Relative Contribution of Nuclear and Membrane Progesterone Receptors in Respiratory Control

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Abstract

Progesterone is a steroid hormone whose physiological effects can affect various systems, including reproductive, immune and cardiorespiratory systems. In fact, there are growing evidences proving that progesterone is potent respiratory stimulant with therapeutic value for sleep-disordered breathing. However there is no clear understanding of how progesterone mediates its stimulant respiratory effects and alleviates apnea. Mechanistically, it was demonstrated that this hormone elicits some of its respiratory effect via the classical mechanism of the nuclear progesterone receptor (nPR), a transcription factor belonging to the super family of steroid hormone receptors. Moreover, experimental results indicate that activation of alternative non-genomic (i.e. non-nuclear) signaling pathways such as the membrane progesterone receptors (mPR) could have a key role in the regulation of the respiratory control system. We provide preliminary results suggesting an important role of mPRβ on respiratory control and ventilatory response to hypoxia in adult female mice.

Keywords

Breathing disorders • Hypoxic ventilatory response • Sex-steroids • Hormonal treatment

30.1 Introduction

Breathing disorders such as obstructive sleep apnea, sudden infant death, and Rett syndromes show several sex differences in their prevalence, indicating that sex is primordial determinant of respiratory health and lending weight to the link of gonadal steroid hormones in respiratory

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control (Kapsimalis and Kryger 2002; Chahrour and Zoghbi 2007). Progesterone is a steroid synthetized primarily in the gonads, the adrenal glands and in the placenta, but progesterone is also synthetized de novo in the central and peripheral nervous system, and can thus be classified as a neurosteroid (Birzniece et al. 2006). Progesterone is well- known as a powerful respiratory stimulant with a potential therapeutic value for the treatment of apnea in adults (Shahar et al. 2003), and it has been suggested that it could also be used for the treatment of apnea in preterm neonates (Finer et al. 2006). However, progesterone is not clinically approved for respiratory disordered breathing, because experimental data are still scarce, and there is no clear mechanical studies explaining how progesterone influences the control of breathing and alleviates apnea. Here, we will briefly focus on progesterone receptors, to highlight that we lack critical knowledge on their relative contributions in the regulation of the respiratory control system. However, our preliminary results clearly indicate that these receptors could play an important role in regulation of breathing.

30.2 Respiratory Effects of Progesterone

Progesterone is a potent respiratory stimulant, (Dempsey et al. 1986). Medroxyprogesterone, an analogue of progesterone, increases minute ventilation and responsiveness to hypercapnia or hypoxia (Skatrud et al. 1978; Zwillich et al. 1978). In addition, menopause is a risk factor for sleep apnea, and hormone replacement with progesterone reduces sleep disordered breathing in post-menopausal women (Shahar et al. 2003). Furthermore, progesterone reduces the occurrence of apnea in newborn and adult rats (Yamazaki et al. 2005; Lefter et al. 2007). Progesterone acts at different levels of the respiratory control system, including areas in central nervous system (Bayliss et al. 1987) and in peripheral chemoreceptors (Hannhart et al. 1990; Joseph et al. 2012). The direct application of progesterone on the dorsal surface of the medulla, at the level of the Nucleus Tractus Solitarius (NTS), increases phrenic nerve activity (Bayliss et al. 1987), and the effects of progesterone on respiratory activity requires intact hypothalamic structures (Bayliss et al. 1990).

30.3 Molecular Mechanisms by Which Progesterone Stimulates Breathing

Progesterone exerts it biological effects by genomic and non-genomic mechanisms (Fig. 30.1). We recently reported that progesterone uses non-genomic mechanisms to increase VO_2 and VCO_2 (Marcouiller et al. 2014), most likely through the truncated form of the nuclear progesterone receptor (nPR) located on the external membrane of the mitochondria (Dai et al. 2013). Moreover, allopregnanolone, a neuroactive metabolite of progesterone, affects breathing in newborn rats and arterial baroreflex responses in adults (Ren and Greer 2006; Heesch 2011). Despite the multiple potential pathways by which progesterone acts, little is known about the role of these pathways in regulating respiration.

