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Distribution of mRNAs Encoding Classical Progestin Receptor, Progesterone Membrane Components 1 and 2, Serpine mRNA Binding Protein 1, and Progestin and AdipoQ Receptor Family Members 7 and 8 in Rat Forebrain

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Abstract

Several lines of evidence suggest the existence of multiple progestin receptors that may account for rapid and delayed effects of progesterone in the central nervous system. The delayed effects have been long attributed to activation of the classical progestin receptor (Pgr). Recent studies have discovered novel progestin signaling molecules that may be responsible for rapid effects. These include, and progesterone receptor membrane component 1 (Pgrmc1), Pgrmc2, progestin and adipoQ receptor 7 (Paqr7) and Paqr8. The functions of these molecules have been investigated extensively in non-neural, but not in neural tissues, partly because it is unclear which are expressed in the brain and where they are expressed. To address these issues, we compared the distributions of mRNAs encoding Pgr, Pgrmc1, Pgrmc2, Paqr7 and Paqr8 using *in situ* hybridization with radiolabeled oligodeoxynucleotidyl probes in forebrain tissues of estradiol-treated female rats. We also examined the distribution of serpine mRNA binding protein 1 (Serbp1), a putative binding partner of Pgrmc1. Analysis of adjacent brain sections showed that the highest expression of mRNAs encoding Pgr, Pgrmc1, Pgrmc2 and Serbp1 was detected in several hypothalamic nuclei important for female reproduction. In contrast, expression patterns of *Paqr7* and *Paqr8* were low and homogeneous in the hypothalamus, and more abundant in thalamic nuclei. The neuroanatomical distributions of these putative progestin signaling molecules suggest that Pgrmc1 and Pgrmc2 may play a role in neuroendocrine functions while Paqr7 and Paqr8 are more likely to regulate sensory and cognitive functions.

Keywords

neuroendocrine; steroid; mPR; progestin; mapping

Introduction

Progesterone (P_4) is widely recognized for its ability to regulate neural functions related to reproduction, but it also affects diverse processes such as cognition and neurogenesis

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(Berman et al., 1997, Giachino et al., 2003). The traditional tenet of P_4 action is that it binds the cognate progestin receptor (Pgr), and functions as a ligand-activated transcription factor to regulate gene expression. However, rapid non-genomic effects have also been reported (Meyerson, 1972, Parsons et al., 1980, Mani et al., 1994), and P_4 can act in the absence of Pgr (Frye et al., 2006). These data support the emerging concept that P_4 actions in the brain may be through the classical Pgr and also through non-classical mechanisms.

Current research suggests that there are several possible candidates for mediating the non-classical effects of P_4. One such protein is progesterone receptor membrane component 1 (Pgrmc1), but it does not appear to function as a traditional receptor because it requires a binding partner known as serpine mRNA binding protein 1 (Serbp1) (Peluso et al., 2005, Peluso et al., 2006). Moreover, the structure of Pgrmc1 does not share homology with either classical steroid receptors or G-coupled protein receptors (Mifsud and Bateman, 2002). Nonetheless, Pgrmc1 mediates several important Pgr-independent effects. For example, P_4 acts through Pgrmc1 to activate phosphoinositide-dependent protein kinase 1 and phosphorylate Akt (Hand and Craven, 2003). In the ovary, Pgrmc1 and Serbp1 form a receptor complex required for the antiapoptotic effects of P_4 in granulosa cells (Peluso et al., 2006, Zhang et al., 2008). Pgrmc2 is a closely related isoform of Pgrmc1, differing mainly in its N-terminus, but there is virtually no information regarding Pgrmc2 function (Falkenstein et al., 1999, Peluso et al., 2005). Pgrmc1 has been localized to several brain regions (Krebs et al., 2000, Sakamoto et al., 2004, Meffre et al., 2005), but no studies have systematically mapped its distribution and the role of this protein in the brain remains unknown. Likewise, no studies have mapped neural expression of *Serbp1* or *Pgrmc2*. Despite these limitations, several lines of evidence indicate that Pgrmc1/Serbp1, and possibly Pgrmc2, may be important for non-classical P_4 actions in the brain.

Two other candidates for mediating the non-genomic effects of P_4 are progestin and adipoQ receptor 7 (Paqr7) and Paqr8. These are G-protein coupled receptors first discovered in spotted sea trout, and subsequently in mammalian tissues (Zhu et al., 2003a, Zhu et al., 2003b). Although activation of these receptors by P_4 regulates cAMP levels and MAPK activity in fish (Hanna et al., 2006), there is some debate about whether they function as *bona fide* P_4 receptors in mammals (Fernandes et al., 2008). Recent reports detected Paqr7 and Paqr8 mRNAs in hypothalamic tissue of mice, but the exact anatomical localization is unknown (Sleiter et al., 2009). Collectively, these findings raise the possibility that Paqr7 and Paqr8 also mediate P_4 neural actions.

