Endocrine (1995) 3, 421-424

Sex-specific changes in preoptic regulatory factor-1 and preoptic regulatory factor-2 mRNA expression in the rat brain during development

S.-B. Hu[1] & F.V. Nowak[2]

[1]Division of Endocrinology, Department of Medicine and [2]Division of Endocrinology, Department of Medicine and Department of Pharmacological and Physiological Sciences, St. Louis University Health Sciences Center, St. Louis, Missouri 63104, U.S.A.

Preoptic regulatory factor-1 (Porf-1) and Preoptic regulatory factor-2 (Porf-2) are two novel peptide genes which are expressed in the central nervous system. Expression is modified by age and by hormones of the reproductive system. In this study nuclease protection assays were employed to investigate Porf-1 and Porf-2 mRNA expression in the cerebral cortex (CC), hippocampus (HIPP), preoptic area (POA) and medial basal hypothalamus (MBH) of male and female rats, aged 15, 30 and 60 days. Porf-1 and Porf-2 mRNA expression tended to decrease from 15 to 60 days, with two exceptions. Porf-2 in the hippocampus of female rats, and Porf-1 in the MBH of the male rats, were found instead to increase with age. There were distinctive sex differences in *porf-1* and *porf-2* gene expression. Consistently higher mRNA levels were measured for both genes in the POA of female rats at all ages examined, and this difference reached statistical significance for Porf-1 at the age of 60 days. In contrast, levels of Porf-2 mRNA were higher in MBH of male than female rat MBH at 15 days, and Porf-1 mRNA was substantially more abundant in male than in female rat MBH at 30 and 60 days of age. These results indicate that there is sexual dimorphism and regional specificity in the developmental expression of these genes.

Keywords: Porf-1; Porf-2; mRNA; central nervous system; sexual dimorphism and development

Introduction

Porf-1 and -2 are two gonadal steroid sensitive neuropeptide genes expressed in the central nervous system and peripheral tissues (Nowak, 1990, 1991). Porf-1 and -2 mRNA expression in the brain of the male rat can be induced upon hypophysectomy or castration (Nowak, 1991). Preliminary results also show that ovariectomy alters Porf-2 mRNA expression in the MBH of the female rat and the ovariectomy-induced changes in Porf-2 expression can be reversed by steroid replacement (Hu & Nowak, 1994a). Distinctive changes in expression of these two genes have been observed in the cerebral cortex (CC), hippocampus (HIPP) and preoptic area (POA) during the aging process (Hu & Nowak, 1994b), suggesting that the preoptic regulatory factor gene products may be acting as neuromodulators with possible neurotrophic functions in these selected brain areas. It thus became of interest to examine the pattern of expression of these two genes at earlier stages of brain development and maturation in both male and female rats.

Sex specific expression of various hypothalamic neuropeptides is well documented (Gabriel *et al.*, 1989; Malik *et al.*, 1991). Although Porf-1 and -2 mRNA expression has been observed in various brain regions in the male rat (Nowak, 1990, 1991; Hu & Nowak, 1994b) and the expression of these two neuropeptide genes has been shown to respond to testicular and pituitary factors (Nowak, 1991) and to sex steroids (Hu & Nowak, 1994a), a direct comparison of the expression of these two genes in male and female rat brain has not been previously reported.

In this study the developmental patterns of *porf-1* and *-2* gene expression in the immature and young rat brain were investigated. Porf-1 and -2 mRNAs were measured in parallel in male and female rats to study the possible sex differential expression of these two genes in the developing brain.

Correspondence: Shuang-Bao Hu, Endocrine Unit, Massachusetts General Hospital/Harvard Medical School, 32 Fruit Street, WEL 501, Boston, MA 02114-2698.
Received 22 December 1994; accepted 3 January 1995

Results

Porf-1 and Porf-2 mRNA expression in the CC, HIPP, MBH and POA during development

In the majority of brain areas examined in this study both Porf-1 and Porf-2 mRNA levels were highest in the 15 day old rats of both genders. However there were exceptions. Porf-2 in the hippocampus of the female rats, and Porf-1 in the MBH of the male rat were seen instead to increase with age ($P<0.05$), while Porf-2 mRNA levels in the HIPP of the male rat remained constant ($P>0.05$).

