Neuropeptide FF Distribution in the Human and Rat Forebrain: a Comparative Immunohistochemical Study

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ABSTRACT

Neuropeptide FF (NPFF) is an octapeptide implicated in a variety of physiological functions, including nociception, cardiovascular responses, and neuroendocrine regulation. The NPFF gene and its mRNA are highly conserved across species. A comparative study of NPFF distribution in the human and rat forebrain was carried out by using single NPFF and double NPFF + vasopressin (VP) immunohistochemistry. NPFF is extensively localized within neurochemical circuits of human and rat forebrain. Semiquantitative analysis revealed that the densities of NPFF cells and fibers in many forebrain nuclei in the human correlate well with those observed for the same structures in the rat. High numbers of NPFF positive neurons in the dorsomedial hypothalamic nucleus and a dense plexus of NPFF fibers surrounding the fornix within the bed nucleus of the stria terminalis were identified in the human and rat forebrain. Within the hypothalamus of both species, dense NPFF innervation was observed in the perinuclear zone of the supraoptic nucleus (SO) just dorsolateral to the VP-positive neurons. Extensive NPFF innervation of ventricular ependyma and brain microvasculature were common for both species. At the same time, obvious differences in NPFF localization between the two species were also apparent. For example, in contrast to the rat SO, no NPFF- or NPFF- + VP-immunostained cells were observed in the human SO. Knowledge of NPFF neuroanatomical localization in the human brain and the relationship of these observations to those in the rat brain may provide insight into the role of this peptide in central cardiovascular and neuroendocrine regulation. J. Comp. Neurol. 496:572–593, 2006. © 2006 Wiley-Liss, Inc.

Indexing terms: RF amide; morphine modulatory peptide; hypothalamus; vasopressin

Neuropeptide FF (NPFF; FLFQPQRF-amide) was one of the first amidated neuropeptides to be discovered in the mammalian brain (Yang et al., 1985). Injections of NPFF into the cerebral ventricles in the rat resulted in a wide range of physiological effects, suggesting a capacity of this peptide to modulate the central autonomic regulatory mechanisms (for review see Panula et al., 1995; see also Murase et al., 1996; Sunter et al., 2001; Jhamandas and MacTavish, 2003). A high concentration of NPFF in the rat hypothalamus (Panula et al., 1995), the presence of NPFFmRNA, NPFF immunoreactivity, and specific binding sites for NPFF in many subcortical nuclei (Vilim et al., 1999; Gouarderes et al., 2000) have added considerable support for this view. The genes encoding for NPFF (Vilim et al., 1999) and for two NPFF receptors (Bonini et al., 1999) and Grant sponsor: Heart and Stroke Foundations of Alberta, N.W.T., Nunavut, and Canada; Grant number: G-01-JH-0293; Grant sponsor: Canada Research Chairs Program.

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2000; Elshourbagy et al., 2000; Hinuma et al., 2000) have been isolated in human tissue. We have recently described the distribution of NPFF receptor 1 (NPFF1, FF1) in the human brain (Goncharuk et al., 2004). Moreover, NPFF was also detected in the human cerebrospinal fluid (CSF; Sundblom et al., 1997) and serum (Sundblom et al., 1995), and its rhythmic release into the circulation implies a hormonal role for this peptide (Sundblom et al., 1998). In addition, a modest rise of circulating NPFF was reported in patients undergoing electroconvulsive therapy (ECT), which also implies leakage from the CNS (Sundblom et al., 1999). Although these findings suggest an intrinsic NPFF system in humans, a systematic immunohistochemical mapping of this peptide in the human brain has not been done, not to mention a correlation of such findings with those in the rat brain, a species in which a majority of the experimental work has been performed on this peptide.

In this study, we report, for the first time, localization of the NPFF system in the human forebrain and further describe the distribution of this peptide in the rat forebrain using immunohistochemistry. Dual immunohistochemical labeling allowed us to clarify interplay of NPFF with the vasopressin (VP) neuropeptidergic system. We show that the intrinsic NPFF system in the human brain bears a close similarity to that in the rat. However, we also demonstrate some notable differences in the distribution of the peptide between the two species, which should be taken into account when attempting to extrapolate experimental effects of NPFF in the rat to a potential role in homeostatic regulation in human.

MATERIALS AND METHODS

Human brain

Nine human brain samples from six males and three females aged from 40 to 63 years who had died due to hypothermia (one case) or myocardial infarction after mechanical trauma of the chest (eight cases) were obtained by autopsy in the morgue of the Institute of Forensic Medicine (Moscow, Russia). Post-mortem delay varied from 4 to 8 hours. Neither neurological nor psychiatric disease was confirmed by clinical history or post-mortem examination. The study was approved by the local Ethics Committee.

Tissue treatment. Once a brain had been removed, the tissue block containing hypothalamus was dissected and fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4 for 14 days at 4°C. The fixed hypothalamus was divided into two symmetrical parts, and the right one was put into buffered 30% sucrose and 0.05% sodium azide for cryoprotection until it sank. It was then frozen on dry ice.

Immunohistochemistry. NPFF immunohistochemistry (five cases) and double (NPFF + VP) immunohistochemistry (four cases) were carried out on floating cryostat sections as described previously (Goncharuk et al., 2004). Briefly, the frozen right part of the hypothalamus was cut coronally into serial sections of 20 μm thickness, and each tenth section was taken for a staining procedure. The protocol included pretreatment with absolute methanol + 3% H2O2 for 10 minutes, followed by a threefold

