Prostaglandins and Other Lipid Mediators xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Prostaglandins and Other Lipid Mediators

journal homepage: www.elsevier.com/locate/prostaglandins

Original Research Article

Intermittent hypoxia alters dose dependent caffeine effects on renal prostanoids and receptors in neonatal rats

Kay D. Beharry[a,b,c,*], Charles L. Cai[a], Kultida Soontarapornchai[a], Taimur Ahmad[a], Gloria B. Valencia[a], Jacob V. Aranda[a,b,c]

[a] Department of Pediatrics, Division of Neonatal-Perinatal Medicine, State University of New York, Downstate Medical Center, Brooklyn, NY, USA
[b] Department of Ophthalmology, State University of New York, Downstate Medical Center, Brooklyn, NY, USA
[c] SUNY Eye Institute, New York, NY, USA

ARTICLE INFO

Keywords:
Aquaporins
Caffeine
Kidneys
Neonatal intermittent hypoxia
Prostanoids
Prostanoid receptors

ABSTRACT

Caffeine, one of the most commonly prescribed drugs in preterm neonates, is given in standard or supra-pharmacologic doses. Although known as a diuretic, its effects in the neonatal kidneys are not well studied. We tested the hypothesis that neonatal intermittent hypoxia (IH) and high caffeine doses (HCD) alter renal regulators of vasomotor tone and water balance. Newborn rats were randomized to room air, hyperoxia, or IH and treated with standard or high caffeine doses; or placebo saline. Renal prostanoids; histopathology; and cyclooxygenase (COX), prostanoid receptor, and aquaporin (AQP) immunoreactivity were determined. HCD in IH caused severe pathological changes in the glomeruli and proximal tubules, consistent with acute kidney injury. This was associated with reductions in anthropometric growth, PGI_2, and IP, DP, and AQP-4 immunoreactivity, well as a robust increase in COX-2, suggesting that the use of HCD should be avoided in preterm infants who experience frequent IH episodes.

1. Introduction

Caffeine citrate was first demonstrated to decrease apnea episodes in premature neonates in the 1970s by Aranda et al. [1]. Since then, caffeine has been shown to reduce the duration of mechanical ventilation and oxygen therapy [2], the incidence of bronchopulmonary dysplasia (BPD), long-term neurodevelopmental impairment, and risk of severe ROP [3,4]. Due to its significant impact on major acute neonatal morbidities and long term outcomes, caffeine is now one of the most commonly prescribed drugs for use in extremely low gestational age neonates (ELGANs) [5–7]. The mechanisms underlying the benefits of caffeine may be due to its anti-oxidant, anti-inflammatory, and anti-apoptotic properties [8]. ELGANs experience numerous episodes of intermittent hypoxia (IH) during supplemental oxygen therapy in the first few weeks of life with fluctuations in PaO_2 [9]. Hyperoxia and IH activate the formation of reactive oxygen species (ROS). Due to poorly developed, immature antioxidant systems, ELGANs are less capable of scavenging ROS, the accumulation of which can result in oxidative stress, DNA damage and cell death [10] leading to “oxygen free radical diseases of the newborn” [8,11–17]. Caffeine has been shown to have antioxidant properties by scavenging ROS, preventing lipid peroxidation and reducing oxidative DNA damage [18–20].

Caffeine produces diuresis. Its elimination is much slower in ELGANs than term neonates due to immature hepatic cytochrome P450 and renal functions [21,22]. Caffeine exerts its diuretic effects through increasing creatinine clearance, and glomerular filtration rate. In neonates, urinary flow rate, water output/input ratio, and creatinine clearance increased significantly after the administration of caffeine [23]. In adults, caffeine decreased renal vascular flow and renal vascular flow response to exogenous angiotensin II [24]. In animals, caffeine exacerbated renal failure in obese diabetic rats [25]. These effects of caffeine on urinary and vascular flow may be due to alterations in renal prostanoids [26].

