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DISTRIBUTION OF MEMBRANE PROGESTERONE RECEPTOR ALPHA IN THE MALE MOUSE AND RAT BRAIN AND ITS REGULATION AFTER TRAUMATIC BRAIN INJURY

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Abstract—Progesterone has been shown to exert pleiotropic actions in the brain of both male and females. In particular, after traumatic brain injury (TBI), progesterone has important neuroprotective effects. In addition to intracellular progesterone receptors, membrane receptors of the hormone such as membrane progesterone receptor (mPR) may also be involved in neuroprotection. Three mPR subtypes (mPRα, mPRβ, and mPRγ) have been described and mPRα is best characterized pharmacologically. In the present study we investigated the distribution, cellular localization and the regulation of mPRα in male mouse and rat brain. We showed by reverse transcription-PCR that mPRα is expressed at similar levels in the male and female mouse brain suggesting that its expression may not be influenced by steroid levels. Treatment of males by estradiol or progesterone did not modify the level of expression of mPRα as shown by Western blot analysis. *In situ* hybridization and immunohistochemistry analysis showed a wide expression of mPRα in particular in the olfactory bulb, striatum, cortex, thalamus, hypothalamus, septum, hippocampus and cerebellum. Double immunofluorescence and confocal microscopy analysis showed that mPRα is expressed by neurons but not by oligodendrocytes and astrocytes. In the rat brain, the distribution of mPRα was similar to that observed in mouse brain; and after TBI, mPRα expression was induced in oligodendrocytes, astrocytes and reactive microglia. The wide neuroanatomical distribution of mPRα suggests that this receptor may play a role beyond neuroendocrine and reproductive functions. However, in the absence of injury its role might be restricted to neurons. The induction of mPRα after TBI in microglia, astrocytes and oligodendrocytes, points to a potential role in mediating the modulatory effects of progesterone in inflammation, ion and water homeostasis and myelin repair in the injured brain.

Key words: membrane progesterone receptor (mPR), neurons, astrocytes, oligodendrocytes, microglia, traumatic brain injury (TBI).

INTRODUCTION

Progesterone has a wide spectrum of actions in the nervous system, although it has generally been thought of as a hormone that influences female reproductive behavior and physiology (Pfaff et al., 1994). Progesterone also modulates various physiological and behavioral functions in both males and females and influences gene sets that modulate social, sexual, and anxiety-related behaviors (Witt et al., 1995; Phelps et al., 1998; Gomez et al., 2002; Schneider et al., 2003; Mong and Pfaff, 2004; Auger and Vanzo, 2006). In addition, progesterone also provides protective and trophic effects (Schumacher et al., 2007; Gibson et al., 2008; Stein, 2008; De Nicola et al., 2009). In the brain, the hormone can modulate synaptogenesis (McEwen and Woolley, 1994; Sakamoto et al., 2001) and stimulate myelination (Ghoumari et al., 2003, 2005; Hussain et al., 2011). The neuroprotective effects of progesterone have been demonstrated in experimental models of neurodegeneration (Vongher and Frye, 1999), brain ischemia (Cervantes et al., 2002; Sayeed et al., 2007; Gibson et al., 2009), and traumatic brain injury (TBI) (Stein, 2001; Stein and Sayeed, 2010). Based on these experimental findings (Schumacher et al., 2007; Brinton et al., 2008; Gibson et al., 2008; Stein, 2008; Stein and Wright, 2010), two phase II trials have already assessed the beneficial effects of progesterone following TBI (Wright et al., 2007; Xiao et al., 2008), and their encouraging outcomes have spurred the launching of two phase III multi-center trials (ProTECT-III, 2011; SyNAPSe, 2011).

Progesterone actions are mediated by its binding to specific receptors; the classical nuclear receptors (PR) which belong to a superfamily of transcription factors (Evans and Hollenberg, 1988). In addition, progesterone

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Abbreviations: 3β-HSD, 3β-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase; ANOVA, analysis of variance; AP, antero-posterior; CFC3, Inflammatory proteins C3 complement; DAB, diaminobenzidine; ER, estrogen receptor; GFAP, Glial fibrillary acidic protein; mPR, membrane progesterone receptor; NeuN, neuronal nuclei; Pgrmc1, progesterone receptor membrane component 1; PBS, phosphate buffered saline; PR, progesterone nuclear receptors; SSC, saline-sodium citrate; RT, reverse transcriptase; SEM, standard error of the mean; SDS, sodium dodecyl sulfate; TBI, traumatic brain injury; TBST, TRIS buffer salin -Tween 20.

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has rapid, non-genomic actions initiated at the cell surface by binding to membrane receptors (Falkenstein and Wehling, 2000; Schmidt et al., 2000). While progesterone effects can occur through the classical PR, a role for other progesterone-binding proteins or receptors in the nervous system has long been postulated. Progesterone and its derivatives bind to cell membranes of various rat brain tissues, suggesting non-classical receptor mechanisms of action (Towle and Sze, 1983; Ke and Ramirez, 1990; Tischkau and Ramirez, 1993; Zuloaga et al., 2012). Different non-genomic and rapid effects of progesterone have been observed within the CNS (Ramirez and Dluzen, 1987; Frye, 2001; Balasubramanian et al., 2008a,b; Hwang et al., 2009).