Although there is abundant evidence that these signaling molecules participate in P_4 signaling, it is unclear which are important in the nervous system. Moreover, while many neural functions are modulated by P_4, there is little information about which functions require Pgr, non-nuclear receptors or both. One obstacle to resolving this question is that neither the classical Pgr nor any of the non-classical P_4 receptor candidates have been systematically mapped in the brain. To address this issue, we used *in situ* hybridization histochemistry (ISHH) to map the expression of mRNAs encoding Pgr, Pgrmc1, Pgrmc2, Serbp1, Paqr7 and Paqr8. In these studies, we used female rats because of the important role P_4 plays in regulation of female-specific physiological functions.

Materials and Methods

Animals and tissue preparation

All protocols were approved by the Institutional Animal Care and Use Committee of the University of Massachusetts and all animals were housed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Six adult female Sprague-Dawley rats (225–250 g; approximately 95 days of age; Harlan Sprague-

Dawley, Madison WI) were individually housed in the Animal Care Facility on a 14:10 light:dark cycle with food and water provided *ad libitum*. To achieve a similar hormonal milieu among rats, we ovariectomized them and implanted two Silastic capsules containing E_2 (150 μg/ml 17β-estradiol in sesame oil) a week later as described previously (Petersen and LaFlamme, 1997). Twenty-four hours later, we collected brains and rapidly froze and stored them at −80 °C until they were cryosectioned (Leica CM3000, Nussloch, Germany).

For three animals, 14-μm coronal forebrain sections were obtained and thaw-mounted onto gelatin-coated slides and stored at −80 °C until ISHH was performed. The remaining three animals were used for RNA isolation in validation studies described below.

Oligodeoxynucleotidyl probe preparation

In these studies, we used oligodeoxynucleotidyl probes of the same length and specific activity. Antisense oligodeoxynucleotide sequences used for end-tailing are provided in Table 1. Both sense and antisense sequences were produced by an automated DNA synthesizer and purified by reverse-phase HPLC by Integrated DNA Technologies (Coralville, IA). Oligodeoxynucleotides were 3'-end labeled with [α^{33}P]-dATP (PerkinElmer, Waltham, MA) using terminal deoxynucleotidyl transferase (Roche, Indianapolis, IN) as described previously (Petersen et al., 1989). Incubation was halted by addition of TE (10mM Tris-HCL; pH 8.0, 1 mM EDTA), and the probe was purified by phenol-chloroform extraction and ethanol precipitation. The resulting pellet was washed with 70% ethanol and resuspended in 25 μl TE.

ISHH

The distribution pattern for each mRNA was determined in separate ISHH runs, and tissue sections were prehybridized as previously described (Ottem et al., 2004). Radioisotopic probes (0.5×10^6 cpm) were applied directly to brain tissue in 20 μl hybridization buffer. This buffer contained 4XSSC (1XSSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.2), 50% (v/v) formamide, 10% (w/v) dextran sulfate, 250 μg/ml yeast tRNA, 1X Denhardt's solution, 500 μg/ml heparin sodium salt, 0.1% sodium pyrophosphate and 0.05 M dithiothreitol added freshly before use. Sections were covered with glass coverslips and hybridized overnight at 37 °C in humidified plastic boxes. Slides were removed from 37 °C and allowed to cool, and coverslips were floated off in 1XSSC. They were washed four times for 15 minutes each in 2XSSC-50% formamide solution at 40 °C, followed by four washes, 15 minutes each, in 1XSSC. Finally, slides were rinsed in water and briefly dehydrated in 70% ethanol. The slides were air-dried and apposed to Kodak BioMax MR film (Rochester, NY) for signal detection. In order to acquire optimum signal, autoradiograms were developed at 1, 3 and 6 weeks by an X-ray film processor and images were acquired using BioQuant Imaging Software (Bio-Quant Inc, Nashville, TN,) and a CCD videocamera (QImaging QICAM *FAST* color).

Validation of probe specificity

To determine the specificity of the hybridization signal, sense strand probes to each target of interest were hybridized to representative sections. To verify specificity of each antisense probe, subsets of adjacent slides were treated with RNAse A solution (100 μg/ml RNAse A in 0.5 M NaCl, 0.05 M EDTA and 0.01 M Tris-HCl) for one hour at 37 °C following prehybridization. An additional set of slides was used for Nissl staining in order to provide reference material for identification of specific brain regions.

Regardless of exposure time, Paqr7 and Paqr8 antisense probes produced diffuse and homogeneous signal, therefore multiple probes (Table 2) were used for each gene to verify signal specificity. To ensure specificity in regions that displayed low ISHH signal for Paqr7,

quantitative polymerase chain reaction (QPCR) was performed using cDNA derived from RNA of the diagonal band of Broca and striatum, regions with two different signal intensities. RNA was isolated from tissue punches using the RNeasy Lipid Tissue Kit (Qiagen, Valencia, CA) and reverse transcribed into cDNA using Quantitect Reverse Transcription Kit (Qiagen) and manufacturer's protocol. Reactions were performed in a Stratagene Mx3000P instrument programmed as follows: 95 °C, 10 min, and 40 cycles of 95 °C for 15 sec and 60 °C for 60 sec. Reactions contained reagents from QuantiTect SYBR Green Kit and manufacturer's protocols were used (Roche Diagnostics, Indianapolis, IN). Specific primer sets were obtained from Integrated DNA Technologies, and the forward and reverse primers used to detect Paqr7 mRNA were TGCACCGCATCATAGTGTC and TGATAGTCCAGCGTCACAGC. Resulting cycle thresholds were normalized using forward and reverse primers for beta-actin: GGGAAATCGTGCGTGACATT and GCGGCAGTGGCCATCTC. Samples with no cDNA were used as negative controls. Products were resolved using 2% agarose gel electrophoresis.

