Alternative splicing of human and mouse NPFF2 receptor genes: Implications to receptor expression

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Abstract   Alternative splicing has an important role in the tissue-specific regulation of gene expression. Here we report that similar to the human NPFF2 receptor, the mouse NPFF2 receptor is alternatively spliced. In human the presence of three alternatively spliced receptor variants were verified, whereas two NPFF2 receptor variants were identified in mouse. The alternative splicing affected the 5′ untranslated region of the mouse receptor and the variants in mouse were differently distributed. The mouse NPFF system may also have species-specific features since the NPFF2 receptor mRNA expression differs from that reported for rat.
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1. Introduction

Neuropeptide FF (NPFF) was discovered 20 years ago [1] and it appears to participate in several physiological functions both in the CNS and non-neuronal tissues. In accordance with its known functions in pain transmission and endocrine functions, NPFF is found in the posterior pituitary, dorsal horn of the spinal cord, the hypothalamus and pons-medulla [2–4]. Besides the distinct distribution in the central nervous system (CNS), NPFF is found in the periphery [5], and it is secreted into blood [6].

The target molecules for NPFF and related peptides, NPFF1 and NPFF2 receptors, were identified recently [7–10]. The expression patterns of the two NPFF receptors are distinct [7,11], suggesting functional differences between them. In neuroblastoma cells NPFF signals through endogenous NPFF2 receptor [12] and currently, it seems that NPFF2 receptor is the receptor mostly responsible for the effects of

NPFF peptide. NPFF2 receptor was first cloned as an orphan receptor [13,14], but a ligand was found soon after the initial discovery ([7,8]; Table S1). Interestingly, the reported NPFF2 receptor sequences are not identical and might represent alternative splice variants of the receptor. The gene for the mouse NPFF2 receptor has been cloned [11,14], but its mRNA distribution has not been described and the mouse NPFF2 receptor splice variants are unknown.

In this study, we show that human and mouse NPFF2 receptors are alternatively spliced. We cloned and analyzed the splice variants and investigated the tissue-specific expression of mouse NPFF2 receptor transcripts.

2. Materials and methods

2.1. Experimental animals

The permission for the mouse experiments was obtained from the Institutional Animal Care and Use committee of Abo Akademi University. All the experiments were conducted in accordance with the European community council directive 68/609/EEC guidelines. Adult male mice (C57B6/6ola or ICR wild type mice) were maintained at a 12-h dark–light cycle with food and water available ad libitum. The animals ($n = 3$) were sacrificed by the administration of CO_2 followed by decapitation. The brains were dissected and frozen in isopentane. The frozen tissues were embedded in Embedding Matrix[R] (Shandon, Pittsburg, PA, USA) and sectioned at $-20\,°C$. Twenty micrometer sections were thaw-mounted on poly-L-lysine slides (Menzel-Gläser, Braunschweig, Germany). For the RNA isolation the tissues were frozen in liquid nitrogen. The human placenta tissue sample was obtained from the Turku University Hospital (Turku, Finland) with the permission of the patient.

2.2. RNA isolation and cDNA synthesis

Total RNA from human placenta and mouse tissues was isolated with RNAwiz protocol (Ambion Inc., Austin, TX, USA). The total RNA was treated with DNaseI (Promega, Maddison, WI, USA) before the cDNA synthesis. cDNA from total RNA was produced with Superscript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA).

2.2.1. Rapid amplification of cDNA 5′ ends (5′ RACE).   Five micrograms of human placental or mouse brain total RNA was used as template material for the GeneRacer kit (Invitrogen). A primary and nested PCR was performed with RNA-oligonucleotide specific forward primers provided by the kit and gene-specific reverse primers (Table S2). The PCR products were cloned into pGEM-T Easy vector (Promega) and several recombinant clones were isolated for sequencing analysis.

2.2.2. RT-PCR analysis of the expression of mouse NPFF2 receptor.   Two sets of mouse NPFF2 receptor specific primers extending over introns were used for the RT-PCR analysis of the mouse NPFF2 receptor transcripts in non-neuronal tissues and different brain areas (Table S2). The samples were also analyzed for the expression of

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glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the case of non-neuronal tissue samples and phorphobilinogen deaminase (PBGD) in the case of CNS samples. The samples were analyzed with agarose gel electrophoresis using ethidium bromide to visualize the PCR products.