30.3.1 Genomic Mechanisms

The nuclear progesterone receptor (nPR) belongs to the superfamily of the steroid hormone receptors. In the classical model of nPR activation, progesterone, a lipophilic molecule, diffuses through the cellular and nuclear membranes to bind to a nPR. When bound by progesterone, nPR undergoes conformational changes, dissociates from chaperone proteins, dimerizes, and directly interacts with specific response elements in the promoter regions of target genes through its DNA-binding domain (Mani 2006). The nPR gene (located on chromosome 11 in humans) encodes a single mRNA that, through alternative splicing, generates two major PR isoforms (nPR-A and nPR-B). These isoforms regulate

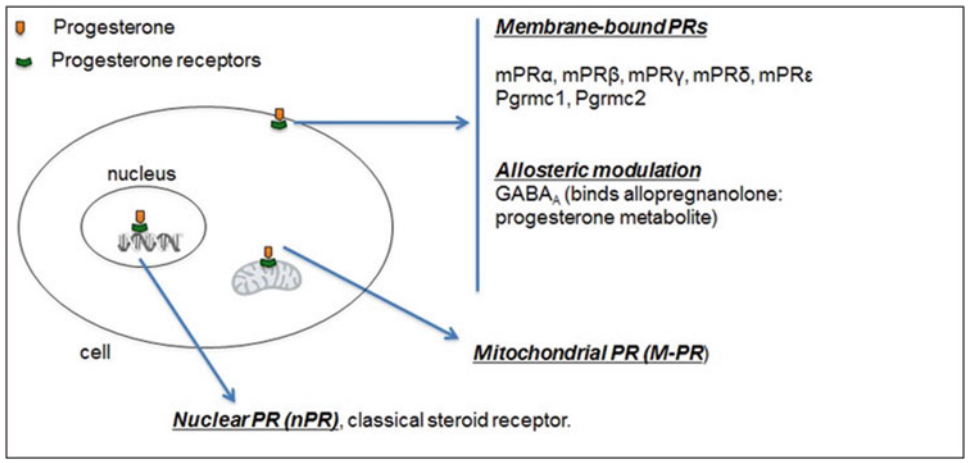

Fig. 30.1 Potential mechanisms of action by which progesterone might produce its respiratory effects. Progesterone, either from systemic circulation or produced locally in the neuronal system, can bind and signal throughout the classical nuclear receptor, membrane progesterone receptor (mPRα to mPRξ), and the Progesterone receptor membrane component (Pgrmc) 1 and 2. In addition, allopregnanolone, a metabolite of progesterone, can bind GABA$_A$ receptor

the expression of different target genes and, therefore, different functions in cells (Mani 2006; Camacho-Arroyo et al. 2007; González-Flores et al. 2011). Several other isoforms have been identified. Because some of these isoforms have a defective DNA-binding domain and lack the nuclear localization signal, they are expected to be localized in the cytosol or on cell membranes, rather than in the nucleus, and activate the mitogen-activated protein kinase (MAPK) signaling cascade (Maller 2003; Brinton et al. 2008).