Data Analysis

Neuroanatomic mapping of Pgrmc1, Pgrmc2, Paqr7, Paqr8, Serbp1 and Pgr was performed with the aid of a rat brain atlas (Swanson, 1998) and Nissl-stained adjacent sections. Relative levels of mRNA were determined by optical densitometric measurements of the autoradiographic signals across different brain regions. Results of this semiquantitative ISHH were obtained by digitizing all autoradiographic images and four ranges of density of labeling were used to determine relative intensities across brain regions and across probes. Signal strength intensity was determined using arbitrary optical density units and denoted by – (background; 0–51), + (low; 52–102), ++ (moderate; 103–153), +++ (154–204), and ++++ (highest signal intensity; 205–255). Digitized images were imported into Adobe Photoshop 8.0 CS (Adobe Systems Inc., San Jose CA) and all figures were cropped to the same size for display.

For QPCR studies, relative levels of Paqr7 mRNA were analyzed using the 2-ΔΔCt method (Livak and Schmittgen, 2001).

Results

Probe specificity

We verified the specificity of the probes used in these studies in several ways. All four oligodeoxynucleotidyl probes for each target produced the same labeling pattern. In addition, neither sense strand controls nor RNAse A-treated sections showed specific signal with the exception of the sense probe for Paqr7. Although Paqr7 sense strand control probes showed signal above background, the signal for antisense probes was abolished by tissue pretreatment with RNAse A (Fig. 1, D3). Moreover, QPCR detected a single band of expected size using primers to Paqr7. Consistent with our ISHH findings, QPCR detected higher mRNA levels in the diagonal band of Broca than in the striatum (data not shown).

Distribution of Pgrmc1, Serbp1, Pgrmc2, Paqr7, Paqr8 and Pgr mRNAs

The neuroanatomical distributions of mRNAs encoding Pgrmc1, Serbp1, Pgrmc2, Paqr7, Paqr8 and Pgr are presented in Table 3. The distributions of mRNAs encoding Pgrmc1, Pgrmc2 and Serbp1 overlapped extensively throughout the brain, and some of these regions displayed high levels of the classical Pgr, most notably in ventral structures. Paqr7 and Paqr8 mRNAs were not highly expressed in these structures, but showed expression patterns similar to one another. Unlike signals for probes to Pgrmc1, Pgrmc2, Serbp1 and Pgr, signals for Paqr7 and Paqr8 probes were characterized by diffuse labeling found predominantly in the thalamus.

Diencephalon

In the rostral diencephalon, high signal intensities for Pgrmc1 mRNA were detected in several olfactory nuclei as indicated in Fig. 1, A1. We found moderate signal for mRNAs encoding Pgrmc1, Paqr7 and Paqr8 in the diagonal band of Broca (Fig. 1; A2, D2 and D4, respectively). In the preoptic area (POA), the AVPV nucleus displayed very high levels of mRNAs encoding Pgrmc1 and Pgr (Fig. 2, A1 and F1). Serbp1 and Pgrmc2 also showed elevated signal intensities in this nucleus (Fig. 2, B1 and C1). Although Paqr7 and Paqr8 mRNAs were found in the POA, clearly defined signal within the AVPV was not apparent (Fig. 2, D1 and E3). The bed nucleus of the stria terminalis contained dense labeling for Pgrmc1, Serbp1 and Pgr (Fig. 2, A2, B2 and F2). Moderate levels of mRNAs encoding Pgrmc1 and Paqr8 were found in the rostral portion of the lateral septal nucleus (Fig. 2, A1 and E1).

The paraventricular nucleus (PVN) and supraoptic nucleus (SON) showed abundant labeling for Pgrmc1, Serbp1, and Pgrmc2 (Fig. 2, A3, B3 and C3). High signal for probes encoding these mRNAs was also detected in the zona incerta, ventromedial nucleus (VMH) and arcuate nucleus (Arc), a finding consistent with the distribution of Pgr (Fig. 3, column 1). The reunions nucleus of the thalamus showed moderate levels of mRNAs encoding Pgrmc1, Serbp1, Pgrmc2 and Pgr mRNAs (Fig. 3, A1, B1, C1 and F1). In contrast, probes for these mRNAs showed low levels of signal intensity in the ventromedial thalamic nuclei while those encoding Paqr7 and Paqr8 were somewhat prominent (Fig. 3, column 1). The anterodorsal and anteroventral thalamic nuclei displayed similarly elevated levels of *Serbp1* and *Paqr8* gene expression (Fig. 3, B1 and E1).