Abbreviations

3v third ventricle
AAA anterior amygdaloid area
AAV anterior amygdaloid area, ventral part
ACo anterior cortical amygdaloid nucleus
ArcD arcuate nucleus, dorsal part
ArcMP arcuate hypothalamic nucleus, medial posterior part
bv blood vessel
ac anterior commissure
aca anterior commissure, anterior part
acp anterior commissure, posterior part
BST bed nucleus of the stria terminals
BSTC bed nucleus of the stria terminals, central division
BSTL bed nucleus of the stria terminals, lateral division
BSTPL bed nucleus of the stria terminals, lateral division, posterior part
BSTM bed nucleus of the stria terminals, medial division
CC corpus callosum
CPu caudate putamen (striatum)
DMC dorsomedial hypothalamic nucleus, central part
DMH dorsomedial hypothalamic nucleus
DMV dorsomedial hypothalamic nucleus, ventral part
DTM dorsal tubero mamillary nucleus
E ependyma and subependymal layer
fx fornix
gcc genu of the corpus callosum
HDB nucleus of the horizontal limb of the diagonal band
inf infundibular nucleus
ic internal capsule
InS infundibular stem
Ld lambda septal zone
lo lateral olfactory tract
LM lateral mammillary nucleus
LSD lateral septal nucleus, dorsal part
LSI lateral septal nucleus, intermediate part
LSV lateral septal nucleus, ventral part
LV lateral ventricle
mfb medial forebrain bundle
ML medial mammillary nucleus, lateral part
MM medial mammillary nucleus, medial part
MMm medial mammillary nucleus, median part
MnPO median preoptic nucleus
MRe mamillary recess of the 3rd ventricle
MS medial septal nucleus
mmt mammotegmental tract
opt optic tract
ox optic chiasm
Pa paraventricular hypothalamic nucleus
PaAP paraventricular hypothalamic nucleus, anterior parvicellular part
PaD paraventricular hypothalamic nucleus, dorsal part
PaDC paraventricular hypothalamic nucleus, dorsal cap
PaLM paraventricular hypothalamic nucleus, lateral magnocellular part
PaM paraventricular hypothalamic nucleus, magnocellular part
PaMP paraventricular hypothalamic nucleus, magnocellular part
PaP paraventricular hypothalamic nucleus, parvicellular part
PaPo paraventricular hypothalamic nucleus, posterior part
Pe periventricular hypothalamic nucleus
PeF perifornical nucleus
PMD pre mamillary nucleus, dorsal part
SCh suprachiasmatic nucleus
SFi septofimbrial nucleus
SHi septohippocampal nucleus
SO supraoptic nucleus
sso supraoptic decussation
VHM ventromedial hypothalamic nucleus
VHC ventromedial hypothalamic nucleus, central part
VMHDM ventromedial hypothalamic nucleus, dorsomedial part
VMHVL ventromedial hypothalamic nucleus, ventrolateral part
VMP vasopressin
VTM ventral tubero mamillary nucleus
ZI zona incerta
rinse in TBS (altogether 5 x 10 minutes; pH 7.6), followed by incubation with the rabbit polyclonal NPFF antibody (1:4,000; overnight at 4°C), rinsing in TBS (3 x 10 minutes), incubation with biotinylated goat anti-rabbit IgG (H + L) (Vector; 1:400; 1.5 hours), rinsing in TBS (3 x 10 minutes), incubation with ABC (Vector; 1:800; 1.5 hours), rinsing in TBS (3 x 10 minutes), incubation in a mixture containing 0.05% 3,3’-diaminobenzidine tetrachloride, 0.2% nickel ammonium sulfate, and 0.001% H2O2 (10 – 15 minutes), and rinsing in TBS (2 x 10 minutes). The final dark blue granular product in cell bodies and processes identified the location of NPFF. For double (NPFF + VP) labeling, NPFF-immunostained sections were further incubated with polyclonal rabbit VP antibody (1:4,000; overnight at 4°C) and then treated with, along with the above-described protocol, exclusion of nickel ammonium sulfate in the final incubation. This procedure resulted in a yellow-brown granular product in cell bodies and processes containing VP. Immunohistochemically stained sections were mounted on Superfrost/Plus (Fisher) microscope slides, air dried, dehydrated in graded alcohols, cleared in xylene, and coverslipped with Cytoseal 60 mounting media (Stephens Scientific). For reference purposes, in the cases of single NPFF staining (three samples), adjacent sections were processed for Nissl staining by using 0.5% thionine. Identification of hypothalamic nuclei and surrounding areas was carried out by using the human brain atlases of Saper (1990), Sakamoto et al. (1999), and surrounding areas was carried out by using the human brain atlases of Saper (1990), Sakamoto et al. (1999), and Paxinos and Watson (1997).

Antibodies

We began this study in 1998–1999 in the Netherlands Institute for Brain Research (Amsterdam) and used the same NPFF antibody (batches 1 and 2), which was kindly provided by Dr. H.-Y.T. Yang (NIMH, Washington, DC) to Prof. Fred van Leeuwen (Netherlands Institute for Brain Research, Amsterdam). This polyclonal rabbit antibody was raised against synthetic NPFF (F8Famide), and its specificity was initially established with standard radioimmunoassay (RIA). Further evidence for its specificity was obtained from identification of immunoreactive NPFF in bovine brain extract by highly sensitive reverse-phase HPLC combined with RIA (HPLC-RIA; Majane and Yang, 1987). In addition, the antibody was extensively tested by using both immunostaining of blot spots and rat brain immunohistochemistry (Boersma et al., 1993). The data obtained showed that this NPFF antibody has no cross-reactivity with NPY, FMRFamide, PYY, γ-MSH, Met3-enk, substance P, Met3-enk-Arg4-Phe7, CCK-8, Arg-PheNH2, AVP, AVP-neurophysin, AVP-glycopeptide 22–39, OXT, DYN 1–8, DYN 1–17, Leu-ENK, YGGFMRF, galanin, angiotensin II, or α-neoendorphin (Majane and Yang, 1987; Boersma et al., 1993). A highly sensitive HPLC-RIA analysis of bovine brain extract showed that the antibody also detected one minor peak in the position of NPAF (A15Famide), which might be due to its weak cross-reactivity with this peptide (Majane and Yang, 1987). In other studies, similarly developed polyclonal rabbit NPFF antibody also demonstrated very low (2% or less) affinity to NPAF (Labrouche et al., 1993; Sundblom et al., 1995). It should be emphasized that experimental data on NPFF in the human brain are not yet available. Therefore, we examined additionally specificity of the NPFF antibody by Western blots of human brain lysate and immunohistochemical staining of human brain sections with blocking controls using preincubation of the antibody with NPFF, NPY, FMRFamide, or NPVF, identified recently by Liu et al. (2001). For a comparison, we carried out the same controls for the rat brain.

To detect VP, we used a rabbit VP antibody (Truus, No. 19895, NIBR) produced in the Netherlands Institute for Brain Research, Amsterdam. For details on staining procedure and specificity controls, see Van der Woude et al. (1995).

Image processing

Brightfield images were acquired digitally on an Axioplan 2 microscope (Zeiss) with an AxioCamMRc camera (Zeiss), using MRGrab software package (Zeiss). The digital images were subsequently edited in CorelDraw 9, and in the process only brightness and contrast were adjusted. Table 1, which depicts the relative densities of NPFF-immunopositive cell bodies and fibers, was constructed by inspection of sections by three independent observers.