2.3. In situ hybridization

The probes (Table S2) were 3′ end labeled with [^{33}P]- or [^{35}S]-deoxy-adenosine 5-(-thio) triphosphate (Amersham Biosciences, Buckinghamshire, UK) using terminal deoxynucleotide transferase (Promega). The hybridization procedure has been described in detail before [5]. A 100-fold excess of unlabeled specific probe was used to inhibit binding of the labeled probe in control samples. The sections were exposed to Kodak BioMax X-ray films (Eastman Kodak, Rochester, NY, USA).

3. Results and discussion

3.1. The splice variants of human NPFF2 receptor

Since its initial discovery, several sequences for the human NPFF2 receptor have been published (Table S1). Apparently, these different NPFF2 receptor sequences represent alternative splice variants. First, we confirmed the identity of the human NPFF2 transcripts. After analyzing a number 5′RACE clones, three distinct receptor variants were verified (Fig. 1A and B). The shortest transcript, NPFF2 receptor variant1, corresponded to the GenBank AF257210 sequence. The variant2 differed from the variant1 at its 5′untranslated region (5′UTR) and it had a putative alternative start codon nine nucleotides upstream of the reported start codon. The variant2 corresponded to AF236083. The NPFF2 receptor variant3 had a long N-terminal extension that was not found in the other variants, and corresponded to AF119815. The exon–intron structures of the variants are shown in Fig. 1A and the exon–intron junctions in detail in Fig. 1B.

Of the three cloned NPFF2 receptor variants only the variant3 differs significantly in protein coding sequence. Laemmle et al. [15] showed that this isoform has much lower affinity to NPFF peptides than NPFF2 receptor. Thus, in human the NPFF2 receptor variant3 may have a distinct function from the other variants. Laemmle et al. [15] detected yet another NPFF2 receptor variant that is lacking exons1–3 and represents a truncated receptor. This truncated transcript was not detected by the primers used in this study. The exon2 found in the variant2 contains an alternative start codon. Whether this start codon upstream of the reported transcription start site is actually used, remains to be studied.

3.2. The splice variants of mouse NPFF2 receptor

Since mouse is an extensively used species to study the NPFF system, we next asked whether the mouse NPFF2 receptor is alternatively spliced. The 5′RACE analysis of mouse whole brain RNA revealed two NPFF2 receptor transcripts in mouse (Fig. 1A and C). The coding regions, but not the 5′UTRs, of the cloned mouse NPFF2 receptor splice variants corresponded to GenBank NM_133192. The 5′UTR reported for NM_133192 was not detected by 5′RACE or traditional cloning. The BLAST search for this sequence showed it to be a repetitive sequence and maybe a cloning artefact. Similar to human, the mouse NPFF2 receptor variants differed only slightly in their coding sequence but the additional exon in variant2 introduced a 5′UTR that is missing in the variant1. The mouse variant2 had a putative alternative start codon but
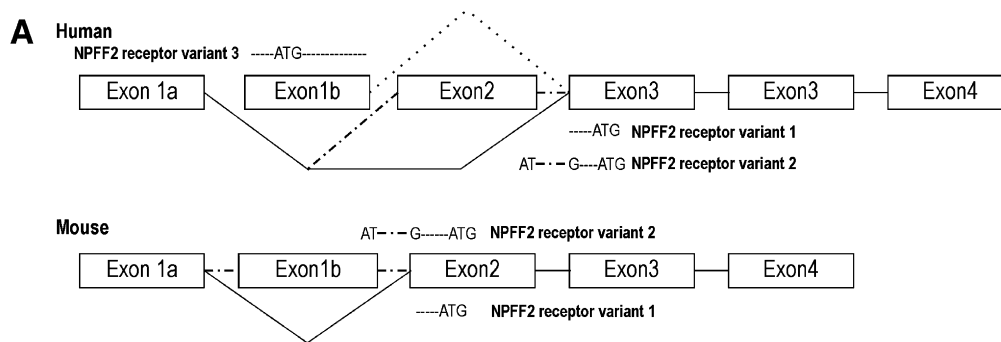
it was followed by a termination codon and, thus, the protein coding sequence of the two mouse NPFF2 receptor variants appears to be the same. The strength of the putative splice sites was analyzed with Alternative Splice Site Predictor [16]. The exon1a and exon2 were found in both mouse variants and in accordance with this, donor splice site of the exon1a and the acceptor and donor sites of the exon2 showed very high scores for constitutive splice sites. The exon1b was found only in the receptor variant2 and it had splice site sequences typical for alternative splicing. In spite of the cloning efforts, a human variant3-like transcript was not detected in mouse. Accordingly, alternative splicing is reported to be more frequent in human than in mouse, both in terms of percentage of genes that are alternatively spliced and the number of alternative splicing events per gene [17]. Although the receptor variants1 and 2 hardly differ in their protein coding sequence, they do have a significantly different 5′UTR sequences. At least 25% of all alternative exons are predicted to regulate transcript abundance and the alternative splicing of the 5′UTR may have drastic effects on the gene expression [18–20]. Thereby, the alternatively spliced 5′UTR may have a role in the regulation of NPFF2 receptor expression.