Telencephalon

The hippocampus showed intense hybridization signals for Pgrmc1, Serbp1, Pgrmc2 and Pgr probes as shown in Fig. 3, columns 1 and 2. Within this region, signal intensities were highest in the CA2 and CA3 for all four mRNAs. In the dentate gyrus, stronger signal was detected for mRNAs encoding Pgrmc1, Serbp1 and Pgrmc2 (Fig. 3, A2, B2 and C2) than for those encoding Pgr (Fig. 3, F2). Paqr8 displayed low to moderate signal intensity within the hippocampus (Fig. 3, E2). In contrast, mRNAs encoding Paqr7 were undetectable in CA1, CA2, CA3 and dentate gyrus (Fig. 3, D1 and D2).

In the cortex, moderate levels of mRNAs were observed for Pgrmc1, Serbp1, Pgrmc2, Paqr8 and Pgr as shown in column 2 of Fig. 1. High signal intensity was detected in cortical regions for Pgrmc1, Serbp1 and Pgr mRNA (Fig. 3, row A, B and F). The medial amygdala showed moderate expression of *Pgrmc1*, *Serbp1*, *Pgrmc2* and *Pgr* (Fig. 3, columns 1 and 2). Alternatively, Paqr7 and Paqr8 signals in the amygdala were low overall (Fig. 3, D2 and E2)

The highest levels of mRNAs encoding Pgrmc1, Serbp1 and Pgr in the caudal forebrain were detected in the periaqueductal gray (PAG) (Fig. 3, A3, B3 and F3). Pgrmc2 mRNA levels were moderately high within this region (Fig. 3, C3), but Paqr7 and Paqr8 mRNAs were very low (Fig. 3, D3 and E3). Interestingly, Paqr8 mRNA levels were highest in the nucleus of the oculomotor tract (Fig. 3, E3). Finally, within the substantia nigra and interpeduncular nucleus, we detected moderately high levels of mRNAs encoding Pgrmc1, Serbp1 and Pgr (Fig. 3, A3, B3 and F3).

Discussion

Collectively, these neuroanatomical mapping data provide important clues as to functions of putative progestin signaling molecules. The high levels of Pgrmc1 and Pgrmc2 mRNAs within virtually all hypothalamic nuclei suggest that these molecules are likely candidates for mediating rapid neuroendocrine effects. Moreover, signals for Pgrmc1 and Pgrmc2

mRNAs were strongest in regions that also contained Pgr and regulate reproductive functions, consistent with the idea that these functions require both rapid and delayed actions of P_4. Pgrmc1 and Serbp1 mRNAs displayed strongly overlapping distribution patterns supporting the idea that these molecules interact to form a functional receptor (Peluso et al., 2004). Our data indicate that Paqr7 and Paqr8 may be important in sensory relay systems and other less well understood P_4 functions regulated by thalamic and cortical brain regions. Our findings suggest that P_4 may act through a number of signaling molecules to influence a much broader array of physiological effects than previously recognized.

The specific neural mechanisms underlying the role of Pgrmc1 have not yet been delineated, but the distinctive and overlapping patterns of Pgrmc1 and Serbp1 mRNAs suggest that these molecules may interact in neurons as they do in other cells. In granulosa cells, P_4 activation of the Pgrmc1/Serbp1 complex stimulates phosphorylation cascades through activation of protein kinase G (Peluso et al., 2007). Protein kinase G can activate additional kinases such as phosphoinositide 3-kinase, resulting in phosphorylation of Akt (Kandel and Hay, 1999, Brazil and Hemmings, 2001). The rapid phosphorylation of Akt in response to P_4 has been observed in cells from cortex and hippocampus (Singh, 2001, Hwang et al., 2009), and our study shows that both brain regions contain Pgrmc1 and Serbp1. Taken together, these observations suggest that phosphorylation events initiated by P_4 may involve the Pgrmc1/Serbp1 complex.

Brain regions rich in Pgrmc1 and Serbp1, as well as Pgrmc2 and Pgr, include the AVPV, medial preoptic (MPN), Arc and suprachiasmatic nuclei (SCN). These findings are in agreement with previous immunocytochemical and ISHH studies of Pgr and Pgrmc1 (Parsons et al., 1982, Krebs et al., 2000, Curran-Rauhut and Petersen, 2002, Meffre et al., 2005). Each of these nuclei contribute to the timing and magnitude of the preovulatory luteinizing hormone (LH) surge, a process in which P_4 exerts rapid and delayed effects. For example, estrogen induction of the LH surge requires Pgr expression in the AVPV/MPN (Chappell and Levine, 2000), and P_4 rapidly advances and augments the LH surge in estradiol-primed female rats (Krey et al., 1973, DePaolo and Barraclough, 1979, Levine and Ramirez, 1980). Our findings that the AVPV/MPN displayed high levels of Pgrmc1, Serbp1 and Pgrmc2 support the idea that these signaling molecules mediate rapid P_4 effects in the LH surge mechanism. P_4 also rapidly represses basal LH levels, and this action may involve the Arc (Banks and Freeman, 1978, Goodman et al., 1981, Richter et al., 2005), where Pgrmc1, Pgrmc2 and Serbp1 mRNA levels were also high. Finally, levels of these mRNAs were elevated in the SCN, and while not directly linked to reproduction, this nucleus sends projections to the AVPV and is critical for the temporal regulation of the LH surge (Paxinos and Watson, 1982, Watts and Swanson, 1987, de la Iglesia et al., 1995). Although the rapid effects of P_4 on LH surge release may be mediated by membrane-associated Pgr (Hammes and Levin, 2007), the current observations suggest that Pgrmc1, Pgrmc2 and Serbp1 may also play a role.