RESULTS

Characterization of NPFF antibody in human and rat brain tissue

Western blot analyses of human and rat brain tissue with the NPFF antibody and NPFF antibody preincubated with the NPFF, NPY, FMRFamide, or NPVF were carried out. Immunoblots using human and rat brain homogenates revealed a single band of molecular weight approximately 28 kDa by using the NPFF antibody, which was not observed when the same brain tissues from either species were probed with NPFF antibody preincubated
TABLE 1. Density of NPFF-Immunopositive Neurons and Fibers in Human and Rat Forebrain

<table>
<thead>
<tr>
<th>Region</th>
<th>Human</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior amygdaloid area</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Basal nucleus, compact part</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Bed nucleus of the stria terminalis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dorsomedial hypothalamic nucleus</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Horizontal limb of the diagonal band (HDB)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Infundibular (arcuate) nucleus (inf)</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Lateral hypothalamic area (LH)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lateral tuberal nucleus (LT)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Medial forebrain bundle (mbf)</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Paraventricular hypothalamic nucleus (Par)</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Perireticular hypothalamic nucleus (PeF)</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Periventricular hypothalamic nucleus (Pe)</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Posterior hypothalamic area (PA)</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Supraoptic nucleus (SO)</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Tuberal hypothalamic nucleus (Tu)</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Ventromedial hypothalamic nucleus (VMH)</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

1Three levels of the density are characterized and marked as: +, scattered cellular profiles and single fibers; +++, single groups in two or three cells and moderate number of fibers; +++, several clusters in four to six or more cellular profiles and dense fiber networks.

For the human brain, we observed NPFF-positive neuronal profiles and fibers in many forebrain nuclei extending from the optic chiasm to the level of thalamic nuclei and mammillary bodies. The number of NPFF neurons varied markedly between different nuclei and even between their subdivisions, but it should be noted that hypothalamic nuclei located close to the wall of the third ventricle and those positioned ventrolaterally below the anterior commissure (ac) demonstrated the highest density of NPFF immunostaining. Thus, in the most rostral sections at the level of anterior end of the third ventricle, only scattered NPFF-positive fibers penetrating ependyma of the lateral ventricle from the edge of septal nuclei were observed (not shown), whereas several NPFF neurons and fibers were found in all divisions of the bed nucleus of the stria terminals (BST; Figs. 2, Table 1). Within the BST, higher density of NPFF immunoreactivity was revealed in the central division (BSTc), especially in the vicinity of the ac (Fig. 2B). It should be pointed out that the bipolar shape and the small size (up to 20 μm) of NPFF neurons observed in the BST are typical for neurons in this nucleus. Also, the random orientation of the NPFF neuronal profiles and fibers in the medial division of the BST (BSTM; Fig. 2C) and in the BSTc at the level of the ac (Fig. 2B,D) is characteristic of neurons located in this area. In more caudal sections, the NPFF neurons and fibers in the posterior division of the BST (BSTP) were also oriented rather randomly (Fig. 2F), whereas those located in the BSTC at this level appeared to course in parallel with the fibers of the stria terminalis (st; Fig. 2G). We also visualized a dense plexus of the NPFF fibers in the subependymal layer covering the fornix (fx) at this level (Fig. 2H). It should be noted that ependymal layer throughout the entire ventricular system was highly innervated by NPFF fibers, with many of them seen to protrude into the ventricular cavity.

Abundant NPFF fibers surrounding the SCh were observed (Fig. 3A). Many of these penetrated deeply into the SCh and were sometimes seen to cover the profiles of the VP-containing neurons (Fig. 3B). Also within the SCh, NPFF-positive fibers were every so often observed to follow capillaries over a long distance, especially in the ventromedial part of the nucleus. We identified scattered NPFF neurons within the human SCh (Fig. 3B). Similarly, great numbers of NPFF fibers were visualized within the medial forebrain bundle (mbf) area, just dorsal and lateral to the suprachiasmatic nucleus (SO; Fig. 3C). These fibers projected to large VP-containing neurosecretory cells positioned ventrally and even some positioned deeper within the SO (Fig. 3D). Moderate numbers of NPFF neurons were identified in the hypothalamic paraventricular nucleus (Pa; Table 1). These neurons were characterized by their small bipolar shape and were distributed either randomly or, more rarely, as small groups within the nucleus (Fig. 3F). NPFF fibers within the Pa were often observed to pass very close to neuronal perikarya of VP-positive cells, seemingly making synaptic-like contacts (Fig. 3G). Interestingly, NPFF fibers together with VP fibers were seen to penetrate deeply into the blood vessel walls (Fig. 3H).

We also observed a moderate number of NPFF neurons in the ventromedial hypothalamic nucleus (VMH; Fig. 4).
Fig. 2. Specificity of the NPFF antibody (NPFFab) in human brain tissue. Serial sections of the human suprachiasmatic nucleus (SCh) were stained immunohistochemically with NPFFab (A,B), NPFFab preincubated with NPFF (NPFFab + NPFF; C,D), neuropeptide Y (NPFFab + NPY; E,F), FMRFamide (NPFFab + FMRF; G,H), or neuropeptide NPVF (NPFFab + NPVF; I,J). Areas boxed in A,C,E,G,I are present at higher magnification in B,D,F,H,J, respectively. Note the absence of any immunoreactivity in sections treated with NPFFab preincubated with the NPFF (C,D). Note also that preincubation of the NPFFab with NPY, FMRF, or NPVF did not affect pattern or intensity of immunostained neuronal elements (arrowheads in F,H,J, respectively), which were indistinguishable from those observed in an adjacent section treated with NPFFab only (arrowhead in B). Scale bars = 200 μm in I (applies to A,C,E,G,I); 20 μm in J (applies to B,D,F,H,J).
Fig. 3. Specificity of the NPFF antibody (NPFFab) in the rat brain tissue. Serial sections of the rat brain at the level of dorsal part of the lateral septal nucleus (LSD) were stained immunohistochemically with NPFFab (A,B), NPFFab preincubated with NPFF (NPFFab + NPFF; C,D), neuropeptide Y (NPFFab + NPY; E,F), NPFFab + FMRFamide (NPFFab + FMRF; G,H), or neuropeptide NPVF (NPFFab + NPVF; I,J). Areas boxed in A,C,E,G,I are present in B,D,F,H,J, respectively. Note that only preincubation of NPFFab with NPFF abolished any immunostaining (C,D). At the same time, the pattern and staining intensity of immunoreactive fibers in sections treated with NPFFab + NPY, NPFFab + FMRF, NPFFab + NPVF (arrowheads in F,H,I, respectively) were not changed compared with those observed in an adjacent section treated with NPFFab only (arrowhead in B). Scale bar = 100 μm in I (applies to A,C,E,G,I); 20 μm in J (applies to B,D,F,H,J).
Somewhat higher numbers of NPFF neuronal profiles were identified in the dorsomedial hypothalamic nucleus (DMH), especially within its caudal portion (Fig. 6A,C), in the perifornical nucleus (PeF; Fig. 6E), and in the infundibular nucleus (inf, Fig. 6F). In general, these cells were bipolar and small (up to 20 μm) and were clustered as small groups, each containing on average three or four cells (Fig. 6C,E,F). Sometimes we observed large bipolar NPFF profiles (about 30 μm) in the PeF, especially in the ventrocaudal part of this nucleus (Fig. 6E). NPFF fibers, which appeared to contact nonstained neuronal profiles (Fig. 6B) and also to innervate blood vessels (Fig. 6F), were observed in all hypothalamic nuclei at this level.