3.3. Expression of NPFF2 receptor splice variants in mouse

Interestingly, the conserved alternative splicing did not change the reading frame of the transcript. Such conserved modular alternative splicing events seem to be associated with tissue-specific regulation of splicing [21]. Therefore, the expression pattern of the mouse receptor variants was analyzed by in situ hybridization and by RT-PCR. Furthermore, since there are significant differences in the NPFF binding between species [11] and even rodent strains [22], it was considered of importance to determine the NPFF2 receptor mRNA expression pattern in mouse. In addition, the differential expression of the receptor variants has not been studied.

The expression of NPFF2 receptor was detected at restricted sites in the mouse CNS (Fig. 2). The highest expression level was detected at the external plexiform layer of the olfactory bulb (EPl) and a strong signal in the nucleus of the solitary tract (Sol), dorsal tegmental nucleus (DTg) and reunions thalamic nucleus (Re). Moderate or low level of expression was detected also at other sites. Mouse NPFF2 receptor variant 2 mRNA was detected only in the olfactory bulb (Fig. 3).

The individual contribution of the receptor variant1 to the NPFF2 receptor expression could be revealed by RT-PCR. To get a more complete view of the contribution of the transcripts in mouse, the expression of the splice variants in peripheral organs was analyzed. Based on the RT-PCR analysis both variants were expressed in several brain regions (Fig. 4A) and non-neuronal tissues (Fig. 4B), but their relative abundance varied markedly between tissues. The analysis of brain regions with RT-PCR showed wider expression of NPFF2 receptor than was expected based on the in situ hybridization data (Data S1). In general, the variant2 was more abundant in the non-neuronal tissues and whole brain. In the CNS, the variant1 was more abundant than the variant2 only in the olfactory bulb. Similar to in situ hybridization analysis, the RT-PCR analysis of the CNS (Fig. 4A) indicated the highest NPFF2 receptor mRNA expression in the olfactory bulb. The role of NPFF in the olfactory system is not known and should be further investigated in detail. The strong mRNA sig-

A Human

NPFF2 receptor variant 3 ------ATG---------------

| Exon 1a | Exon1b | Exon2 | Exon3 | Exon3 | Exon4 |

------ATG **NPFF2 receptor variant 1**

AT--·--G----ATG **NPFF2 receptor variant 2**

Mouse

AT--·--G------ATG **NPFF2 receptor variant 2**

| Exon 1a | Exon1b | Exon2 | Exon3 | Exon4 |

------ATG **NPFF2 receptor variant 1**

B Human NPFF2 receptor variants

Exon 1b (variant3)
```
75541 aTCTGGAGCC AAGTAATGGT GATACTgATG CTTCCTTTTC TTTGCCGCGC TCGGATTCTG
75601 AGTTTCACAA GAATGTACCT GGGTGCCCCT TAGCGGGATA TGAATAGCTT CTTCGGAACC
75661 CCAGCGGCCA GCTGGTGCCT CCTGGAAAGT GACGTCTCAT CTGCACCGGA CAAGGAGGCG
75721 GGGAGGGAGC GCAGAGCACT CAGCGTCCAG CAGCGCGGCG GGCCAGCCTG GAGCGGAAGC
75781 CTGGAGTGGA GCAGGCAGTC CGCGGGGGAC AGACGTCGGC TGGGATTGAG CCGGCAGACT
75841 GCGAAAAGTA GCTGGAGCCG GAGCAGGGAC AGAACCTGTT GCTGCAGACG GGCTTGGTGG
75901 ATTCTGGTTC CTGCCGCCGA CAGGGCTCGC CGGGAGAGgt aacagcatgg gccagtttgt
```