Cerebral cortex In the CC, Porf-1 and -2 mRNA expression in both male and female rats followed a similar pattern (Figure 1). Levels of Porf-1 and Porf-2 mRNAs at the age of 15 days were the highest, decreased dramatically by the age of 30 days and remained constant at 60 days. The mRNA levels recorded at 15 days were approximately 4-fold higher than those at 30 and 60 days ($P<0.05$).

Hippocampus The highest levels of Porf-1 mRNA in the hippocampus of both male and female rats were observed at 15 days of age. Porf-2 mRNA in the female rats rose significantly ($P<0.05$) between 15 and 60 days, while in the male rats, there was no statistically significant difference from 15 to 60 days ($P>0.05$) (Figure 2).

Medial basal hypothalamus Porf-1 mRNA levels in the female rats decreased from 15 to 60 days of age ($P<0.05$), while in the male rats, Porf-1 mRNA expression steadily increased from 15 to 60 days ($P<0.05$) (Figure 3). Porf-2 mRNA in both male and female rats decreased from 15 to 60 days of age ($P<0.05$).

Preoptic area Expression of Porf-1 mRNA in the POA decreased between 15 and 60 days in the female rats ($P<0.05$) (Figure 4), while Porf-2 mRNA did not change. Neither Porf-1 nor Porf-2 mRNA levels in the male rats changed with age in the POA.

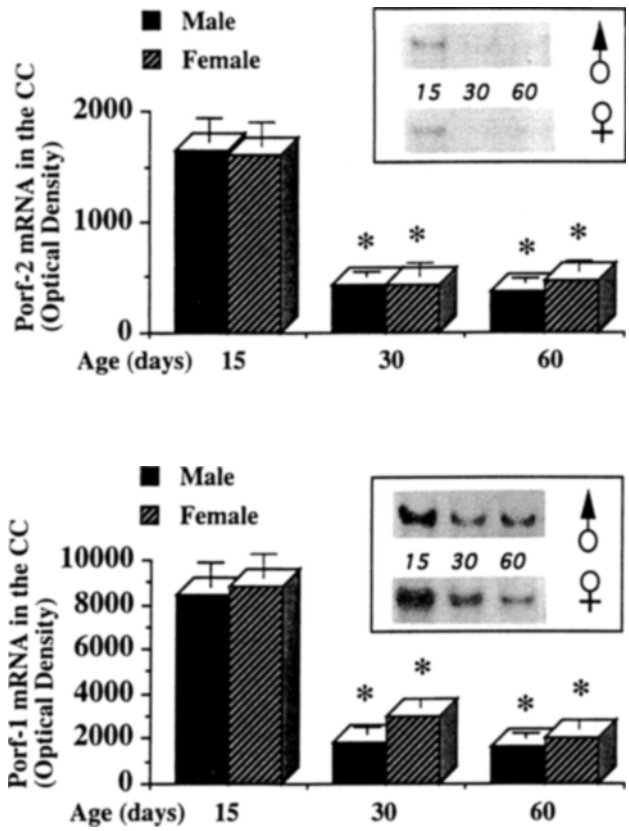


Figure 1 Porf-1 and Porf-2 mRNA expression in the cerebral cortex (CC) of male and female rats at 15, 30 and 60 days of age. The inset shows a representative example of the corresponding autoradiograph of protected Porf-1 or Porf-2 mRNA-probe hybrids from male and female rats. Each value in the bar graph represents the mean ± SEM of four individual samples. Statistics were performed with ANOVA followed by Duncan's multiple range test for the comparison of different ages of the same gender. Unpaired Student *t*-test was used for the comparison between male and female rats at the same age. $*P<0.05$ compared with the 15 day group of the same gender