Relatively high numbers of NPFF neurons were identified in the horizontal limb of the diagonal band (HDB), the compact part of the nucleus basalis of Meynert (BC), and the anterior amygdaloid area (AAA). Considerably fewer NPFF-positive neurons were seen in the zona incerta (ZI; Table 1). In all these nuclei, multipolar NPFF cellular profiles with a marked number of branching processes were observed (Fig. 6G,H). Finally, only occasionally were NPFF neurons present in the lateral tuberal nucleus (Ltu) or tuberomamillary nucleus (TM), and we did not observe any NPFF immunoreactivity in the medial and lateral mammillary nuclei. Data concerning the overall distribution of NPFF-immunoreactive neuronal cells and fibers in the human forebrain are summarized and presented in a set of maps (Fig. 7).

NPFF immunoreactivity in the rat hypothalamus and adjacent structures

For the rat forebrain, we observed NPFF-immunoreactive cells and fibers mainly in the septum, hypothalamus, and extended amygdala. Within the septum, NPFF immunoreactivity was not distributed evenly. The medial part of the septum was characterized by poor NPFF immunostaining, whereas lateral and ventral parts contained more NPFF-positive cells and fibers. Indeed, the septohippocampal nucleus (SHi), lamboid septal zone (Ld) and medial septal nucleus (MS) all contained very few NPFF-immunostained cells and fibers. Moreover, within all three parts of the lateral septal nucleus, dorsal (LSD), intermediate (LID), and ventral (LSV), as well as in the septomamilibrum nucleus (SFi), high numbers of NPFF fibers were observed close to the internal wall of the lateral ventricle, with many of the fibers penetrating into the ependymal layer (Fig. 8A,B). The vertical limb of the diagonal band (VDB) contained a few NPFF neurons, whereas the HDB contained many more NPFF cells and fibers (Fig. 8C,D, Table 1).

The most caudal forebrain structures, at the level of the preoptic region of hypothalamus, were characterized by significant amounts of NPFF immunostaining. Thus, a number of NPFF-positive cells and especially fibers were observed in the shell of the accumbens nucleus (AcbSh), in the islands of Calleja (both ICj and ICjM; not shown), in all divisions of bed nucleus of the stria terminalis (BST; Fig. 8E, Table 1), and in the interstitial nucleus of the posterior limb of the anterior commissure (IPAC). Unlike the lack of intense NPFF immunostaining in medially placed septal nuclei, the median preoptic nucleus (MnPO) of the hypothalamus was characterized by a high density of NPFF-immunoreactive fibers (Fig. 8E). Moreover, high numbers of NPFF fibers were identified in the dorsal part of lateral olfactory tract (lo; not shown). In addition, the NPFF-positive cellular profiles were revealed in the cortex–amygdala transition zone (CxA), anterior cortical amygdaloid nucleus (ACo), and ventral part of the anterior amygdaloid area (AAV; Fig. 8G,H).

In the anterior region of the hypothalamus, prominent NPFF immunoreactivity was revealed in the Sch, supraoptic (SO), and paraventricular hypothalamic (Pa) nuclei (Fig. 9). Dense plexi of NPFF fibers were observed in the Sch (Table 1). The fibers were located mainly in its ventrolateral part. Only sparse NPFF fibers were found among numerous VP neurons in the dorsomedial part of the nucleus (Fig. 9A,B). We did not observe any NPFF-positive perikarya within the rat Sch. The SO, however, had a moderate number of NPFF and double NPFF + VP immunostained cellular profiles (Fig. 9C,D). In addition, a dense network of NPFF fibers was observed in the area located immediately dorsal to the SO. NPFF fibers could also be seen ending on neurosecretory neurons and making synaptic-like contacts with VP cells (Fig. 9F). All parts of the Pa contained dense networks of NPFF fibers ending locally with synaptic boutons (Fig. 9G,H). Scattered perikarya containing NPFF or coexpressing NPFF and VP (Fig. 9H) were revealed in the Pa. Sometimes, small clusters of NPFF-positive neurons were observed in the most lateral posterior part of the Pa (PaPo; Fig. 10A,B).

In the tuberal region of the rat hypothalamus, NPFF immunoreactivity was revealed in the dorso- and ventromedial hypothalamic nuclei (DMH and VMH, respectively) and arcuate (Arc) and periventricular (Pe) nuclei. The NPFF-immunopositive cellular profiles were located mainly close to the boundaries of the DMH, VMH, or Arc (Fig. 11A–D). Sometimes, NPFF cells belonging to these three distinct but neighboring nuclei were observed to converge in large, densely packed neuronal cell clusters (Fig. 11B,D). This tendency for the NPFF cells to cluster was present up to the mostly caudal portions of the tuberal region. As an example, a cellular cluster of NPFF-immunopositive neurons belonging to the ventral part of dorsomedial hypothalamic nucleus (DMV), central part of the ventromedial hypothalamic nucleus (VMHC), and dorsal part of the arcuate nucleus (ArcD) at the level of infundibular stalk is shown in Figure 11E,G,H. The rat Pe

