THE USE OF CAFFEINE in preterm infants continues to be an exciting and controversial area of neonatal medicine. Caffeine was first used to manage apnea of prematurity in 1977 (3), and commencement of caffeine therapy during the first 10 days of life in very low birth weight of preterm infants (weighing 500 to 1,250 g at birth) was associated with a reduction in the frequency of bronchopulmonary dysplasia (BPD) and a reduction in the duration of assisted mechanical ventilation (30). While there is general consensus that early caffeine administration is associated with a reduction in the rates of death or BPD and patent ductus arteriosus (21), some concerns remain regarding possible adverse effects, such as increased risk of necrotizing enterocolitis (34). Some investigators have called for further randomized clinical trials to examine early versus late therapy, before caffeine prophylaxis can be universally recommended for infants under 29 wk of gestational age (16).

Controversy around the use of caffeine in preterm infants is not restricted to the timing of caffeine therapy. Apart from the management of apnea, caffeine has also been suggested to influence postnatal lung maturation, in particular, to stimulate secondary septation and thereby drive lung alveolarization (32). Since preterm infants with “new” BPD exhibit pronounced alveolar simplification as a consequence of stunted secondary septation, the possibility of driving postnatal lung development using a pharmacological intervention that is already established in a neonatal intensive care setting is very exciting indeed. Whether or not caffeine is able to drive lung alveolarization or protect the developing lung from injurious insults remains a matter of debate (32).

BPD results from a combination of oxygen toxicity, inflammation, and mechanical stress during respiratory support of affected patients (19). These same injurious stimuli are employed to model BPD in experimental animals (2, 24), including mice (5, 25), rats (27), rabbits (9), pigs (4, 6), lambs (1), and nonhuman primates (39). At this junction, it is important to recognize the limitation of term animal, particularly rodent, BPD models. The primary utility of caffeine in a neonatal intensive care setting is to reduce apnea, which reduces the need for mechanical ventilation and oxygen supplementation by stimulating respiratory efforts. As there is no apnea in mouse pups exposed to hyperoxia, the impact of caffeine on this very important aspect of the clinical consequences of preterm birth is never addressed in animal models (2, 24), even though caffeine also increases the respiratory rate in mouse pups (28). The discussion that follows here is restricted exclusively to the more controversial question of whether as a side effect of the use of caffeine to manage apnea, Does caffeine administration also influence postnatal lung maturation, in particular, lung alveolarization?

Encouraging data evaluating caffeine as an intervention to drive lung alveolarization in animal models of BPD have been obtained. Studies reporting the use of caffeine and related methylxanthines as pharmacological interventions in experimental animal models of BPD are summarized in the scheme in Fig. 1. Among the reports from the “pro-caffeine lobby” are observations that caffeine administration to neonatal rats blunted the lung inflammation that was provoked by hyperoxia (38) and that caffeine administration to preterm rabbits limited the damaging effects of hyperoxia on lung alveolarization, ostensibly also by blunting hyperoxia-provoked inflammation (23). When intra-amniotic bacterial lipopolysaccharide (LPS) was used to mimic choorioamnionitis in pregnant rats, leading to arrested lung alveolarization in offspring, the administration of caffeine (20) or the related methylxanthine theophylline (17, 26), appreciably attenuated the blunted alveolarization, most likely by limiting inflammation. The utility of theophylline to attenuate arrested lung alveolarization in rats caused by hyperoxia exposure has also been demonstrated, also by limiting inflammation (40). Thus a body of evidence exists highlighting the utility of caffeine to protect or promote lung alveolarization in rat or rabbit models of BPD. However, two other reports in preclinical studies either do not support or actually discourage the idea that caffeine administration promotes lung alveolarization. One report has documented the deleterious impact of caffeine on lung alveolarization in a hyperoxia-based mouse model of BPD, where caffeine treatment worsened lung structure (10), while another study reported no impact at all of caffeine administration in the same model (28). Thus, considerable controversy currently exists about whether caffeine has a positive or negative effect, or indeed, no effect, on postnatal lung alveolarization.

A recent report (35) published in the American Journal of Physiology-Lung Cellular and Molecular Physiology provides more ammunition to the “pro-caffeine lobby.” The report of Teng and coworkers (35) identified endoplasmic reticulum (ER) stress (22) as a possible target of caffeine in a hyperoxia-based BPD model in rats. In their study, Teng and coworkers...
arrested alveolarization in Sprague-Dawley rat pups by exposure to 90% O\textsubscript{2} from postnatal day 1 (P1) to P10, with concomitant daily administration of caffeine (20 mg/kg ip) from P2. Changes in lung structure were assessed by morphometry, where caffeine administration decreased the mean linear intercept and increased both the number of secondary septa per high-power field and the radial alveolar count. Additionally, lung vascularization was improved, as assessed by the number of rat endothelial cell antigen-stained vessels per high-power field.

Teng and coworkers (35) noted that hyperoxia exposure caused increased expression of binding immunoglobulin protein (BiP) in the lungs of affected rat pups. As BiP is a master regulator of ER stress and induction of BiP is regarded as a sign of increased ER stress, this led Teng and coworkers to conclude that hyperoxia drove ER stress. Treatment of newborn rat pups with caffeine concomitant with hyperoxia exposure prevented the hyperoxia-driven expression of BiP. Additionally, the authors provided convincing data that hyperoxia drove the activation of the transducers protein kinase R-like endoplasmic reticulum kinase (PERK; also called eukaryotic translation initiation factor 2-alpha kinase 3) and inositol-requiring enzyme 1-alpha by phosphorylation and drove activation of activating transcription factor-6 by proteolytic cleavage. These data suggested engagement of the unfolded protein response (UPR). Consistent with the activation of UPR transducers, the expression of two UPR effector molecules, C/EBP homologous protein (CHOP) and X-box binding protein (XBP1), was increased, although immunoblot data supplied to support changes in uXBP1 expression were not convincing, and there is no indication that an outlier test was applied to the densitometry data. The activation of UPR transducers and increased expression of CHOP and XBP1 in response to hyperoxia exposure were attenuated by caffeine treatment concomitant with hyperoxia exposure. In addition to activation of the UPR by hyperoxia, Teng and coworkers also claimed that hyperoxia drove increased COX-2 expression and increased apoptosis (assessed from increased caspase-12 expression) and that both increased COX-2 expression and increased apoptosis were attenuated by caffeine treatment; however, the COX-2 and cleaved caspase-12 immunoblots are difficult to reconcile with the densitometry data provided.