We found high levels of mRNAs encoding Pgrmc1, Pgrmc2, Serbp1 and Pgr in the VMH and in PAG, brain regions important for P_4-dependent female sex behaviors (Pfaff and Sakuma, 1979, Rubin and Barfield, 1983). This confirms a previous report of Pgrmc1 in the VMH (Krebs et al., 2000). Rat copulatory behavior requires activation of the Pgr within the VMH (Pollio et al., 1993, Ogawa et al., 1994), a nucleus that sends projections to many brain regions including the PAG (Saper et al., 1976). In the PAG, P_4 rapidly elicits female sexual receptivity (Gorski, 1974), an effect originally attributed to Pgr expression in this region. However, work in Pgr-null mice shows that sexual receptivity can be rapidly induced by midbrain injection of P_4, indicating a role for other signaling mechanisms (Beyer et al., 1988, Frye and Vongher, 1999). One such mechanism involves P_4 metabolism to allopregnanolone, a molecule that induces feminine sex behaviors when injected into the

midbrain (Beyer et al., 1988, Pfaff et al., 1994). The present work suggests another possibility, namely that Pgrmc1 contributes to elicitation of sexual receptivity by mediating rapid P_4 signaling through phosphorylation events as described above. Alternatively, Pgrmc1 may influence sex behavior through actions on neurosteroid production in the midbrain, a process that may be required for feminine sex behavior (Akesson et al., 1988, Frye et al., 2007). Consistent with this idea, Pgrmc1 interacts with key regulators of cholesterol homeostasis (Suchanek et al., 2005) and enhances steroidogenesis in diverse cell types (Laird et al., 1988, Min et al., 2004).

The present study identified high levels of Pgrmc1, Pgrmc2 and Serbp1 mRNAs in regions not directly linked to reproduction. For example, we confirmed previous findings that *Pgrmc1* is expressed in the PVN and SON (Krebs et al., 2000, Meffre et al., 2005), and extend these findings to show that Pgrmc2 and Serbp1 mRNAs were also found in these nuclei. The physiological significance of these findings are unclear, but Meffre *et al.* found that Pgrmc1 protein was expressed specifically within vasopressinergic neurons of the PVN and SON, suggesting that Pgrmc1 may play a role in regulation of water and ion homeostasis (Meffre et al., 2005). We also found Pgrmc1, Pgrmc2 and Serbp1 mRNAs in CA1, CA2, CA3 and dentate gyrus. These findings may be relevant to the observation that P_4 enhances hippocampal-dependent cognitive performance (Hoshina et al., 1994, Sandstrom and Williams, 2001). Our data also compliment previous findings of Pgrmc1 and Pgrmc2 mRNAs in neuroprogenitor cells derived from adult hippocampus (Liu et al., 2009). In these cells, the authors showed that P_4 induces neurogenesis through Pgrmc1-dependent phosphorylation cascades that do not require Pgr. These findings suggest that non-genomic signaling events involving Pgrmc1, and possibly Pgrmc2 and Serbp1, underlie P_4 actions in diverse neural functions.

Neither *Paqr7* nor *Paqr8* genes were strongly expressed in neural regions that control gonadotropin release and contain abundant Pgrmc1 mRNA. This was somewhat surprising because these genes are colocalized in other parts of the reproductive system. For example, Paqr7, Paqr8 and Pgrmc1 have been detected in cells of the ovary, uterus, and placenta in rats, sheep, and humans (Zhu et al., 2003a, Cai and Stocco, 2005, Peluso et al., 2006, Zhang et al., 2008, Ashley et al., 2009). Moreover, in the rat corpus luteum, the expression of each of these genes changes during pregnancy, and the patterns of change for *Paqr7* and *Pgrmc1* expression are strikingly similar (Cai and Stocco, 2005). In contrast to Pgrmc1, neither Paqr7 nor Paqr8 mRNA levels are found at levels much above background in AVPV/MPN. However, the AVPV provides afferent projections to GnRH neurons and results of recent studies show that Paqr7 and Paqr8 are functional in GT1-7 immortalized GnRH neurons (Sleiter et al., 2009). Thus, it is possible that Paqr7 and Paqr8 impact the neural control of reproduction, but through GnRH neurons scattered in the rostral preoptic area that also receive signals from Pgrmc1-containing neurons of the AVPV.