Exon 1a (variants 1 & 2)
```
82921 TGAGTAGCTG GGACTACAGG CGCCCGCCAC CACGCCTGGC TAATTTTTTT GTATTTTTAG
82981 TAGGGACGGC GTTTCACTGT GTTAGCCAGA TGGTCTCCAT CTCCCGACCT CGTGATCCAC
83041 CCACCTCGGC CTCCCAAAGT GCTGGGATTA CAGGCGTGAG CCACCGCGCC CGGCCAATTT
83101 CCTTTCTTAG TTGCCTCTGC CCACCTCTTC TCTTCTGCTT CCATATTACA Ggtaaccaat
```

Exon 2 (variant2)
```
112621 tgcctgaggt tcttagtttt tttttttttt tatcttatct tttttcctgc atggctctag
112621 acacatatgg tttgatttct ttatttccag GTCTCCTCAG TTGCGAAATT AGGATGTTAA
112681 TTATAGCTTT TGACATACAA GAAACATCAA AAAGATTGAA TGTCTTAATA AGAGTGAAGC
112741 ATGTAGATCA GTGACTGCTA Tgtaagtatt ctaatattat tttgatcttt tgaaaaagag
```

Exon 3 (variants1-3)
```
2401 tcttaaactc aggcacagaa tttatgcttt actggttcct aattatgttt ttcttttctg
2461 tctcttcttt attaagGTTC ATCATGAATG AGAAATGGGA CACAAACTCT TCAGAAAACT
2521 GGCATCCCAT CTGGAATGTC AATGACACAA AGCATCATCT GTACTCAGAT ATTAATATTA
2581 CCTATGTGAA CTACTATCTT CACCAGCCTC AAGTGGCAGC AATCTTCATT ATTTCCTACT
2641 TTCTGATCTT CTTTTTGTGC ATGATGGGAA ATACTGTGGT TTGCTTTATT GTAATGAGGA
2701 ACAAACATAT GCACACAGTC ACTAATCTCT TCATCTTAAA CCTGGCCATA AGTGATTTAC
2761 TAGTTGGCAT ATTCTGCATG CCTATAACAC TGCTGGACAA TATTATAGCA Ggtatgttg
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C Mouse NPFF2 receptor variants

Exon 1a (variants 1 & 2)
```
38761 ctgtgACTAA CCAGCAGCTG ACCGACCAGT GCAGTTCTGA GAGTGCAGCA AGAGCGGAGA
38821 GTGCAGAGCC CGCCGGCGTC AGCCCCGCTG CAGAGGGAAG TGGCTAGCCA GTCGCACCTA
38881 GGTCACCTGG TAGAGCAGAG CCCGCGAACC AGACAGAACC TGTTGCTCAG GCTCCGCTTG
38941 GCTTCTGCTT TCAACAGGGT TGGGAATCTG TCCTTGCCCA GGAACAGgtg agggcacggg
```

Exon 1b (variant2)
```
66961 cctgacagtt ccctggcttc ctgcctttgt acgtgagtca gcatttaata cttttcctatc
67021 ccttccttta ttctcagGCT TTCCAGTAAC CTATTCCCAG TCCTTGGATG ACTCTGCCTC
67081 ATCTGCCTCC CTTCCTAAGA CTATAATCTA GTGGAGACTA ACTCACAATG TGACTGAGgt
67141 aattggaata tgagattaat aagctacatc tgactgaagg tggaagacag atttgtatag
```

Exon 2 (variants 1 & 2)
```
79561 tctaccaaaa ttgtattaat cttttttttt tcctgcttct cctttgttaa gGTCCACCAT
79621 GAGCGAGAAA TGGGACTCAA ACTCTTCAGA AAGCTGGAAT CACATCTGGA GTGGCAATGA
79681 TACACAGCAT CACTGGTATT CAGATATCAA CATTACCTAT GTGAACTACT ATCTCCACCA
79741 GCCCCAAGTG GCAGCTGTCT TCATCAGCTC CTACCTCCTG ATCTTTGTCT TGTGCATGGT
79801 GGGAAATACT GTCGTTTGCT TTATTGTGAT AAGGAATAGA CACATGCACA CAGTCACTAA
79861 TTTCTTCATC TTAAACCTTG CCATAAGTGA TTTACTGGTT GGAATATTCT GTATGCCTAT
79921 CACATTGCTG GACAACATCA TAGCAGgtat gttggcttgt gcgctgtata atggtgatct