Early speculation regarding the identity of the receptors mediating the rapid effects of progesterone and its metabolites in the brain led to the discovery of steroid binding sites on the $GABA_A$/benzodiazepine receptor chloride channel complex (Lan et al., 1991; Belelli and Lambert, 2005; Hosie et al., 2006). These binding sites have affinity for multiple steroid metabolites, including the progesterone derivative 3α, 5α-tetrahydroprogesterone found in blood and brain tissues and following stress (Rupprecht, 2003; Gunn et al., 2011). A potential membrane receptor of progesterone was first cloned by Wehling's group from porcine liver microsomes (Meyer et al., 1996). Its rat analog is the protein 25-Dx (Selmin et al., 1996) renamed progesterone receptor membrane component 1 (Pgrmc1). Pgrmc1 does not appear to function as a traditional receptor because it requires a binding partner known as serpine mRNA binding protein 1 (Peluso et al., 2005). Pgrmc1 is expressed in the brain (Krebs et al., 2000; Meffre et al., 2005), and in the spinal cord (Labombarda et al., 2003) and its expression is up-regulated after injury (Labombarda et al., 2003; Meffre et al., 2005).

The recent discovery of a family of membrane progesterone receptors (mPRs) offers an alternative mediator of non-genomic progesterone effects in the nervous system. mPR cDNA was first cloned in a fish (Zhu et al., 2003b). Then, other members of this family were identified from several vertebrates including human and mouse (Zhu et al., 2003a). Phylogenetic analysis indicated that these cDNAs comprise three groups: α, β and γ. These proteins have the characteristics of G-protein-coupled receptors with seven transmembrane domains. Using a reverse transcriptase (RT)-PCR, Tang et al. found mRNA of all three mPRs (α, β, and γ) in the human brain (Tang et al., 2005). An mPR with 91% amino acid homology to human mPRα has been cloned from sheep and is expressed in both hypothalamus and pituitary tissues (Ashley et al., 2006). Both mPRα and mPRβ have been detected in the mouse brain (Thomas, 2004). Expression of their mRNAs has been more specifically localized to the medio-basal hypothalamus of both the rat (Liu and Arbogast, 2009) and mouse (Sleiter et al., 2009). mPRα and mPRβ expression in the brain vary with hormonal changes during estrus, with highest mPR expression during proestrus, suggesting receptor up-regulation by estrogen and down-regulation by progesterone (Liu and Arbogast, 2009). However, a recent study showed that the expression of genes encoding mPRα and mPRβ mRNA levels were most robust in the thalamic nuclei and cortex rather than in the hypothalamus (Intlekofer and Petersen, 2011b). The same authors showed that mPRα mRNA was not regulated by estradiol or progesterone treatment (Intlekofer and Petersen, 2011a). A more recent study showed a wide distribution of mPRβ in the female rat brain and the stimulation of its expression by estradiol within the medial septum (Zuloaga et al., 2012).

To date, studies on mPR expression in the brain were focused on females and regions mainly involved in neuroendocrine functions (Liu and Arbogast, 2009; Sleiter et al., 2009; Intlekofer and Petersen, 2011b,a; Zuloaga et al., 2012). Because of the well-documented and multiple effects of progesterone in the brain, some of which are likely to involve membrane actions, the aims of the present study were to precisely map the regional and cellular distributions of mPRα at both the mRNA and protein levels. We also sought to determine whether mPRα expression is influenced by sex, steroids or TBI. Our results suggest that this receptor may be implicated in different biological effects of progesterone in the brain and particularly in modulating myelination, water homeostasis, and inflammation after TBI.

EXPERIMENTAL PROCEDURES

Animals

Adult (12-week-old) male and female C57BL6 mice and adult male Sprague-Dawley rats (300 g) were obtained from Janvier, France. Animals were housed in a temperature-controlled room on a 12-h light, 12-h dark cycle with food and water *ad libitum*. Experimental protocols were approved by the Direction départementale de la protection des populations du Val-de-Marne, France, authorization number 94-345 to R.G., accredited establishment number 94-043-13). Experiments were performed in accordance with French ethical laws (Act 87-848; Ministère de l'Agriculture et de la Forêt) and the European Communities Council Directives of November 24, 1986 (86/609/EEC) guidelines for the care and use of laboratory animals.

Semi-quantitative RT-PCR and sequencing

RNA extraction. Adult male ($n = 6$) and female ($n = 6$) mice were killed by decapitation. Total brain and spinal cord (positive control) were dissected out, frozen on dry ice and stored at −80 °C until use. Total RNA was extracted using Trizol reagent (Life Technologies, Invitrogen, France) according to the manufacturer's instructions. Frozen samples were directly homogenized in Trizol using a glass–glass homogenizer. The concentration and purity of total RNA were determined by measuring optical density at 260 and 280 nm. All samples were precipitated with ethanol, and then dissolved in distilled water at a concentration of l μg/μl; their quality was verified by gel electrophoresis.

RT. Total RNA was subjected to DNAse 1 (Stratagene, La Jolla, CA, USA) treatment (10 U for 15 min at 37 °C) to remove residual contaminating genomic DNA. cDNA templates for PCR amplification were synthesized from 2 μg of total RNA using a