Fig. 4. NPFF-immunoreactive neuronal cells and fibers in various divisions of the bed nucleus of the stria terminalis in two consecutive coronal sections of the human brain. Areas in A boxed in the bed nucleus of the stria terminalis, medial division (BSTM), and in the bed nucleus of the stria terminalis, central division (BSTC), are shown at high-power magnification in C,D, respectively. Area marked by an asterisk in A is shown at higher magnification in B. Boxes in E, covering the area in the bed nucleus of the stria terminalis, posterior division (BSTP), and a part of the fornix (fx) with ependymal layer (E) are shown at high-power magnification in F,H, respectively. G demonstrates NPFF-positive neuronal profile in the ventrolateral part of the BSTC. Notice that NPFF-positive neuronal profiles differ markedly in size and shape from VP neurons (compare positively stained neurons in B and strongly stained neurons in D,G,H). Note random orientation of NPFF-positive neurons and fibers with punctate variabilities on the surface of the cell bodies (compare C with D). H shows NPFF-positive cells in the BSTC and BSTM. Figure 4 illustrates the possible coexistence of NPFF and VP immunoreactivities in the same cell.
LOCALIZATION OF NPFF IN THE HUMAN AND RAT FOREBRAIN

is one of the hypothalamic nuclei that contained the highest numbers of the NPFF-positive neurons (Table 1), and throughout its entire extent in the tuberal region it appeared as a narrow band of NPFF-immunopositive profiles located very close to the wall of the third ventricle (Fig. 11H).

In the mamillary region of the hypothalamus, NPFF immunoreactivity was observed in the dorsal and ventral tuberomamillary nuclei (DTM and VTM, respectively) and in the medial posterior part of the arcuate nucleus (ArcMP). High numbers of NPFF-immunostained neurons were observed in the DTM on both sides of the mamillary recess of the third ventricle (MRe; Fig. 12A–C). Only scattered, mainly small to medium-sized bipolar, but sometimes larger multipolar, NPFF-positive, neurons were observed dorsally in the ArcMP (Fig. 12D). In the most caudal area of this hypothalamic region, only networks of NPFF fibers of a moderate density were visualized in the ArcMP and VTM (Fig. 12E,F). We did not identify any NPFF immunoreactivity in any of the mamillary nuclei (Fig. 12E).

We observed NPFF-immunostained neurons also in parts of the brain other than the hypothalamus and the forebrain areas adjacent to it. Thus, scattered or occasionally a few clusters of NPFF-positive cells were observed in the hippocampus or somatosensory cortex, the morphology of the neurons being typical for these brain structures. An overwhelming majority of blood vessels in the rat forebrain were strongly innervated by NPFF fibers. These fibers penetrated deeply into the vascular wall and in some cases even reached the endothelial layer. They were observed in blood vessels of various calibers within the many septal and especially hypothalamic nuclei (Fig. 13A–D).

DISCUSSION
NPFF antibody specificity

The specificity of NPFF antibody used in our study was established previously by standard RIA, immunostaining of spot blots, and HPLC-RIA analysis of bovine brain extracts and by rat brain immunohistochemistry (Majane and Yang, 1987; Boersma et al., 1993). Nevertheless, because we used human brain tissue in the present study for the first time, and because of the potential for cross-reactivity among several related RFamide peptides (Majane and Yang, 1987; Boersma et al., 1993; Labrouche et al., 1993; Sundblom et al., 1995; Yano et al., 2003), we carried out additional controls by Western blot of both human and rat brain lysates and immunohistochemistry on human and rat brain sections, using NPFF antibody preincubated with NPFF, NPY, FMRFamide, or NPVF. In Western blots, the NPFF antibody revealed a band of molecular weight approximately 28 kDa. This band was not observed in either species if the antibody was preincubated with NPFF. At the same time, it was easily detectable when the antibody was preincubated with NPY, FMRFamide, or NPVF. Although these data demonstrate, first of all, a presence of endogeneous NPFF in both human and rat brain, the immunostained band at 28 kDa in our Western blots indicates that the molecular weight of immunoreactive material is about twice as high as that calculated for the amino acid sequence of NPFF peptide precursor and predicted from both human and rat genomic databases by Vilim et al. (1999). There are several possible explanations for this discrepancy. First, the exact information on molecular weights is known to be difficult to obtain from proteins separated on gels because of gel-induced protein modifications. For example, it has been reported that residual acrylamide monomers in the gel can covalently bind with cysteine residues on proteins during electrophoresis (for reviews see Jeannot et al., 1999; Wall et al., 2001; Bronstrup, 2004), and NPFF precursor was found to contain cysteine residue (Vilim et al., 1999). Also, posttranslational modifications may shift the molecular weight of a polypeptide to the high mass end, making it markedly different from that expected from the database (for review see Williams et al., 2004). Furthermore, NPFF precursor might exist as a dimer, thereby doubling its molecular weight calculated from the predicted amino acid sequence. Unfortunately, experimental data on the processing of the NPFF precursor are not yet available, but posttranslational dimerization of a neuropeptide precursor has been reported to be an important factor for further proteolytic cleavage (Hekimi et al., 1989, 1991; Hidaka et al., 2000), so we cannot exclude such a possibility for NPFF precursor. In spite of these unresolved issues regarding the molecular size of the NPFF precursor, it is clear that the immunohistochemical staining observed in human brain sections is abolished only with preabsorption of the antibody using NPFF and not with other related peptides.

Furthermore, our immunohistochemical controls demonstrated a blockade of staining in both human and rat brain sections after preabsorption of antibody with NPFF but not with NPY, FMRFamide, or NPVF. Yano et al. (2003) tried to distinguish NPFF from RFRP-3/NPVF in the rat brain sections by using specific antibodies against each peptide and in situ hybridization. These authors reported that distributions of NPFF and RFR-3/NPVF neurons were different, although NPFF is known to contain the same structure at the C-terminus, PQRFamide, as RFRP-3/NPVF. With double-label immunocytochemistry, they did not demonstrate a colocalization of NPFF with RFRP-3/NPVF. Moreover, preabsorption of RFRP-3/NPVF antibody with NPFF did not change the pattern of RFRP-3/NPVF immunostaining. Overall, we conclude