The report of Teng and coworkers (35) is interesting, given the observations that both COX-2 and the ER stress/UPR pathways are activated in the lungs of preterm infants with BPD (7). In pioneering work from the Bhandari laboratory (7), COX-2 inhibition, using celecoxib, as well as genetic interference with CHOP expression, attenuated the deleterious impact of hyperoxia on postnatal lung alveolarization in C57BL/6J mice. These studies validated a causal role for both COX-2 and CHOP-mediated pathways in hyperoxia-induced arrest of postnatal lung alveolarization. Building on these studies, with the use of a conditional knockout of the ER chaperone 78-kDa glucose-regulated protein (GRP78) in lung epithelial cells, the UPR has been proposed as a potential therapeutic target in BPD (15).

The study of Teng and coworkers (35) is limited in that the study reported two separate sets of observations in parallel but did not functionally connect the two arms of the study. First, the report of Teng and coworkers confirmed the observations of the Bhandari laboratory that COX-2 expression and ER stress and the UPR are engaged during hyperoxia-induced arrest of lung alveolarization. In parallel, Teng and coworkers also confirmed the observations of Zhu and coworkers (40), who documented the ability of methylxanthine derivatives to attenuate the impact of hyperoxia on lung alveolarization in
rats. However, no direct functional link was made between hyperoxia-induced arrest of alveolarization and the ability of caffeine to target ER stress and the UPR to attenuate this impact of hyperoxia on alveolarization. To this end, the use of a genetic model, chemical inhibitors, or chemical chaperones such as tauroursodeoxycholic acid (31), in combination with hyperoxia and caffeine administration, would strengthen the suggestions that ER stress and UPR are targeted by caffeine to attenuate the impact of hyperoxia on lung alveolarization.

To the latter point, a vast number of effects of caffeine on the lung have been described, both in vivo and in vitro. In vivo, caffeine has been documented to drive alveolar epithelial cell apoptosis (10) and to modulate transforming growth factor-β (TGF-β) signaling (28) in animals models of BPD. The effects of caffeine on limiting inflammation and other physiological processes in adult animals in response to proinflammatory stimuli such as hyperoxia (18), LPS application (29), and ischemia-reperfusion injury (8) have also been described. Further information has been gleaned from in vitro studies, where in epithelial cells, caffeine promotes cell-cycle arrest (14, 36), drives surfactant protein production (11–13), promotes apoptosis (37), and modulates TGF-β signaling (14, 28). In lung fibroblasts and fibroblast-like cell lines, caffeine impacts peroxynitrite-mediated matrix metalloproteinase production (33), modulates glucocorticoid effects in the lungs (12), and modulates TGF-β signaling (12, 28). Thus there are many avenues described in lung cells by which caffeine may function to protect lung development from the damaging effects of hyperoxia (summarized in Fig. 2). It remains for Teng and coworkers to demonstrate that the protective effects of caffeine administration in rats is due to caffeine directly targeting ER stress and the UPR and not via any of the other caffeine-driven physiological effects highlighted above.

The report of Teng and coworkers (35), which employed rats as an animal model, raised another interesting issue. A screen of the literature revealed that all of the beneficial effects of caffeine that have been noted in rodent models of BPD have been made in rats, which also holds true for studies in nonhuman primates and rabbits (Fig. 1, blue routes). In contrast, all caffeine administration studies undertaken in mouse models of BPD have failed to demonstrate any protective effects of caffeine (Fig. 1, red routes): in one study, caffeine worsened the impact of hyperoxia on lung alveolarization in FVB/N mice (10), whereas in another study, caffeine did not influence the impact of hyperoxia on lung alveolarization in C57BL/6J mice (28). The study of Teng and coworkers in rats was able to document a protective effect of caffeine (by an as-yet-undetermined mechanism) on hyperoxia-induced arrest of lung alveolarization. This protective effect of caffeine and other methylxanthine derivatives has been noted in a variety of rat BPD models (17, 20, 23, 26, 38, 40). Taken together, these reports beg the question: Is there a fundamental difference in the responsiveness of rats versus mice to caffeine, which is relevant to lung alveolarization? These discordant effects comparing rats to mice might, for example, be attributable to dosing (which is an important consideration in the clinical use of caffeine to manage preterm infants) or to differential expression of caffeine target molecules (such as adenosine receptors and phosphodiesterases) in rats versus mice.

In sum, it is clear that the controversy surrounding the utility of caffeine to manage preterm infants is not restricted to concerns about the timing of the initiation of therapy in the neonatal intensive care unit. In a preclinical setting, many open questions remain concerning whether or not—and how—caffeine may influence postnatal lung maturation in experimental animal models of BPD. Among the key questions still to be answered are as follows. What are the in vivo targets and pathways that caffeine modulates to influence aberrant lung...
development in animal models of BPD? Furthermore, what underlies the discordant effects of caffeine on lung alveolarization in rat versus mouse models of BPD?

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