Though we did not find strong evidence for *Paqr7* or *Paqr8* gene expression in neuroendocrine nuclei, their expression was high in thalamic and cortical brain regions suggesting that these may participate in less well-studied actions of P_4. The thalamus is known primarily as a sensory relay system but few studies have examined P_4 actions within this brain region. However, P_4 binding occurs in the lateral septal nucleus of the thalamus (Shughrue et al., 1992) where we found high levels of mRNA encoding Paqr8. This is important because present and previous studies have found that the Pgr is absent in this brain region (Parsons et al., 1982). P_4 binding within the lateral septum decreases anxiety and depression-like behaviors (Pesold and Treit, 1992, Estrada-Camarena et al., 2002), and these effects have been presumed to be mediated by P_4 metabolites acting on $GABA_A$ receptors (Majewska et al., 1986). Similarly, $GABA_A$ receptors are implicated in the neuroprotective effects of P_4 in the cortex, a region in which we observed high levels of Paqr7, Paqr8 and

Pgr mRNAs. In light of the present findings, it is also possible that Paqr8 mediates P_4 actions in the lateral septum and cortex. This idea is supported by findings that in the cortex, maximal neuroprotective effects of P_4 require activation of MAPK and phosphoinositide 3-kinase pathways (Kaur et al., 2007), suggesting a role for membrane-initiated P_4 signaling, possibly involving Paqr7 and Paqr8.

In summary, we found that genes encoding Pgrmc1, Serbp1, Pgrmc2 and Pgr, but not Paqr7 and Paqr8, were highly expressed in many preoptic and hypothalamic nuclei and their projection sites. In contrast, Paqr7 and Paqr8 mRNA levels were most robust in thalamic nuclei and cortex. In view of these neuroanatomical findings, it is tempting to speculate that Pgrmc1 and Pgrmc2 control neuroendocrine functions, while Paqr7 and Paqr8 regulate affect and cognition. Additionally, the extensive overlap of Pgrmc1, Pgrmc2 and the classical Pgr mRNAs raise the intriguing possibility that these molecules interact within the same cells to regulate rapid and delayed P_4 effects in the brain. However, there were some regions in which Pgrmc1 and Pgrmc2 may exert effects independently of the Pgr. Together, these data provide anatomical information that will be important for determining the functional roles of these novel P_4 signaling molecules.

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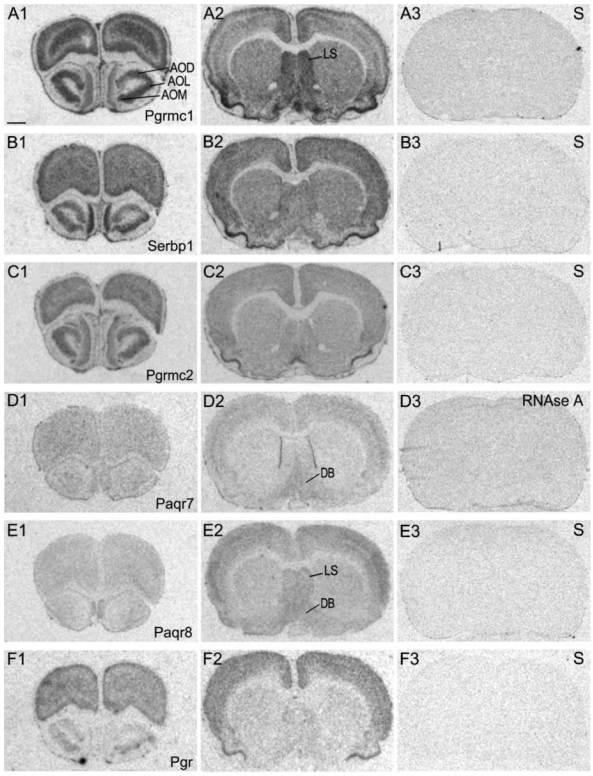

Figure 1.

Photomicrographs of film autoradiograms of hybridized rat brain sections to ^{33}P-labeled oligodeoxynucleotidyl probes for A) Pgrmc1 B) Serbp1 C) Pgrmc2 D) Paqr7 E) Paqr8 and F) Pgr. For Pgrmc1, Serbp1, Pgrmc2, Paqr8 and Pgr, the sense (S) strand probe is shown in the third image of each series. For Paqr7, the section was hybridized to the antisense probe and then treated with RNAse A. Scale bar = 200 μm

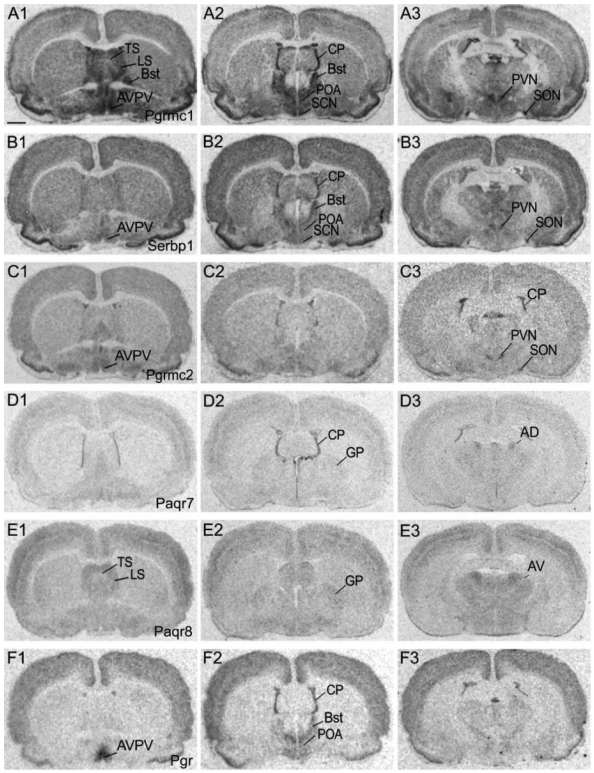

Figure 2.