**Fig. 5. Neuropeptide FF (NPFF; blue)- and vasopressin (VP; yellow)-stained cellular profiles revealed by double immunohistochemistry in coronal sections from the preoptic region of the human hypothalamus.** B shows higher magnification of the area marked by an asterisk in A. D and F represent areas boxed in C and E, respectively. Note a dense network of NPFF fibers (arrowheads) surrounding VP neurons of the ScH (A). Single NPFF-positive neurons (arrow) and fibers (arrowheads) among numerous VP-immunoreactive neurons within the ScH are observed (B). A dense network of the NPFF fibers within the medial forebrain bundle (mfb) is located just dorsal to the supraoptic nucleus (SO; C). Note the NPFF fiber (arrow) terminating with synaptic-like contact on a VP neuropecretoary neuron within the dorsal region of SO (D). Note the NPFF neurons and fibers (blue) among VP fibers (brown) in the dorsal part of the paraventricular hypothalamic nucleus (PaD; E,F). Note also the NPFF fiber (arrow) making synapse on a perisynaptic-like contact with a VP neuron in the magnocellular part of the hypothalamic paraventricular nucleus (PaM; G), as well as the NPFF (blue) and VP (yellow) fibers (arrowheads) penetrating deeply into the wall of a blood vessel from the posterior part of the paraventricular hypothalamic nucleus (H). Scale bars = 100 μm in E (applies to A,C,E); 50 μm in H (applies to B,H); 20 μm in F (applies to D,F,G).
Fig. 6. NPFF-immunoreactive neuronal profiles and fibers in a coronal section from midtuberal region of the human hypothalamus at the level of the ventromedial nucleus (VMH). In A, a boxed area in the dorsomedial hypothalamic nucleus (DMH) and the perifornical nucleus (PeF) is shown at high-power magnification in C. Note clustered NPFF-positive small bipolar profiles in the DMH (C), VMH (D), and infundibular nucleus (inf; F). Note also both small and large NPFF bipolar neurons in the PeF (E) and multipolar NPFF neurons in the zona incerta (ZI; G) and in the anterior amygdaloid area (AAA; H). Also note NPFF fibers covering an unstained profile of the large cell in the DMH (B) and NPFF fibers penetrating the blood vessel wall in the inf (F, arrowheads). Scale bars = 1 mm in A; 20 μm in H (applies to B–H).
that the NPFF antibody used in our study recognized specifically the polypeptide chain containing NPFF.

**Distribution of NPFF in the human forebrain**

We demonstrate in this study that NPFF-containing neurons are widely distributed in the human forebrain. The distribution of this peptide, in general, coincides with that of neurons expressing NPFF1 receptor (FF1), which we described recently (Goncharuk et al., 2004). For example, a very high density of NPFF-positive fibers surrounding the ac in frontal sections of the central division of the bed nucleus of the stria terminalis (BSTC) corresponds to a very high density of neuronal fibers containing FF1 (Goncharuk et al., 2004), which suggests the possibility of a high number of NPFF synapses in this region. It should

![Fig. 7. Drawings summarizing the distribution of NPFF immunoreactivity in the human forebrain. Increasing degrees of stippling in the maps reflect the progressively higher concentrations of NPFF-positive neurons (A–C) and fibers (D–F) in each region. The maps depict rostral (A,D) to caudal (C,F) extent of NPFF distribution.](image-url)
Fig. 8. Neuropeptide FF (NPFF)-immunoreactive cells and fibers in a series of coronal sections through the rostrocaudal extent of the septum and preoptic region of the rat hypothalamus. Note penetration of NPFF fibers from the dorsal part of the lateral septal nucleus (LSD) into the ependymal layer (E) of the lateral ventricle (LV) wall (A,B), NPFF bipolar cellular profiles in the horizontal limb of the diagonal band (HDB; C,D), NPFF fibers in the median preoptic nucleus (MnPO) and bed nucleus of the stria terminalis (BST; E,F), and multipolar NPFF neurons in the anterior amygdaloid area, ventral part (AAV; G,H). B,D,F,H represent high-power magnification of boxed areas in A,C,E,G, respectively. Scale bar = 200 μm in G (applies to A,C,E,G); 50 μm in H (applies to B,F,H); 20 μm in D.
be noted that the existence of NPFF synapses has been demonstrated at the electron microscopic level (Kivipelto, 1991) and confirmed in electrophysiological studies (Miller and Lupica, 1997; Chen et al., 2000). Similarly, NPFF fibers penetrating into the SCh appear to form synapses within the nucleus, insofar as neuronal somata and fibers expressing FF1 were found here (Goncharuk et al., 2004). Also, a dense network of NPFF fibers observed just dorsal to the SO, in the so-called perinuclear zone (PNZ), overlapped FF1-expressing fibers (Goncharuk et al., 2004), which implies a high level of NPFF synaptic activity in this area as well. The numerous NPFF fibers in the PNZ region could originate from NPFF neurons that we observed in the HDB. Tracing experiments in animals have identified direct projections from the diagonal band of Broca (DBB) to the PNZ (Jhamandas et al., 1989). Moreover, projections from the DBB were shown electronmicroscopically to form axodendritic synapses in the PNZ (Jhamandas et al., 1989). We did not observe any NPFF neurons in the SO, nor were any FF1-expressing neurons identified there (Goncharuk et al., 2004). In addition, another receptor with a high affinity for NPFF, namely, NPF (FF2; Bonini et al., 2000; Elshourbagy et al., 2000), also is not expressed in the human SO (Goncharuk and Jhamandas, 2004). However, high numbers of NPFF fibers in the PNZ might be involved in indirect regulation of SO neurons, in that studies in the rat have shown that an interneuronal network located in the PNZ affects the activity of neurosecretory neurons within the SO (Jhamandas et al., 1991; Cunningham et al., 1994; Herbison, 1994).

We did not observe any FF1 expression in large multipolar (vasopressinergic) neurons in either the magnocellular (PaM) or the dorsal (PaD) part of the human hypothalamic Pa (Goncharuk et al., 2004). These cells were, however, found to be surrounded by small bipolar NPFF neurons and NPFF fibers. The data suggest that large neurosecretory cells in the PaM and PaD are regulated indirectly by neighbouring NPFF cells, in a manner similar to that observed for the SO. In support of this proposal, we have recently performed electrophysiological experiments in rat hypothalamic slices, which revealed NPFF to modulate γ-aminobutyric acid (GABA)-ergic inhibitory activity of magnocellular Pa neurons at a presynaptic level (Jhamandas and Harris, 2003).

Small bipolar NPFF neurons and NPFF fibers were evenly distributed in all parts of the human Pa. Some innervate closely positioned nonstained cellular profiles or blood vessels, which suggests that they are probably interneurons. On the other hand, small bipolar NPFF neurons located in the parvicellular part of the Pa (PaP) might project to the anterior part of the pituitary or autonomic regulatory centers in the brainstem. This is supported by findings of Koutcherov et al. (2000), who consider the human PaP to represent a homolog of medial parvicellular subnucleus of the rat Pa, whose neurons project to the anterior hypophysis and caudal brainstem autonomic nuclei (Swanson and Kuypers, 1980; Sawchenko et al., 1993).

