effects of IL-1β on the respiratory response in neonatal mice, we cannot exclude an effect of carotid body sensitivity or vagal input on the observed respiratory patterns. However, the rapid time course, as well as the mPGES-1 dependency, is consistent with our hypothesis that IL-1β exerts its effects via direct PGE_2-mediated action on the brainstem respiratory centers (**Figure 6**) (8).

mPGES-1 and PGE_2 Release Is Involved in the Immediate Response to Severe Hypoxia

PGE_2 causes hypoventilation by decreasing respiratory frequency, induces apnea, and alters central respiratory rhythm in newborn animals (5,12). PGE_2 can decrease neocortical as well as brainstem network activity by postsynaptically reducing excitatory synaptic transmission (24) via EP3R (8,20). Of note, mice heterozygous for mPGES-1 gasp longer than WT mice but retain their ability to respond to intracerebroventricular administration of PGE_2 (**Figures 3–5**). Thus the prolonged gasping is not due to a change in brainstem sensitivity to PGE_2 but is instead related to reduced production of PGE_2 resulting from attenuated mPGES-1 expression. Anoxia itself has been proposed to induce mPGES-1 and PGE_2 release (15). Our data suggest that acute hypoxia leads to rapid mPGES-1 activation and immediate release of PGE_2 (**Figure 6**).

PGE_2, released in the vicinity of brainstem central pattern generators for cardiorespiratory control, subsequently activates EP3R, which, in turn, reduces cyclic adenosine monophosphate. This leads to a decreased firing amplitude and rate in respiration-related brainstem neurons, thereby depressing respiratory activity. Using WT and EP3R-knockout mice, we previously showed that EP3-receptors are expressed in the rostral ventrolateral medulla oblongata, including putative pacemaker neurons in pre-Bötzinger and the parafascial respiratory group regions (8). Dysfunction of these pacemaker neurons and their neural network, which generate breathing rhythm, can induce apnea. Furthermore, intracerebroventricular injection of PGE_2 in EP3R-knockout mice did not result in respiratory depression or apnea (8). Therefore, PGE_2 exerts its effects on the respiratory control via brainstem EP3R (17).

In the prenatal period, PGE_2 from the placenta inhibits fetal breathing movements, and PGE_2 released at birth may have acute neuroprotective effects during perinatal hypoxia (17). In our experimental setup, attenuation of mPGES-1 has no detrimental, but only beneficial, effects when neonatal animals are exposed to proinflammatory cytokines or severe hypoxia.

Therefore, decreasing immediate PGE_2 release in the initial phase of inflammation and acute hypoxia is favorable for the acute hypoxic respiratory response. The role of mPGES-1 and PGE_2 release during less severe hypoxic challenge and chronic inflammation, as well as the long-term effects of altering this neuro-immune pathway, remain to be investigated.

To our knowledge, the mechanism behind acute PGE_2 release during severe hypoxia has not been elucidated. Biosynthesis of PGE_2 occurs via three sequential steps in the COX pathway. Cytosolic phospholipase A2 catalyzes the release of arachidonic acid (25), which serves as a substrate for either of the two COX isoenzymes, COX-1 or COX-2. The COX metabolite PGH_2 is then isomerized to PGE_2 via microsomal PGE_2 (mPGES-1) and possibly via cytosolic PGE_2 synthase. The constitutive COX-1/cytosolic PGE_2 synthase pathway has been suggested to regulate the immediate and the inducible COX-2/mPGES-1 pathway to regulate the delayed PGE_2-biosynthetic responses (26). However, our data indicate that the COX-2/mPGES-1 pathway is directly involved in the immediate response to hypoxia via PGE_2 release.

Apnea, Respiratory Response to Hypoxia, and mPGES-1 Attenuation

Of note, attenuated mPGES-1 expression in mice is more protective following exposure to IL-1β and severe hypoxia than a complete absence of mPGES-1 expression, resulting in prolonged respiratory efforts and survival. This could have important treatment implications for neonatal apnea related to infections. Indomethacin has been used previously to treat apnea of prematurity, attenuating the effects of IL-1β on basal respiration by blocking prostaglandin synthesis (5). However, indomethacin, a nonselective inhibitor of both COX-1 and COX-2, causes multiple adverse effects in neonates (27). Therefore, treatment modalities selectively targeting mPGES-1 or COX-2 could possibly be more beneficial.