Rostral to caudal arrangement of photomicrographs showing film autoradiograms of hybridized rat forebrain sections to ^{33}P-labeled oligodeoxynucleotidyl probes for A) Pgrmc1, B) Serbp1, C) Pgrmc2, D) Paqr7, E) Paqr8, and F) Pgr. Scale bar = 200 μm

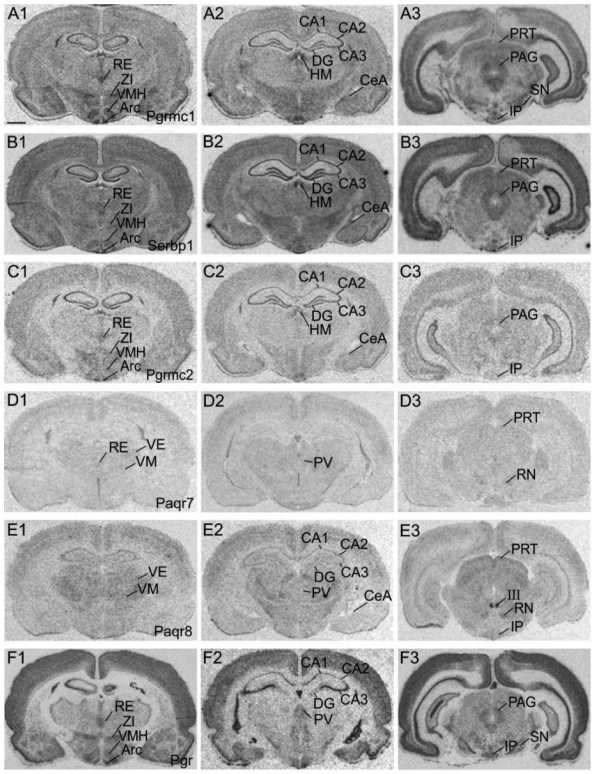

Figure 3.

Rostral to caudal arrangement of photomicrographs showing film autoradiograms of hybridized rat forebrain sections to ^{33}P-labeled oligodeoxynucleotidyl probes for A) Pgrmc1, B) Serbp1, C) Pgrmc2, D) Paqr7, E) Paqr8, and F) Pgr. Scale bar = 200 μm

Table 1

Sequences for Oligodeoxynucleotidyl Probes Used in ISHH Studies

NCBI Gene Name and Accession #	Oligodeoxynucleotide Sequences 5'- 3'	Antisense to bases:
Pgrmc1 NM_021766	TGTAGTTCCAACCCAATTACCAGGTGTGTGAGAGTTACTGTGTGTGGG	1354-1305
Pgrmc2 NM_001008374	CAAAGTTCAGTCCTGTTTACTGTGATCCTTGGTGTCCTCCTCGTCTGT	667-619
Serbp1 NM_145086	AAGGAACCAGTGTTGTATTATGGCATCCAGTTAGGCCAGAGCGGGGAA	1274-1227
Paqr7 NM_001034081	ATAGTCCAGCGTCACAGCTTCTAGCTGGGCTAAAGTGCACAGCACC	888-843
Paqr8 NM_001014099	GCCAGCCCCGCTGGTACCACTTGACAGATCTTCCGCATAACTGGATAA	1112-1065
PgR NM_022847	CACATGGTAAGGCACAGCGAGTAGAATGACAACTCCTTCATCCTCTGC	2375-2328

Table 2

Sequences for Additional Oligodeoxynucleotidyl Probes Used in ISHH Validation Studies

NCBI Gene Name and Accession #	Oligodeoxynucleotide sequences 5'- 3'	Antisense to bases:
PAQR7 NM_001034081	GGTACACGGCCACACCCACATAGTCCAAGAAGAAGAAGCTGTAATGCC	445-398
	GGGGCCAACGGGCGTGCAGAGGCTTATAGATGGCTCCCCGAGCCTGAT	934-887
	CAGGCTGGACAGCAGGTGGCTGAACTTCTGGGCTACTGCCATCGCCAT	48-1
PAQR8 NM_001014099	TTCTGGGACGGTGCATGGCATCTTGGGAAGCCCTTCTTCCAGAATCTT	570-523
	GAGCCAGGCCGGTGGTGAGTTTGAGGCCAGCCTGCTCTATAGAGTGAG	238-191
	GTGAGCCAAGGCGCTGCCATACTGGTAGACACTGACCCCGACGTAGTC	486-439