Several NPFF-positive neurons of various shapes and sizes and high density of NPFF fibers in the BST and the anterior amygdaloid area (AAA) also correlate well with the high expression of FF1 and FF2 that we have described for these areas (Goncharuk et al., 2004; Goncharuk and Jhamandas, 2004). Such a correlation suggests that subnuclei of the extended amygdala might also be actively regulated by NPFF input. In addition, these structures might also serve as a source of NPFF fibers to adjacent structures.

Nuclei located more caudally, such as the DMH and VMH and the infundibular (inf) and perifornical (PeF) nuclei were characterized by the highest density of NPFF neurons and fibers. A similar very high expression of FF1 has also been identified within these structures (Goncharuk et al., 2004). At this level, we often observed NPFF axons even between lobes of the fornix in the very same region where FF1 immunoreactivity was present (Goncharuk et al., 2004). Also, good agreement with density values of NPFF and FF1 expression within these nuclei implies an important role for NPFF in neurotransmission within this region of the hypothalamus.

At the same time, we also noted certain discrepancies in the distribution of NPFF and FF1. This was most noticeable for nuclei, such as the zona incerta (ZI) and the lateral tuberal (LTu) and tuberomammillary (TM) nuclei. For example, we observed small numbers of NPFF neurons in these nuclei whereas they contained a high number of FF1-containing neurons and fibers (Goncharuk et al., 2004). One possible explanation could be that the few NPFF neurons within these nuclei serve as local circuit interneurons. On the other hand, these nuclei could contain neuronal cells expressing other NPFF-related peptides that demonstrate a higher affinity with FF1, for example, NPAF (Elshourbagy et al., 2000) or NPVF (Liu et al., 2001).

Also, it is worthwhile noting that both medial and lateral mammillary nuclei seem to be devoid of any NPFF or FF1 (Goncharuk et al., 2004) or FF2 (Goncharuk and Jhamandas, 2004) immunoreactivity. Many axon-like NPFF-immunostained fibers were observed to penetrate the ventricular lumen, and this anatomical configuration lends itself to the release of peptide into the CSF. Detection of NPFF in the human CSF (Sundblom et al., 1997) supports such a hypothesis. For all the human forebrain nuclei examined in this study, we showed NPFF fibers massively innervating numerous blood vessels of various calibers. These observations are particularly interesting in the light of our previous demonstration of FF1 within walls of vessels in identical forebrain nuclei. These anatomical data provide strong support for the notion that NPFF is a strong regulator of the microvascular tone in the human brain.

**Distribution of NPFF in the rat forebrain**

A closer examination of the NPFF distribution in the rat forebrain in our study is in good agreement with prior studies (Kivipelto et al., 1989; Boersma et al., 1993; Vilim et al., 1999) but also reveals interesting and important data not previously reported. First, we identified that NPFF fibers massively innervate ependyma throughout the ventricular system. These fibers were often smooth and probably consisted of dendrites protruding into the ventricular lumen, which could contribute to formation of the supraependymal plexus. On the other hand, some of these processes could also represent axons capable of releasing NPFF into the ventricle, thereby altering the composition of the CSF. Finally, those NPFF fibers seen to terminate in sub- or intraependymal layer may be involved in the regulation of the activity of ependymal cells. In this regard, neurons that give rise to a supraependymal plexus may play an important role in nonsynaptic signaling mediated by CSF and intercellular fluid of the brain.
Figure 9
Moreover, neuroactive substances contained in axon terminals innervating the ependyma have been shown to regulate the secretory activity of ependymal cells (Schoniger et al., 2002). Furthermore, our dual immunohistochemical study elucidated the relationship between the NPFF and the VP neuronal system in the rat hypothalamus. We showed that NPFF fibers and terminals were concentrated in the ventrolateral part of the SCh, where mainly vasoactive intestinal polypeptide (VIP)-synthesizing neurons are known to be located. Nevertheless, these NPFF fibers slightly overlapped the area occupied by VP neurons, and fine-caliber NPFF fibers were observed also to cover VP profiles. These data suggest that NPFF might synthetically regulate the activity of both VIP and, to a lesser degree, VP neurons within the rat SCh. This suggestion is supported by the data from in situ hybridization analysis (Liu et al., 2001) and binding studies (Gouarderes et al., 2004), which demonstrate the presence of specific receptors for NPFF in the rat SCh.

By using double immunohistochemical labeling, we observed a number of VP neurosecretory neurons coexpressing NPFF within the SO. It should be noted that possible coexistence of NPFF and VP in the SO was proposed by Boersma et al. (1993). These authors performed immunostaining for either VP or NPFF in adjacent serial sections and identified a few neurons that appeared to contain both peptides. These cells may transport both VP and NPFF to the posterior lobe of the pituitary, insofar as immunohistochemistry at the electron microscopic level demonstrated NPFF-containing nerve terminals contacting pituicytes (Boersma et al., 1993), and tract-tracing experiments demonstrated that at least some of these terminals originate from the SO (Majane et al., 1993). Many NPFF fibers were observed in the region immediately dorsolateral to the SO (PNZ), and occasional NPFF fibers were also noted to penetrate the SO, forming synaptic-like contacts with magnocellular VP neurons. In the vicinity of the NPFF fibers within the PNZ, numerous axosomatic synapses on GABAergic neurons have been identified at the electron microscopic level (Jhamandas et al., 1989). Although in situ hybridization (Liu et al., 2001) and receptor binding studies (Gouarderes et al., 2004) in the rat SO did not reveal expression of NPFF receptors NPFF1 and NPFF2, these studies did not specifically examine the PNZ region, which is where we identified dense NPFF fiber networks. Collectively, these data suggest that magnocellular VP neurons in the SO might be regulated indirectly by NPFF via GABAergic neurons in the PNZ. The few VP cells that were noted to be surrounded by NPFF fibers might express other receptors capable of binding NPFF; recent data demonstrated that NPFF may interact directly with, for example, the delta-opioid recep-

Fig. 10. Coronal section from the anterior region of the rat hypothalamus through the caudal level of the hypothalamic paraventricular nucleus. Boxed area in A is shown in B at high-power magnification. Note the NPFF cellular profiles in the posterior part of the hypothalamic paraventricular nucleus (PaPo; B). Scale bars = 200 μm in A; 50 μm in B.