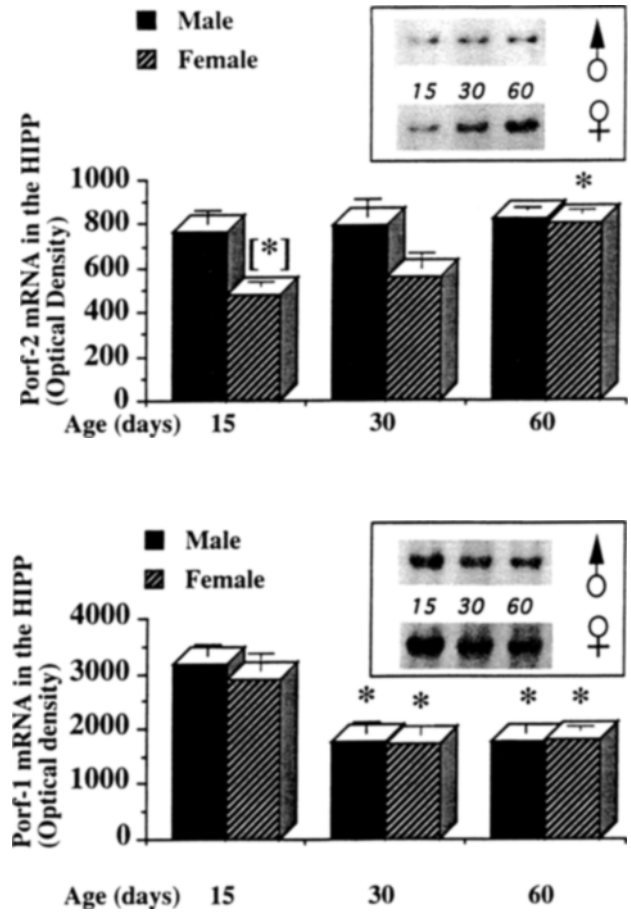


Figure 2 Porf-1 and Porf-2 mRNA expression in the hippocampus (HIPP) of male and female rats at 15, 30 and 60 days of age. The inset shows a representative example of the corresponding autograph of protected Porf-1 or Porf-2 mRNA-probe hybrids from male and female rats. Each value represents the mean ± SEM of three or four individual samples ($n = 4$ except for 15 day male and female groups for Porf-2 and 15 day female group for Porf-1). Statistics were performed as described in Figure 1. $*P<0.05$ compared with the 15 day group of the same gender. $[*]P<0.05$ compared between male and female groups of the same age

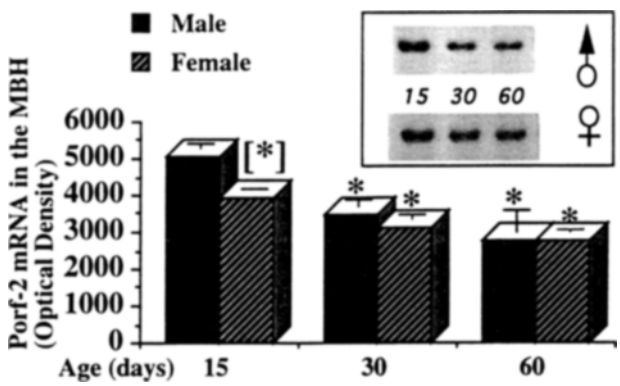
Sexual differential changes of Porf-1 and Porf-2 mRNA expression in the brain during development

We observed region specific and sex specific changes of both Porf-1 and Porf-2 mRNA expression in the brain during development. In the HIPP, Porf-2 mRNA levels were significantly higher ($P<0.05$) in the male than in the female at 15 days. In contrast, both Porf-1 and Porf-2 mRNA levels in the POA were, in general, higher in the female than male rats, and this difference was significant ($P<0.05$) for Porf-1 at the age of 60 days. In the MBH, Porf-1 mRNA expression changed in the opposite direction in males and females, beginning at about the same levels at 15 days, then increasing in the male and decreasing in the female rats from 15 to 60 days. As a result, by 60 days of age, levels of Porf-1 were significantly higher in the male rats compared with 60 day female rats ($P<0.05$).

Discussion

Porf-1 and -2 mRNAs are expressed in the rat brain in response to several types of physiological signals. We previously studied Porf-1 and -2 mRNA levels in the CC, MBH, POA and HIPP of adult male rats at different life stages. The observed tendency of *prof-1* and *-2* gene expression to decline in the CC, MBH and POA between 2 and 24 months (Hu & Nowak, 1994b) suggested a role for these genes in early brain maturation and the possibility of even higher levels of mRNA in the sexually immature rat brain. We have now shown in this study that both Porf-1 and -2 mRNAs were expressed at the highest levels in the youngest rats examined (15 days) and tended to decrease at the ages of 30 and 60 days in the CC and POA of both sexes and in the MBH of females. Age-related expression of Porf-1 in the male rat MBH and Porf-2 in female HIPP were exceptions to this general tendency, showing an increase in expression during the time period studied. We have previously shown that Porf-1 mRNA remains stable between 2 and 12 months of age in male rat HIPP, then decreases slightly in the aged rat (24 months). Thus Porf-1 expression appears to peak in the MBH of the male rat during the peripubertal and reproductively active life stages.