```

Fig. 1. Structure and sequence of the 5′ end of the human and mouse NPFF2 receptor genes. (A) Schematic presentation of the 5′ ends of the cloned human and mouse NPFF2 receptor transcripts. The solid line represents human/mouse NPFF2 receptor variant1, dashed-dotted line human/mouse NPFF2 receptor varian2 and dotted line human NPFF2 receptor variant3. (B) The genomic sequence of the 5′ end of the human NPFF2 receptor gene (EMBL genomic database AC106898 and AC106051, Homo sapiens chromosome 4), and (C) mouse NPFF2 receptor gene (EMBL genomic database AC134840, Mus musculus chromosome 5). Introns at lower case and exons at upper case, the splice sites underlined and in bold face, and putative start codons in bold.
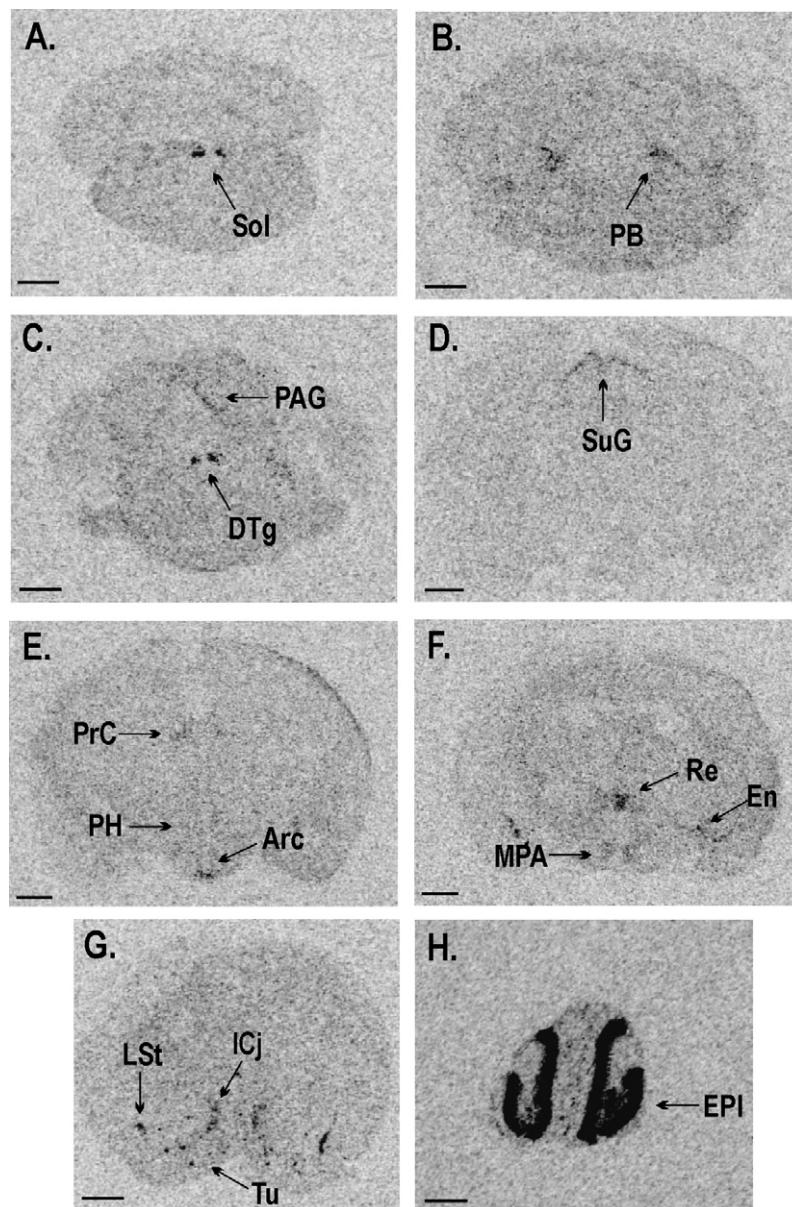
Fig. 2. The distribution of the NPFF2 receptor mRNA in the mouse CNS analyzed with in situ hybridization (A–H). Scale bar 0.1 mm. Arc, arcuate hypothalamic nucleus; DTg, dorsal tegmental nucleus; En, endopiriform nucleus; EPl, external plexiform layer of olfactory bulb; ICj, islands of Calleja; LSt, lateral striatal stripe; MPA, medial preoptic area; PAG, periaqueductal gray; PB, parabrachial nucleus; PH, posterior hypothalamic area; PrC, precommisural nucleus; Re, reunions thalamic nucleus; Sol, nucleus of the solitary tract; SuG, superficial gray, superior colliculus; Tu, olfactory tubercle.

nal in the external plexiform layer suggests that NPFF2 receptor may modulate olfactory singalling on the level of mitral or granule cells.

The results from the RT-PCR analysis are well in accordance with the in situ hybridization data. The only exception is the hippocampus where no signal was detected with in situ hybridization but a clear band was observed with RT-PCR. The results are also in accordance with NPFF receptor binding studies. The NPFF2 receptor mRNA was detected only at sites where also receptor binding has been described [11,23]. However, the receptor binding studies have shown signals at sites where no NPFF2 receptor mRNA was detected in this study. As the NPFF mRNA expressing neurons do not project to these binding sites, some other receptor(s) may contribute to

signal detected with the NPFF radioligands. The comparison of the mouse NPFF2 receptor mRNA expression with rat shows that there are distinct species differences. In accordance with this, the distribution of NPFF2 receptor ligand binding sites is different in mouse and rat [11]. There are also differences in the NPFF2 receptor mRNA expression between human and rat [7], implying that the NPFF system may have distinct species-specific features.