The prostanoid system is vital to normal renal development and contributes to changes during the transition from fetal to neonatal life [27,28]. In cases where the prostanoid synthesis is impaired, major renal abnormalities and function may occur [29]. The kidney is an important organ for prostanoid excretion. It is responsible for removing 50–80% of the body's prostanoids by the urine [30]. Prostanoids are key modulators and mediators of physiological as well as pathological renal conditions. In the kidneys, five primary prostanoids are metabolized via cyclooxygenase (COX), namely prostaglandin E_2 (PGE_2), PGF_{2a},

* Corresponding author at: Department of Pediatrics & Ophthalmology, Director, Neonatal-Perinatal Medicine Clinical & Translational Research Labs, Dept. Pediatrics/Div. Neonatal-Perinatal Medicine, State University of New York, Downstate Medical Center, 450 Clarkson Avenue, Box 49, Room BSB 4-23, Brooklyn, NY, 11203, USA.
E-mail address: kbeharry@downstate.edu (K.D. Beharry).

http://dx.doi.org/10.1016/j.prostaglandins.2017.10.004
Received 4 April 2017; Received in revised form 2 October 2017; Accepted 18 October 2017

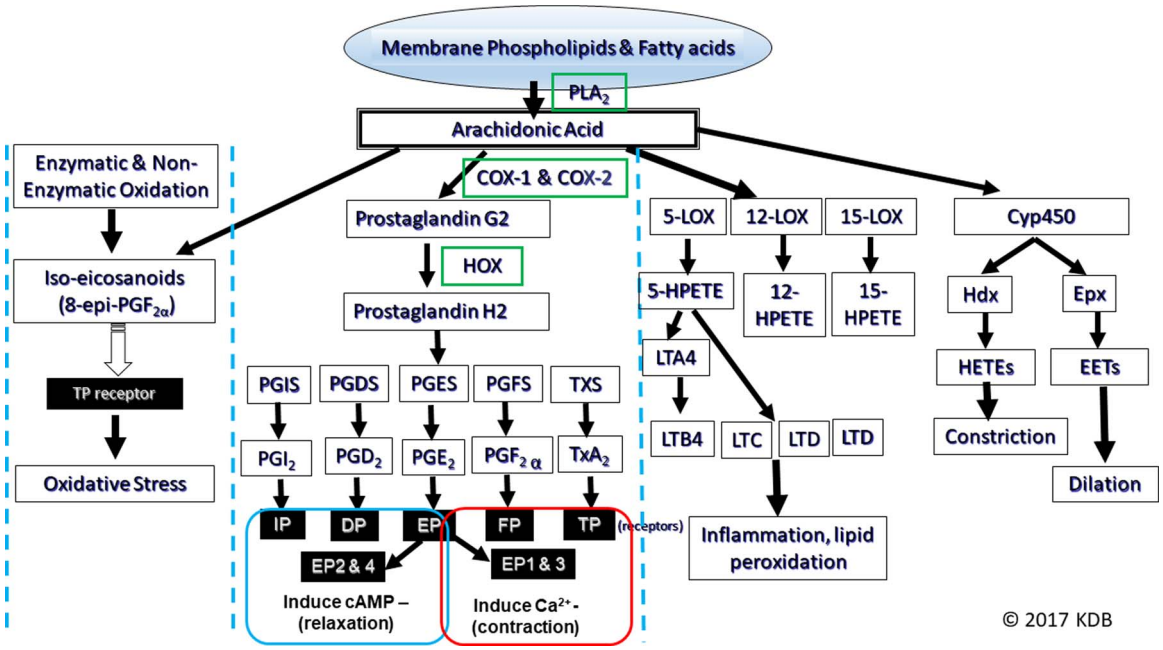

Fig. 1. Arachidonic acid (AA), abundant in cell membranes phospholipids, is the precursor eicosanoids, including prostaglandins and thromboxane (collectively known as prostanoids), and leukotrienes. In response to a wide variety of stimuli, phospholipase A2 is activated and hydrolyzes the phospholipids of the cell membranes to release AA into the cytoplasm. Once released, free AA is converted to prostaglandin G2 (PGG_2) which is then converted by a peroxidase reaction to the unstable prostaglandin endoperoxide (PGH_2) by cytosolic prostaglandin G/H synthase which has cyclooxygenase (COX) and hydroperoxidase (HOX) activities. PGH_2 serves as a substrate for isomerases and synthases to produce prostanoids. AA is also acted upon by lipoxygenases (LOX), to form leukotrienes and lipoxins which are highly involved in inflammation; and epoxygenases to form epoxyeicosatrienoic acids (cytochrome P450 metabolites). A parallel family of free radical catalyzed isomers, the isoeicosanoids are formed by nonenzymatic peroxidation of AA and are biomarkers of oxidative stress.