Developmental plasticity in the mammalian brain including neuronal selection and establishment of interneuronal circuitry, extends well into post-partum life. The age-dependent developmental expression patterns of certain neuropeptides and neurotransmitters are relevant to their functions at developmental stages (Payne *et al.*, 1977; McGregor *et al.*, 1982; Bernstein *et al.*, 1984; Aubert *et al.*, 1985; Macho *et al.*, 1986; Gabriel *et al.*, 1989; Grino *et al.*, 1989; Wiemann *et al.*, 1989). The precise physiological functions of Porf-1 and -2 genes in the central nervous system are still not known.



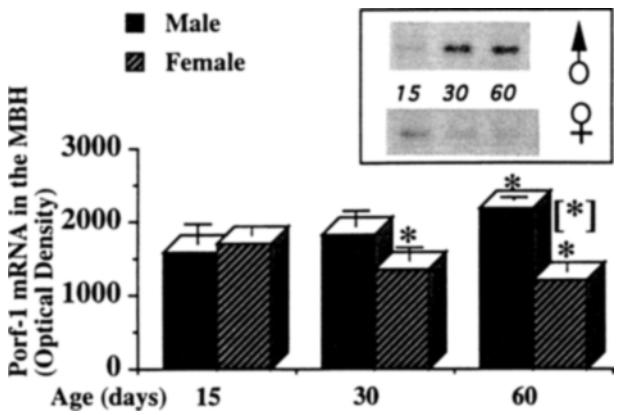
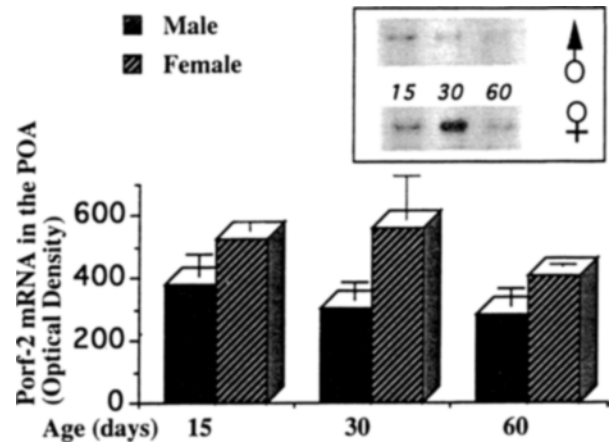


Figure 3 Porf-1 and porf-2 mRNA expression in the medial basal hypothalamus (MBH) of male and female rats at 15, 30 and 60 days. The inset shows a representative example of the corresponding autoradiograph of protected Porf-1 or Porf-2 mRNA-probe hybrids from male and female rats. Each value represents the mean ± SEM of three or four individual samples ($n = 4$ except for 15 and 60 day male groups for Porf-1). Statistics were performed as described in Figure 1. $*P < 0.05$ compared with the 15 day group of the same gender. $[*]P < 0.05$ compared between male and female groups of the same age