In the central nervous system, nPR is localized in the preoptic, paraventricular, ventromedial, dorsomedial, and arcuate hypothalamic nuclei and more importantly, in brainstem areas involved in respiratory control, such as the NTS (the major site of peripheral chemoreceptors integration in the brainstem), the motor nuclei of the Xth and XIIth cranial nerves, and the locus coeruleus (Romeo et al. 2005; Helena et al. 2006; Brinton et al. 2008). In the peripheral nervous system, nPR immunostaining is localized in cells in the carotid bodies of adults, newborn and fetal rats (Joseph et al. 2006). Thus, localization of nPR in these areas of the peripheral and central nervous system are highly suggestive that nPR are involved in respiratory control. Indeed, intraperitoneal injection of progesterone decreases the frequency of apneic episodes recorded during sleep (identified by behavioral criteria) by 50 % in 14-week-old male rats and by almost 80 % in 26- week-old rats. This effect is abolished when mifepristone, an nPR antagonist, is injected 1.5 h before progesterone injection (Yamazaki et al. 2005). Moreover, in anesthetized cats, i.v. progesterone administration enhances phrenic nerve activity, this effect is not elicited by other steroids, and is blocked by pre-treatment with mifepristone suggesting that it is mediated by nPR. Progesterone also enhances the peripheral chemoreceptor response to hypoxia (Hannhart et al. 1990) and enhances ventilatory activity by decreasing the synthesis of dopamine, an inhibitory neurotransmitter in peripheral chemoreceptors (Joseph et al. 2002); nPR-A signaling regulates the expression of tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis (González-Flores et al. 2011). Our recent data suggest that nPR is an important modulator of respiratory control during sleep and in chemoreflex sensitivity. Specifically, adult female mice in which nPR is deleted (PRKO mice) have a

higher frequency of sighs and post-sigh apneas during non-REM sleep and reduced responses to hypercapnia after chronic treatment with administration of progesterone (Marcouiller et al. 2014)

30.3.2 Non-genomic Mechanisms

Activation of the nuclear progesterone receptor is expected to induce measurable physiological responses coinciding with *de novo* protein synthesis (within 30–45 min). However, steroid-induced effects may occur rapidly (within 2–3 min); these effects are likely initiated by cell surface receptors (Revelli et al. 1998). Based on these data, the search for membrane progesterone receptors (mPR) has been of great interest, culminating in the discovery of a gene and a corresponding protein with the characteristics of a fully functional mPR in sea trout ovaries (Zhu et al. 2003b). Structural and phylogenetic analyses have revealed the presence of similar genes in other species, including humans and mice (Zhu et al. 2003a), and three distinct mPR genes (mPRα, mPRβ, and mPRγ) have been originally identified. The family of mPR include five different members (mPRα, mPRβ, mPRγ, mPRδ and mPRξ) (Singh et al. 2013), which belong to the progestin and adipoQ receptor (PAQR) family. mPR proteins are located in the plasma membrane and have seven transmembrane domains with extracellular and intracellular terminals that respectively confer selective progesterone binding and activation of intracellular Gi proteins. When bound by progesterone, mPRα inhibits cAMP production to activate MAPK, which in turns activates the extracellular signal-regulated kinase (Erk1 and Erk2) signaling pathway. mPRα is predominantly expressed in reproductive tissues, mPRβ in the brain, and mPRγ in the kidneys (Zhu et al. 2003b). In mice, mPRα and mPRβ proteins and mRNA are expressed in the spinal cord with a distinct staining pattern that likely underlies the important trophic and protective effects of progesterone at this level (Labombarda et al. 2010). In rats, the mRNAs for mPRα and mPRβ are expressed in the cortex and thalamic nuclei (Intlekofer and Petersen 2011).

The pioneer study conducted by Pascual et al. (2002) showed that progesterone is able to restore the decreased transmission of afferent signals in the NTS during hypoxia, which could explain the stimulatory effect of progesterone in response to this stimulus. Because these effects required between 2 and 3 min to occur, it was suggested that a non-genomic mechanism of action was involved (Pascual et al. 2002). Based on these preliminary data, we sought to determine the expression of mPRβ and mPRα in the brainstem of adult female mice by immunohistochemistry (see (Labombarda et al. 2010), and found staining in the NTS, and the motor nuclei of the Xth and XIIth cranial nerves (Fig. 30.2). We have tested the functional role of mPRβ in adult female mice that were treated for 14 days with an intracerebro-ventricular infusion of a specific siRNA against mPRβ (0.04 mg/day; Stealth Select RNAi™, Life Technologies, Burlington, ON, Canada) in the IVth ventricle, to abolish its expression in central areas of respiratory control. Preliminary results indicate that the deletion of mPRβ induces a depression of the ventilatory response to hypoxia as shown in Fig. 30.3. Interestingly, while hypoxic exposure induced a rapid increase of respiratory frequency in control mice, we observed a mean delay of 4.5 ± 1.1 min before any observable response occurred in mice treated with the siRNA against mPRβ. The efficiency of the knock-down of mPRβ has been verified by immunohistochemistry, showing a wide-spread absence of staining in the brainstem (not shown). These preliminary results support a key role for mPRβ, in respiratory control in adult mice.