4. Conclusions

These data together with the previously published reports support the finding that human and mouse NPFF2 receptors
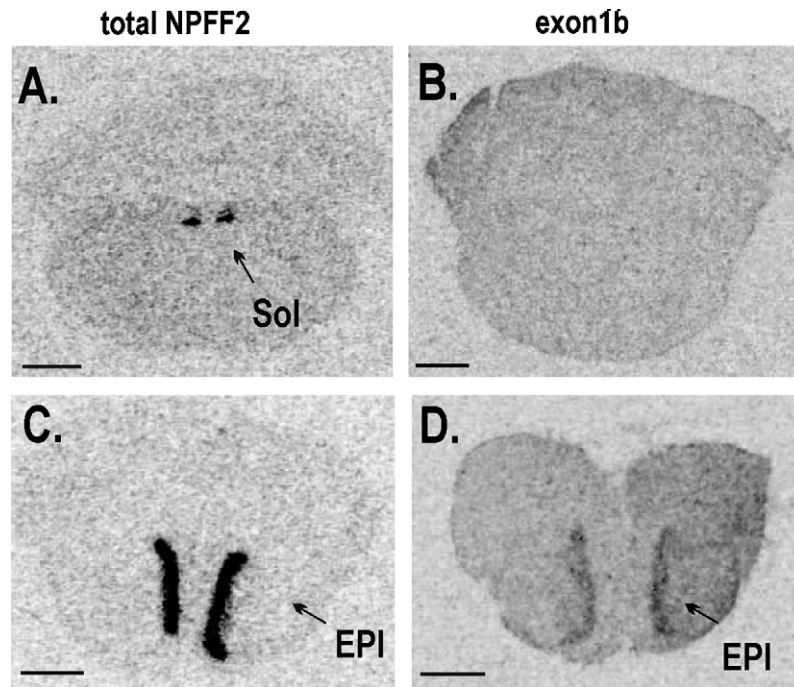
Fig. 3. The distribution of NPFF2 receptor splice variants in the mouse CNS analyzed with in situ hybridization. Representative sites are shown. (A and C) Probe detecting the total NPFF2 receptor; (B and D) probe specific for variant2. Scale bar 0.1 mm. EPl, external plexiform layer of olfactory bulb; Sol, nucleus of the solitary tract.
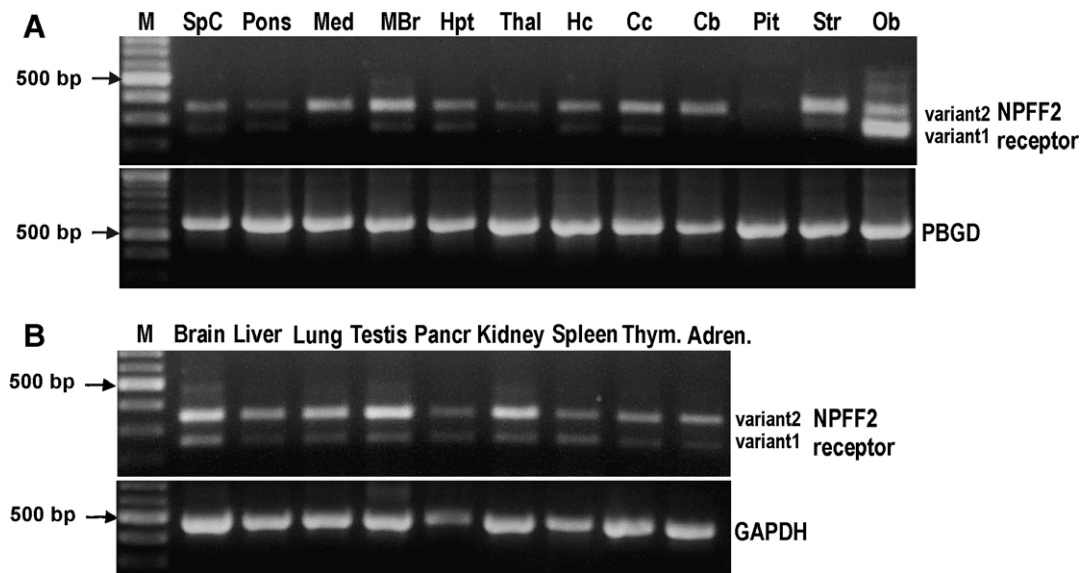

Fig. 4. The distribution of the mouse NPFF2 receptor splice variants analyzed with RT-PCR. (A) CNS. *Upper panel*: NPFF2 receptor splice variants. *Lower panel*: PBGD. Samples from left to right: marker (M), spinal cord (Sc), pons, medulla oblongata (Med), midbrain (MBr), hypothalamus (Hpt), thalamus (Thal), hippocampus (Hc), cerebral cortex (Cc), cerebellum (Cb), pituitary (Pit), striatum (Str), and olfactory bulb (Ob). (B) Non-neuronal tissues. *Upper panel*: NPFF2 receptor splice variants. *Lower panel*: GAPDH. Samples from left to right: marker (M), whole brain, liver, lung, testis, pancreas (pancr), kidney, spleen, thymus, and adrenals.

are alternatively spliced. In human, there are at least three receptor variants compared to two found in mouse. The variants1 and 2 have a similar exon–intron structure in mouse and human. The alternative splicing may be involved in the regulation of the tissue-specific expression of the receptor since the mouse NPFF2 variants have distinct expression patterns. The mouse NPFF2 receptor expression pattern differs from that of rat and, thus, the NPFF system may have species-specific functions.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2006.11.063.

References

[1] Yang, H.-Y.T., Fratta, W., Majane, E.A. and Costa, E. (1985) Isolation, sequencing, synthesis, and pharmacological characterization of two brain neuropeptides that modulate the action of morphine. Proc. Natl. Acad. Sci. USA 82, 7757–7761.

[2] Kivipelto, L. and Panula, P. (1991) Origin and distribution of neuropeptide-FF-like immunoreactivity in the spinal cord of rats. J. Comp. Neurol. 307, 107–119.