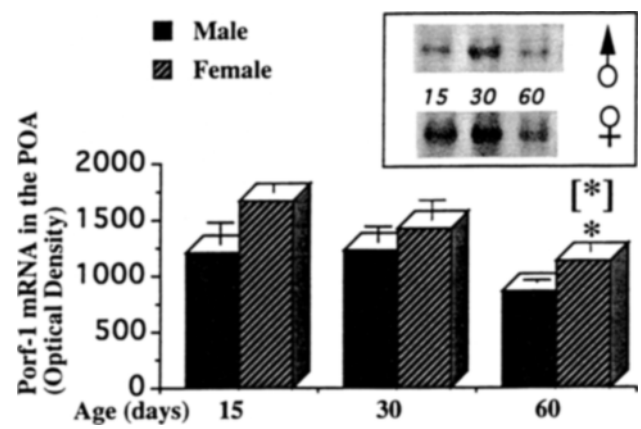


Figure 4 Porf-1 and Porf-2 mRNA expression in the preoptic area (POA) of male and female rats at 15, 30 and 60 days of age. The inset shows a representative example of the corresponding autoradiograph of protected Porf-1 or Porf-2 mRNA-probe hybrids from male and female rats. Each value represents the mean ± SEM of three or four individual samples ($n = 4$ except for 60 day male group for Porf-2). Statistics were performed as described in Figure 1. $*P < 0.05$ compared with the 15 day groups of the same gender. $[*]P < 0.05$ compared between male and female groups of the same age

However the relatively high levels of Porf-1 and Porf-2 expression at an early age suggest that they may play important roles in the early postnatal period of development and maturation in the CC and POA of both sexes and the MBH of the female rat.

Porf-2 in the HIPP of female rats, unlike Porf-1 and Porf-2 mRNA expression in most of the brain areas examined in this study, increased steadily from 15 to 60 days. We had previously observed that Porf-2 mRNA expression in the male rat HIPP increased from 2 to 6 months, remained high at the age of 12 months and declined in the aged rats. Hippocampus is important in the control of central nervous system and hypothalamic neuroendocrine function including GnRH expression (Brown-Grant & Raisman, 1972) and crucial for certain types of short term memory. The pattern of Porf-2 mRNA expression in the HIPP suggests it may function here in the adult brain as a stable neurotransmitter or neuromodulator rather than a developmental factor.

Both Porf-1 and Porf-2 mRNA expression in the neuroendocrine hypothalamus may be regulated by steroid hormones (Nowak, 1990, 1991; Hu & Nowak, 1994a). In this study there was a trend toward higher levels of Porf-1 and Porf-2 mRNA expression in the POA in the female compared to male rats. In addition MBH Porf-1 mRNA expression in the male and female rats changes in the opposite direction, decreasing in the female rats and increasing in the male rats from 15 to 60 days of age. Sexual differentiation expression has been observed for other neuropeptides and neurotransmitters in the central nervous system. Some of these neuropeptides including GnRH (Malik *et al.*, 1991), galanin (Gabriel *et al.*, 1989) and vasopressin (De Vries *et al.*, 1984) have been demonstrated to play a critical role in development of nervous system sexually determined responses. The significance of the marked difference of Porf-1 mRNA expression in the MBH of male and female rats remains to be investigated. However the demonstration of a sexual dimorphism in *porf-1* and *-2* gene expression in the hypothalamus suggests that these peptides may play a role in development of sexual differentiation of the nervous system and may participate in the control of reproductive function or behavior.

In summary, Porf-1 and Porf-2 mRNA expression in the maturing rat brain show regionally distinctive changes between 15 and 60 days of age. Porf-1 and Porf-2 mRNA expression also show sexually dimorphic changes in certain critical regions of the CNS including the MBH and HIPP. These findings support a role for these genes as possible mediators of brain-reproductive organ interaction.

Materials and methods

Animals

Sprague-Dawley CD rats were obtained from Charles River (Portage, WI), and housed under conditions of 12 h dark (lights off: 6.00 pm to 6.00 am) with food and water ad libitum. Rats were sacrificed at 15 days (prepubertal), 30 days (peripubertal) and 60 days of age (postpubertal). Tissues from the POA, CC, MBH and HIPP were harvested as described previously (Nowak, 1990, 1991; Hu & Nowak, 1994b). All tissues were immediately frozen on dry ice. Tissue samples were kept at −80°C until used for RNA preparation.

RNA extraction

Total RNA from individual rat brain regions from four rats in each group was isolated by a guanidine thiocyanate/phenol/chloroform/isoamyl alcohol procedure (Chomczynsky & Sacchi, 1987). RNA samples were quantitated by spectrophotometry at a wavelength of 260 nm. The RNA yielded from all brain regions averaged between 0.7 and 0.9 μg RNA/mg tissues. The variance of recovery within each group was less than 15%. Isolated RNA was stored at −80°C or immediately subjected to nuclease protection assay.