Table 3

Localization and abundance of mRNA encoding Pgr, Pgrmcl, Serbp1, Pgrmc2, Paqr7 and Paqr8 in the female rat forebrain[a]

Anatomical region	Pgr	Pgrmc1	Serbp1	Pgrmc2	Paqr7	Paqr8
Telencephalon						
Olfactory cortex						
Anterior olfactory nucleus external part	+	++	+	++	–	–
Anterior olfactory nucleus lateral part	+	+++	+++	++	–	–
Anterior olfactory nucleus medial part	+	+++	+++	+++	+	–
Anterior olfactory nucleus dorsal part	–	+++	++	++	–	–
Olfactory tubercle	+	+	+	+	+	+
Piriform cortex	++	++++	++++	+++	+	+
Hippocampal formation						
Taenia tecta	+	+	++	+	+	+
Dentate gyrus	+	++	++++	++	–	+
CA1 pyramidal layer	++	+	+++	+	–	+
CA2 pyramidal layer	++	++	+++	++	–	+
CA3 pyramidal layer	+++	++	+++	++	–	+
Amygdala						
Medial nucleus of the amygdala	+++	+++	+++	+	–	–
Posterolateral cortical amygdaloid nucleus	++	+	+	+	+	+
Central nucleus of the amygdala	+	++	+	+	–	–
Basolateral nucleus of the amygdala	++	+	+	+	–	–
Basomedial nucleus of the amygdala	++	+	+	+	–	–
Septal region						
Triangular septal nucleus	–	++	+	+	–	–
Lateral septal nucleus	–	+++	++	+	–	++
Medial septal nucleus	–	++	+	+	–	++
Horizontal limb of the diagonal band of Broca	+	++	+	+	+	+
Vertical limb of the diagonal band of Broca	+	++	+	+	+	+
Bed nucleus of the stria terminalis, anterior	+	+++	++	+	–	–
Bed nucleus of the stria terminalis, posterior	+	++	+	+	+	+
Accumbens nucleus	+	+++	++	+	+	+

Anatomical region	Pgr	Pgrmc1	Serbp1	Pgrmc2	Paqr7	Paqr8
Diencephalon						
Thalamus						
Medial habenular nucleus	+	+++	+++	++	–	+
Lateral habenular nucleus	+	++	+	–	–	+
Paraventricular nucleus	++	++	++	+	–	++
Zona incerta	+++	+++	++	++	+	+
Medial geniculate nucleus	+	++	++	–	+	+
Dorsal lateral geniculate nucleus	+	+	+	–	+	+
Anterodorsal thalamic nucleus	+	++	++	+	++	++
Anteroventral thalamic nucleus	+	++	+	–	++	++
Anteromedial thalamic nucleus	+	+	++	++	+	++
Ventral posteromedial thalamic nucleus	+	+	++	++	+	+
Lateralis	+	+	++	++	–	+
Lateralis posterior	+	+	++	++	+	+
Ventralis	+	+	++	–	+	+
Paracentral thalamic nucleus	+	+	+	+	+	++
Central medial thalamic nucleus	++	+	+	+	+	+
Reuniens thalamic nucleus	++	++	++	++	+	+
Hypothalamus/preoptic region						
Anteroventral periventricular nucleus	++++	++++	+++	+++	+	+
Suprachiasmatic preoptic nucleus	+	++++	+++	++	–	+
Supraoptic nucleus	+	++++	+++	++	+	+
Paraventricular nucleus	++	++++	+++	++	+	+
Rostral arcuate nucleus	++	++++	+++	++	+	–
Caudal arcuate nucleus	+++	+++	+++	++	+	+
Medial preoptic area	+++	+++	+++	++	+	+
Median preoptic nucleus	++++	+++	+++	++	+	+
Anterior hypothalamic area	+++	++	++	+	+	+
Ventromedial nucleus	+++	+++	+++	++	+	+
Dorsal hypothalamic area	++	+++	+++	+	+	+
Premamillary nucleus, dorsal	++	+++	+++	+	+	+

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Anatomical region	Pgr	Pgrmc1	Serbp1	Pgrmc2	Paqr7	Paqr8
Premamillary nucleus ventral	++	++	++	+	+	+
Medial mammillary nucleus	++	+++	+++	+	+	–
Dorsomedial nucleus	++	+++	++	+	+	–
Lateral preoptic area	+	++	++	+	+	+
Lateral hypothalamic area	+	+	++	+	+	++
Mesencephalon						
Interpeduncular nucleus	+++	+++	+++	+	++	+
Substantia nigra	++	++	++	+	+	+
Superior colliculus	+	++	++	+	+	+
Periaqueductal gray	+++	+++	+++	+	+	+
Ventral tegmental nucleus	+	++	++	+	+	+
Nucleus of the oculomotor cranial nerve	+++	+++	+++	+	+	++++
Pretectum	+	++	+++	+	+	++++
Red nucleus	++	++	++	++	++	++

[a]The ratings reflect the relative signal strength for each probe.