To date, management of premature infants with apnea often includes drugs such as methylxanthines (theophylline and caffeine). Caffeine acts on adenosine-A1 and adenosine A2 receptors (A1R and A2R) (28). The A1R, expressed on neuronal cells, has a direct effect on the rostral ventrolateral medulla respiratory-related neurons, central pattern generation, and breathing output (29,30). Therefore, part of the stabilizing effect that caffeine has on neonatal respiration is attributed to its capability to directly antagonize A1R-mediated depression of critical respiration-related neuronal activity. However, in addition to its direct A1R antagonizing effects, caffeine also modulates both innate and adaptive immune responses (31). Indeed, two of the main effects of caffeine treatment in very preterm infants are that it reduces the frequency of patent ductus arteriosus and the incidence of bronchopulmonary dysplasia, both related to prostaglandin and inflammation (32). Therefore, part of the beneficial effects of methylxanthine on apnea might be through its ability to reduce inflammation.

As an alternative to methylxanthines, mPGES-1 has been suggested as a novel therapeutic target (33). Since inhibition of PGE_2 formation by COX-2 inhibitors is effective in ameliorating symptoms of inflammation, specific COX-2 inhibition has

been proposed. However, the use of COX-2 inhibitors is complicated by potentially adverse cardiovascular effects related to COX-2 expression in vascular endothelial cells under normal conditions (33). Instead, specific mPGES-1 inhibition may be a plausible therapeutic target. Like COX-2, mPGES-1 is sparsely expressed during normal conditions, but it is inducible in many tissues after inflammation (33). However, specific mPGES-1 inhibition may have an advantage over COX-2 inhibitors because only hypoxia- or inflammation-induced PGE_2 will be targeted. This could ameliorate central respiratory control and possibly reduce hypoxic apneas as well as improve ability to autoresuscitate from hypoxic events. Moreover, the production of other prostaglandins that maybe advantageous in the resolution of inflammation (34) would be unaffected.

Prostaglandin synthesis inhibitors, which block endogenous prostaglandin production, increase breathing movements and central respiration during early postnatal life (12,14). During the perinatal period, endogenous PGE_2 has a tonic effect on respiratory rhythmogenesis and breathing (8). Moreover, during periods of vulnerability, newborns may be at risk of hypoventilation and/or apneas in response to pathophysiological events that increase central PGE_2 (1,12). Thus, should nonsteroidal anti-inflammatory drugs or more specific mPGES-1 inhibitors or EP3R antagonists be administered to all vulnerable infants in the neonatal intensive care units to potentially improve respiratory outcomes? At present, we can only speculate. Further investigation in animal models and human neonates is warranted.

This study provides evidence that inflammatory-mediated activation of the mPGES-1 pathway, as well as severe hypoxia, rapidly induces PGE_2 release in the vicinity of brainstem cardiorespiratory centers, thereby compromising the respiratory response to hypoxia and the ability to autoresuscitate.

METHODS

Subjects

Neonatal male and female inbred DBA/1lacJ mice (Jackson Laboratory, Bar Harbor, ME) were used ($n = 154$). The *mPGES-1* gene was selectively deleted in mPGES-1 heterozygote (mPGES-1$^{+/-}$) and knockout (mPGES-1$^{-/-}$) mice as described previously (35). Experiments were performed and analyzed before genotype was known. Data from some of the WT and knockout DBA/1lacJ mice were included in the characterization of respiratory behavior in neonatal DBA/1lacJ mice (6,8). All mice were reared by their mothers under standardized conditions with a 12h light:12h dark cycle. Food and water were provided *ad libitum*. The studies were performed in accordance with European Community Guidelines and approved by the regional ethic committee at the Swedish Board of Agriculture. The animals were reared and housed in the Department of Comparative Medicine, Karolinska Institutet.