Nuclease protection assay

Sense RNA and riboprobes were prepared as described previously (Nowak, 1990, 1991) using T7 and SP6 RNA polymerase according to the instruction by the supplier (Promega). ^{32}P-CTP was used for probe synthesis. The assay was performed as described previously (Hu *et al.*, 1993) with some modifications. RNA was dissolved (20 μg for the samples and different concentrations of synthetic mRNA for the standard assay) in 30 μl of hybridization mixture [60% formamide, 0.9 M NaCl, 6 mM EDTA, 60 mM Tris-HCl (pH 7.6), 30 μg of carrier tRNA and riboprobes (0.2 ng, about 2×10^5 c.p.m. of each probe)], and hybridized at 50°C overnight. Following digestion with RNase A (40 μg/ml), and RNase T1 (2 μg/ml) for 60 min at 30°C and proteinase K at 37°C for 15 min, the protected RNA fragments were analysed by 6% non-denaturing polyacrylamide gel electrophoresis. Autoradiograms were quantified by densitometry. A wide range of concentrations of synthetic sense mRNA was processed in each assay so that the optical density of each sample was within the linear range of that of the standard curve (Hu & Nowak, 1994b).

Reagents

RNase T1 and RNase A were obtained from Sigma (St. Louis, USA); T7 RNA polymerase and SP6 RNA polymerase were from Promega (Madison, WI, USA); ^{32}P-CTP was from Amersham (Arlington Heights, Illinois, USA).

Statistics

Results are expressed as mean ± SEM throughout the study. Each data group is the result of analysis of three or four individual tissue sample as indicated. All tissues are from the same set of rats. Statistics were performed with ANOVA followed by Duncan's multiple range test for the comparison of different ages of the same gender. Unpaired Student *t*-test was used for the comparison between male and female rats at the same ages. Significance was accepted at $P < 0.05$. All samples for comparison were analysed in the same nuclease protection assay.

Acknowledgements
The authors wish to thank Dr A.D. Mooradian for his critical comments on this manuscript.

References

Aubert, M.L., Begeot, M., Winiger, B.P., Morel, G., Sizonenko, P.C. & Dubois, P.M. (1985). *Endocrinology*, **116,** 1565–1576.
Bernstein, H.G., Dorn, A., Reiser, M. & Ziegler, M. (1984). *Acta Histochem.*, **74,** 33–36.
Brown-Grant, K. & Raisman, G. (1972). *Brain Res.*, **46,** 23–42.
Chomczynsky, P. & Sacchi, N. (1987). *Anal. Biochem.*, **162,** 156–159.
De Vries, G.J., Buijs, R.M. & Van Leeuwen, F.W. (1984). *Prog. Brain Res.*, **61,** 185–203.
Gabriel, S.M., Kaplan, L.M., Martin, J.B. & Koenig, J.I. (1989). *Peptides*, **10,** 369–374.
Grino M, Young III, W.S. & Burgunder, J.M. (1989). *Endocrinology*, **124,** 60–68.
Hu, S.-B. & Nowak, F.V. (1994a). 75th Endocrine Meeting Program and Abstract, p 375.
Hu, S.-B. & Nowak, F.V. (1994b). *Mol. Cell. Neurosci.*, **5,** 375–381.
Hu, S.B., Tannahill, L.A. & Lightman, S.L. (1993). *J. Mol. Endocrinol.*, **10,** 51–57.
Macho, L., Kvetnansky, R., Culman, J. & Fickova, M. (1986). *Exp. Clin. Endocrinol.*, **88,** 142–150.
Malik, K.F., Silverman, A.-J. & Morrell, J.I. (1991). *Anatom. Rec.*, **231,** 457–466.
McGregor, G.P., Woodhams, P.L., O'Shaughnessy, D.J., Ghatei, M.A., Polak, J.M. & Bloom, S.R. (1982). *Neurosci. Lett.*, **28,** 21–27.
Nowak, F.V. (1990). *Mol. Endocrinol.*, **4,** 1205–1210.
Nowak, F.V. (1991). *Mol. Cell. Neurosci.*, **2,** 395–401.
Payne, A.H., Kelch, R.P., Murono, E.P. & Kerlan, J.T. (1977). *J. Endocrinol.*, **72:** 17–26.
Wiemann, J.N., Clifton, D.K. & Steiner, R.A. (1989). *Endocrinology*, **124,** 1760–1767.