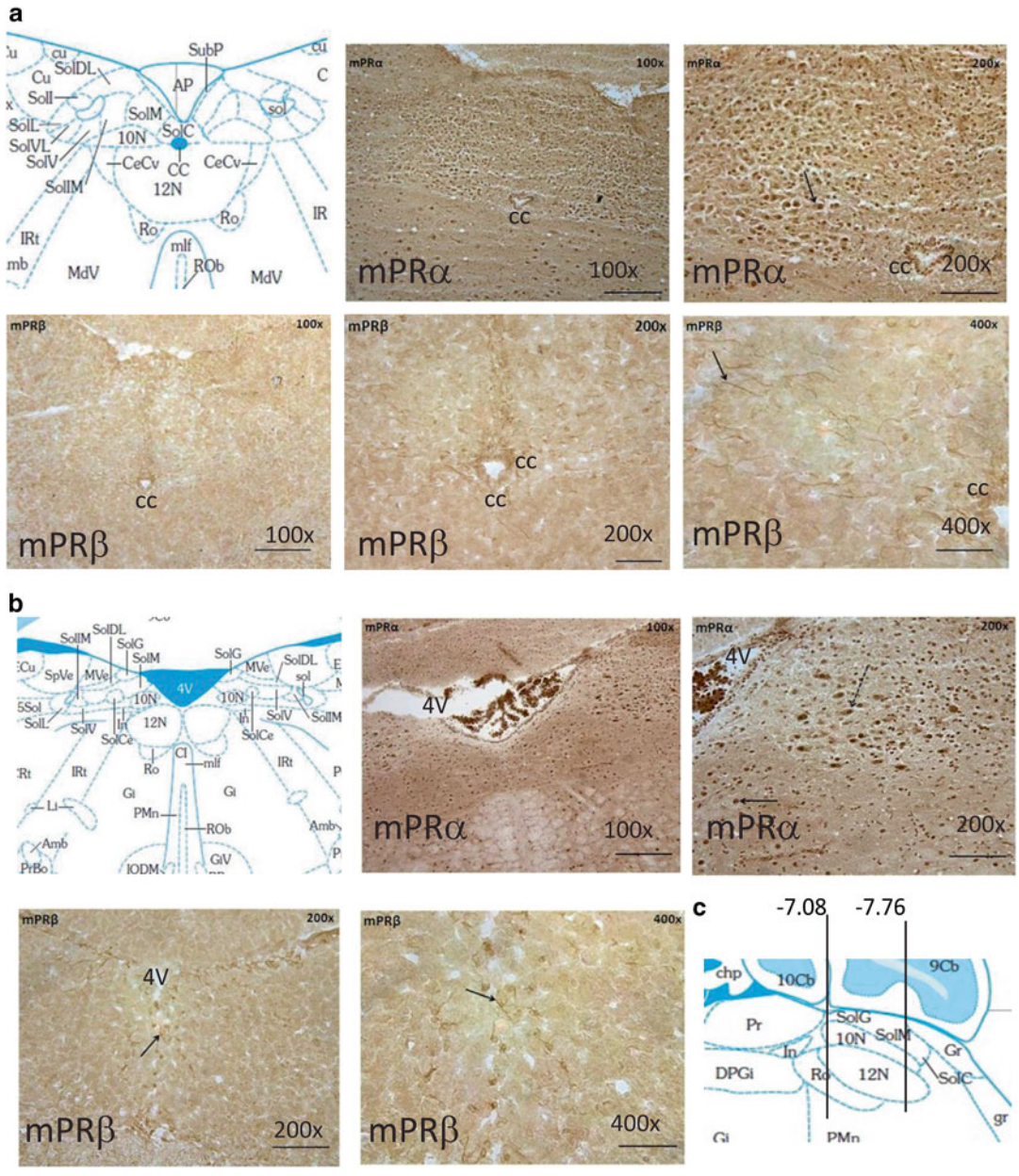

Fig. 30.2 Immunohistochemistry for mPRα and mPRβ in the dorsal region of the adult mouse brainstem. (**a**) Neuroanatomical drawing at bregma – 7.76 mm (from Franklin and Paxinos) and corresponding staining at this level (100, 200, or 400×). (**b**) Neuroanatomical drawing at bregma – 7.08 mm and corresponding staining (100, 200, or 400×). Note the staining for mPRα in cell bodies on the NTS (SolM, SolC) and XIIthMN (12 N) at both bregma levels, and for mPRβ on neurites in the NTS. (**c**) Sagittal view (lateral 0.12 mm) of the brainstem; coronal planes at bregma −7.76 and −7.08 mm. CC: central canal, SolM, SolC, SolG, SolDL, SollM, SolV,… subdivision of the NTS. 12 N: hypoglos-sal nucleus, 10 N: vagal nucleus. Scale bar at 100× = 100 μm; at 200× = 50 μm

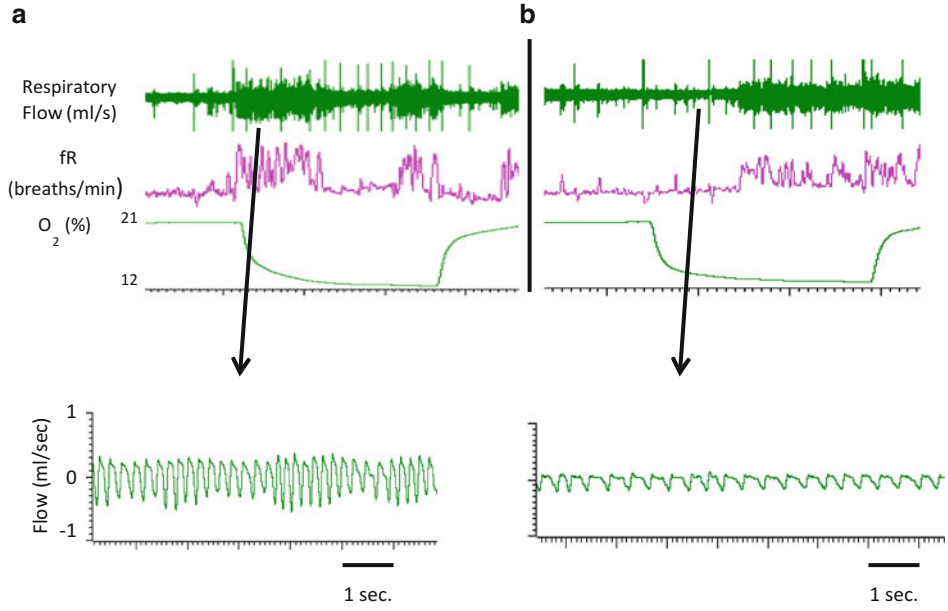

Fig. 30.3 Example of respiratory recordings (Flow – ml/s, and frequency: fR – breaths/min) under normoxia and in response to hypoxia in control adult female mice (**a**) and after infusion of a specific siRNA against mPRβ (**b**). Note the delay of the response in the treated mice. The lower traces show for each animal a typical breathing pattern during the onset of hypoxic exposure (time scale: 1 s)

30.4 Conclusion

With growing awareness of the potent respiratory effects of progesterone, further experiments are required to elucidate its mechanisms of action with the perspective to develop novel pharmacological approaches for the treatment of respiratory disordered due to unstable respiratory control system. Specific knockdown of PR in-¯vivo with siRNA infusion, or knock-out models appear as promising tools to elucidate the relative contributions of the different members from the large PR family.

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