[3] Panula, P., Aarnisalo, A.A. and Wasowicz, K. (1996) Neuropeptide FF, a mammalian neuropeptide with multiple functions. Prog. Neurobiol. 48, 461–487.

[4] Vilim, F.S., Aarnisalo, A.A., Nieminen, M.L., Lintunen, M., Karlstedt, K., Kontinen, V.K., Kalso, E., States, B., Panula, P. and Ziff, E. (1999) Gene for pain modulatory neuropeptide NPFF: induction in spinal cord by noxious stimuli. Mol. Pharmacol. 55, 804–811.

[5] Nieminen, M.L., Brandt, A., Pietila, P. and Panula, P. (2000) Expression of mammalian RF-amide peptides neuropeptide FF (NPFF), prolactin-releasing peptide (PrRP) and the PrRP receptor in the peripheral tissues of the rat. Peptides 21, 1695–1701.

[6] Sundblom, D.M., Hyrkko, A. and Fyhrquist, F. (1998) Pulsatile secretion of neuropeptide FF into human blood. Peptides 19, 1165–1170.

[7] Bonini, J.A., Jones, K.A., Adham, N., Forray, C., Artymyshyn, R., Durkin, M.M., Smith, K.E., Tamm, J.A., Boteju, L.W., Lakhlani, P.P., Raddatz, R., Yoa, W.-J., Ogozalek, K.L., Boyle, N., Kouranova, E.V., Quan, Y., Vaysse, P.J., Wetzel, J.M., Branchek, T.A., Gerald, C. and Borowsky, B. (2000) Identification and characterization of two G protein-coupled receptors for neuropeptide FF. J. Biol. Chem. 275, 39324–39331.

[8] Elshourbagy, N.A., Ames, R.S., Fitzgerald, L.R., Foley, J.J., Chambers, J.K., Szekeres, P.G., Evans, N.A., Schmitdt, D.B., Buckley, P.T., Dytko, G.M., Murdock, P.R., Tan, K.B., Shabon, U., Nuthulaganti, P., Wang, D.Y., Wilson, S., Bergsma, D.J. and Sarau, H.M. (2000) Receptor for the pain modulatory neuropeptides FF and AF is an orphan G protein-coupled receptor. J. Biol. Chem. 275, 25965–25971.

[9] Hinuma, S., Shintani, Y., Fukusumi, S., Iijima, N., Matsumoto, Y., Hosoya, M., Fujii, R., Watanabe, T., Kikuchi, K., Terao, Y., Yano, T., Yamamoto, T., Kawamata, Y., Habata, Y., Asada, M., Kitada, C., Kurokawa, T., Onda, H., Nishimura, O., Tanaka, M., Ibata, Y. and Fujino, M. (2000) New neuropeptides containing carboxy-terminal RFamide and their receptor in mammals. Nat. Cell. Biol. 2, 703–708.

[10] Kotani, M., Mollereau, C., Detheux, M., Le Poul, E., Brezillon, S., Vakili, J., Mazarguil, H., Vassart, G., Zajac, J.M. and Parmentier, M. (2001) Functional characterization of a human receptor for neuropeptide FF and related peptides. Br. J. Pharmacol. 133, 138–144.