prostacyclin (PGI_2), PGD_2, and thromboxane A_2 (TxA_2) which mediate their action via specific receptors (Fig. 1). PGE_2, PGI_2 and PGD_2 are potent vasodilators while $PGF_{2\alpha}$ and TxA_2 are vasoconstrictors. PGE_2 has dual opposing effects which are exerted via its actions on EP receptors, EP1-EP4, to promote renal perfusion, glomerular filtration rate, and water and electrolyte balance [31]. EP1 stimulates calcium to promote constriction, regulates blood pressure, and contributes to the natriuretic and diuretic effects of PGE_2 [32]. EP2 and EP4 activates adenylate cyclase and increase cAMP to promote dilation and increase water reabsorption whereas EP3 increases water excretion [33,34]. PGF_{2a}, is the most abundant prostanoid detected in the urine and mediates its actions via FP receptors [35]. It is predominant in the distal convoluted tubules and cortical collecting duct and promotes natriuretic and diuretic effects [36]. PGI_2 is a platelet de-aggregating factor and renin agonist, and is the most abundant and potent prostanoid in the cortex. It mediates its action on reduction of blood pressure via the IP receptor [37]. The role of PGD_2 in the kidneys is not clearly defined, but studies have shown that PGD_2 mediates increased renal artery flow, urine output, creatinine clearance, and sodium and potassium excretion via the DP receptor [33,38]. The vasoconstrictor TxA_2 is a potent platelet aggregating factor that constricts glomerular capillaries, decreases renal blood flow and decreases glomerular filtration rate via its actions on the TP receptor. Generally TxA_2 levels are low in the kidneys, but are increased during hypertension [39].

Aquaporins (AQPs) are important regulators of water balance and excretion of concentrated urine, particularly, AQP-1 and -2 are important for maintaining water-electrolyte and acid-base balance [40]. AQP-4 is restricted to the distal regions of the renal tubules and is critically involved in the regulation of water balance of the body [41]. Since caffeine is a diuretic, it was important to understand the effects of caffeine exposure on AQPs in the neonatal kidneys. Chronic neonatal IH has detrimental effects on the kidneys [42], which may be exacerbated with caffeine treatment. One clinical trial supported the need for higher concentrations of caffeine to facilitate extubation [2]. However, the effects of high caffeine doses coincident with neonatal IH and oxidative stress on renal prostanoids and their receptor expression have not been addressed. The current study examined the hypothesis that the combination of neonatal IH and high caffeine doses substantially alters renal regulators of vasomotor tone and water balance. To prove our hypothesis, we exposed neonatal rats from birth (P0) to P14 to hyperoxia or neonatal IH, during which they received standard or pharmacologic caffeine doses. Control animals were raised in room air (RA) and/or treated with sterile normal saline.

2. Materials & methods

2.1. Experimental design

All experiments were approved by the State University of New York, Downstate Medical Center Animal Care and Use Committee. Animals were managed according to the guidelines outlined by the United States Department of Agriculture and the Guide for the Care and Use of Laboratory Animals. Certified infection-free pregnant Sprague Dawley rats were purchased from Charles River Laboratories (Wilmington, MA, USA) at 18 days gestation, and remained in a standard environment with food and water provided ad libitum. A total of 54 Sprague Dawley rat pups were randomly assigned to three oxygen groups: 1) room air (RA); 2) hyperoxia (50%O_2); and 3) neonatal intermittent hypoxia, IH (50% O_2 with brief 1-min episodes of 12% O_2,) within 2 h of life (P0)–P14. Within each oxygen environment, pups were randomized to receive either: 1) standard caffeine doses (SCD) of 20 mg/kg IP loading on P0 followed by maintenance doses of 5 mg/kg/day from P1 to P14; 2) high caffeine doses (HCD) of 80 mg/kg loading on P0 and maintenance doses of 20 mg/kg/day from P1-P14; or 3) equivalent volume saline (Sal) on P0–P14. The pups were weighed on P0, P7, and P14 for caffeine dose adjustment.

2.2. Neonatal intermittent hypoxia profile

Pups randomized to hyperoxia or neonatal IH and their mothers were placed into specialized O_2 chambers (Biospherix Ltd., Lacona, NY) connected to an oxycycler. The O_2 content inside the chambers was