Drugs

Recombinant mouse IL-1β (Nordic Biosite AB, Täby, Sweden) was reconstituted in sterile pyrogen-free 0.9% NaCl to provide a 1 μg/ml working solution and then stored at –80 °C until use. A PGE_2 stock solution (20×) was prepared in ethanol and sterile H_2O and stored at –20 °C. On the day of experimentation, the PGE_2 was diluted in artificial cerebrospinal fluid to 5 μg/l (9 μmol/l).

Unrestricted Whole-Body Flow Plethysmography in Mice

The whole-body plethysmography has been described previously (6,8). Briefly, a Plexiglas chamber (35 ml) was connected to a highly sensitive direct airflow sensor. The flow signal was amplified by a four-channel amplifier converted to digital signal and recorded at 100 Hz

by an online computer using DasyLab software. Ambient temperature within the chamber was measured and maintained at 30.1 ± 0.05 °C in accordance with the documented thermoneutral range for mice of similar age (36). The chamber was calibrated by repeatedly injecting standardized volumes with preset precision syringes (Hamilton, Bonaduz, Switzerland) (6).

Moderate Anoxia (5 min)
Each mouse received an intraperitoneal injection (0.01 ml/g) of IL-1β (10 µg/kg) or vehicle. Baseline skin temperature was recorded immediately before injection. At 70 min, skin temperature was remeasured, and the mouse was placed unrestrained into the plethysmograph chamber. Respiration was assessed during 4 min of normoxia (21% O_2) followed by a 1-min hyperoxic challenge (100% O_2). After a 5-min recovery period in normoxia, the chamber was flushed with 100% N_2 for 5 min, and the anoxic response was examined. Finally, 100% O_2 was administered for 8 min, and the ability to autoresuscitate was evaluated. Skin temperature was recorded at the end of each experiment.

Severe Anoxia (1 min After Last Gasp)
Each mouse was exposed to the same protocol as described above with the exception that anoxic exposure (100% N_2) continued until 1 min after the animal's last gasp.

Plethysmography After Intracerebroventricular Injection of PGE$_2$ or Vehicle
After administration of sevoflurane anesthesia for 60 s, neonatal mice were held so that the head was transilluminated with a fiber-optic light on a flexible stem to visualize the lateral ventricles. PGE$_2$ (4 nmol in 2–4 µl of NaCl, $n = 8$) or vehicle (NaCl, $n = 7$) was slowly injected into the lateral ventricle by using a thin pulled glass pipette attached to polyethylene tubing (8). The mouse was then placed immediately into the plethysmograph chamber. After a 10-min recovery period in normoxia, the mouse was exposed to hyperoxic and anoxic challenge as described above. Skin temperatures were measured at baseline time points through experimentation and after removal from the chamber by using a thermistor temperature probe. After experiments, animals were dissected at the injection site and examined for the possible presence of intracerebroventricular bleeding. In 14 of 15 mice no bleeding was detected and only 1 of 15 mice had minor but detectable bleeding.

Plethysmograph Data Analysis
Periods of calm respiration without movement artifact were selected for analysis based on visual observations during experimentation. f_R, V_T, and V_E during normoxia and hyperoxia as well as the anoxic response (i.e., hyperpnea, primary apnea, gasping, secondary apnea, and autoresuscitation) were analyzed as described previously (6). Survival was recorded for all animals. Apnea was defined as cessation of breathing for more than or equal to three respiratory cycles. In some animals regularity of breathing was quantified using the coefficient of variation (i.e., SD divided by mean of breath-by-breath interval during 60-s periods).

Statistics
Factorial ANOVA compared those parameters with normal distribution and equal variance, and multiple comparisons were accounted for using the Tukey–Kramer method. Wilcoxon χ^2-test was used for nonparametric measurements, continuous data with non-Gaussian distributions, and correlations between two numerical parameters. Logistic regression was used for categorical data, as well as for correlations between continuous and categorical data. The Spearman's ρ rank test determined correlations between two continuous variables. Data are presented as mean ± SEM. A value of $P < 0.05$ was considered statistically significant.

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