[11] Gouarderes, C., Puget, A. and Zajac, J.M. (2004) Detailed distribution of neuropeptide FF receptors (NPFF1 and NPFF2) in the rat, mouse, octodon, rabbit, guinea pig, and marmoset monkey brains: a comparative autoradiographic study. Synapse 51, 249–269.

[12] Änkö, M.-L. and Panula, P. (2005) Regulation of endogenous human NPFF2 receptor by neuropeptide FF in SK-N-MC neuroblastoma cell line. J. Neurochem. 96, 573–584.

[13] Cikos, S., Gregor, P. and Koppel, J. (1999) Sequence and tissue distribution of a novel G-protein-coupled receptor expressed prominently in human placenta. Biochem. Biophys. Res. Commun. 256, 352–356.

[14] Parker, R.M., Copeland, N.G., Eyre, H.J., Liu, M., Gilbert, D.J., Crawford, J., Couzens, M., Sutherland, G.R., Jenkins, N.A. and Herzog, H. (2000) Molecular cloning and characterisation of GPR74 a novel G-protein coupled receptor closest related to the Y-receptor family. Brain Res. Mol. Brain Res. 77, 199–208.

[15] Laemmle, B., Schindler, M., Beilmann, M., Hamilton, B.S., Doods, H.N. and Wieland, H.A. (2003) Characterization of the NPGP receptor and identification of a novel short mRNA isoform in human hypothalamus. Regul. Pept. 111, 21–29.

[16] Wang, M. and Marín, A. (2006) Characterization and prediction of alternative splice sites. Gene 366, 219–227.

[17] Pan, Q., Bakowski, M.A., Morris, Q., Zhang, W., Frey, B.J., Hughes, T.R. and Blencowe, B.J. (2005) Alternative splicing of conserved exons is frequently species-specific in human and mouse. Trends Genet. 21, 73–77.

[18] Philips, A.V. and Cooper, T.A. (2000) RNA processing and human disease. Cell. Mol. Life Sci. 57, 235–249.

[19] Faustino, N.A. and Cooper, T.A. (2003) Pre-mRNA splicing and human disease. Genes Dev. 17, 419–437.

[20] Stamm, S., Ben-Arib, S., Rafalska, I., Tang, Y., Zhang, Z., Toiber, D., Thanaraj, T.A. and Soreq, H. (2005) Function of alternative splicing. Gene 344, 1–20.

[21] Xing, Y. and Lee, C.J. (2005) Protein modularity of alternatively spliced exons is associated with tissue-specific regulation of alternative splicing. PLoS Genet. 1, e34.

[22] Gouarderes, C., Faura, C.C. and Zajac, J.M. (2004) Rodent strain differences in the NPFF1 and NPFF2 receptor distribution and density in the central nervous system. Brain Res. 16 (1014), 61–70.

[23] Dupouy, V., Puget, A., Eschalier, A. and Zajac, J.M. (1996) Species differences in the localization of neuropeptide FF receptors in rodent and lagomorph brain and spinal cord. Peptides 17, 399–405.