Fig. 9. Series of coronal sections through the anterior region of the rat hypothalamus. Neuropeptide FF (NPFF; blue) and vasopressin (VP; brown) cellular profiles and processes are revealed by double immunohistochemistry in dorsal cap (PaDC); lateral magnocellular part (PaLM), medial parvcellular part (PaMP), and ventral part (PaV) of the hypothalamic paraventricular nucleus (A,B); supraoptic (SO; C,D) nuclei, and suprachiasmatic nucleus (SCh; G,H). The areas marked by asterisks in A and D are shown, at a higher magnification, in B and F, respectively. D and G represent boxed areas in C and E, respectively. Note the dense NPFF innervation of the periventricular hypothalamic nucleus (Pe) and ventrolateral part of the SCh (A,B), whereas only scattered NPFF fibers are observed among VP cells in dorsal part of the SCh (arrowheads in B). Note also double NPFF- + VP-immunostained cells in the SO (arrows in D), one of which is shown at higher magnification in F (arrow). An NPFF-positive fiber synapsing on a VP neurosecretory neuron in SO is indicated by an arrowhead in F. Note the dense network of NPFF fibers in the PaDC, PaLM, PaMP, and PaV (E) and the numerous synaptic-like contacts on large VP neurons in the PaLM (G). A double NPFF- + VP-immunostained cell in anterior parvcellular part of the hypothalamic paraventricular nucleus (PaAP) is shown by arrow in H. Scale bars = 100 μm in E (applies to A,C,E); 50 μm in G (applies to B,G); 20 μm in H (applies to D,F,H).
Fig. 11. Series of frontal sections through the tuberal region of the rat hypothalamus. B represents higher magnification of hypothalamic structures surrounding the upper part of the third ventricle. Black boxed areas in A,E are shown at higher power magnification in C,H, respectively, white boxed area in E is shown at high magnification in F. The area marked by an asterisk in B is shown at higher magnification in D. The area indicated by an asterisk in E is shown at high magnification in G. Note the predominant location of NPFF-positive cells at the edges of DMD, VMHDM, ArcD (A,B,C,E,G), and VMHC (E,F). Also note two clusters of NPFF cells located in DMD and VMHDM, respectively, which are contiguous through the boundary between these nuclei (B,D). Scale bars = 200 μm in E (applies to A,E); 100 μm in B; 50 μm in H (applies to C,D,F–H).
Fig. 12. Set of frontal sections from caudal portion of the tuberal region of the rat hypothalamus at the level of the mamillary recess of third ventricle (MRe). B represents higher magnification of the upper part of MRe seen in A (asterisk). White and black boxed areas in A and boxed area in E are shown at higher power magnification in C,D,F, respectively. Note numerous small bipolar NPFF cells in the dorsal tuberomamillary nucleus (DTM) in B,C and a single large multipolar NPFF cell in the medial posterior part of arcuate hypothalamic nucleus (ArcMP; D). Note also the dense network of NPFF fibers in the mostly caudal part of ArcMP and in the ventral tuberomamillary nucleus (VTM; E,F). Also note the absence of any NPFF immunoreactivity dorsal to the MRe, in median and medial mamillary nuclei (MMn and MM, respectively), and in the dorsal part of premamillary nucleus (PMD; E). Scale bars = 200 μm in E (applies to A,E); 100 μm in B; 50 μm in F (applies to C,D,F).
tor (Anko and Panula, 2005). Finally, we observed, in the mostly ventral part of the SO, a dense plexus of NPFF and VP fibers passing in the mediolateral direction very close to the ependyma, which suggests that both peptides may be capable of regulating activity of the ependymal layer.

High numbers of NPFF fibers and NPFF axon-like terminal boutons surrounding and covering VP neuronal profiles were observed in all parts of the rat Pa. High numbers of NPFF1 receptors, as judged by mRNA expression (Hinuma et al., 2000) and binding (Gouarderes et al., 2004), have been reported for this nucleus in the rat. Taken together these data suggest a high level of NPFF participation in synaptic transmission within the rat Pa. Our double labeling revealed only few VP profiles coexpressing NPFF, considerably fewer than those reported in a previous study using single immunostaining for VP or NPFF on adjacent sections (Boersma et al., 1993). This discrepancy might be caused by pretreatment of animals with colchicine, used in the aforementioned study, which can cause an accumulation of the peptide in perikarya, thereby increasing the number of detectable cells. The NPFF network within the Pa thus seems to be actively involved in the regulation of VP neurons projecting to both the brainstem autonomic centers and the pituitary gland. Several studies using injection of NPFF into cerebral ventricles appear to support this conclusion (Arima et al., 1996; Jhamandas and MacTavish, 2003). At the cellular level, NPFF modulates synaptic transmission to both the parvocellular and the magnocellular cells of the rat Pa (Jhamandas and Harris, 2003).

In the posterior hypothalamus, the distribution of NPFF cells in general also resembled that described previously (Kivipelto et al., 1989; Boersma et al., 1993). Additionally, however, we noted that NPFF neurons were
localized exclusively in peripheral parts of the DMH, VMH, and ARC, but never in central parts of these nuclei. In some sections, these cells formed a large, unitary cluster, covering the dorsal part of the ARC and area described by Panula et al. (1986) as the “region between DMH and VMH.” In conjunction with neurons within the Pe, the NPFF network of cells covered an area where Hinuma et al. (2000) also noted a high expression of NPFF receptor (OT7T022) mRNA. High levels of NPFF synaptic activity in this region might be involved in regulation of feeding, insofar as the ARC, DMH, and VMH are known as important participants in the control of food intake (for reviews see Schwartz et al., 2000; Horvath, 2005; Muntzberg and Myers, 2005). Moreover, i.c.v. injection of NPFF has been shown to modulate feeding behavior (Murase et al., 1996; Sunter et al., 2001). In addition, NPFF neurons located here might be involved in a much wider spectrum of adaptive reactions, because they project to the Pa, which is known to be a main integrative and regulatory hypothalamic center, and the brainstem autonomic nuclei (Jhamandas et al., 2001).

Finally, for many forebrain nuclei, we observed NPFF fibers to innervate blood vessels of various calibers. Intravenous injection of NPFF has been shown to result in a significant increase in blood pressure (Roth et al., 1987; Allard et al., 1995). Allard et al. suggested that NPFF might act through the stimulation of specific NPFF receptors located on vascular sympathetic terminals to evoke a pressor response. At the same time, the increase in blood pressure was attenuated only by 30% in catecholamine-depleted rats and by 50% in beta-receptor antagonist-pretreated rats. Moreover, these authors did not observe any increase in plasma noradrenaline and adrenaline levels during the peak pressor response after systemic NPFF injections. The authors suggested, on the basis of these collective data, that NPFF pressor effect is in part mediated by catecholamine-independent mechanisms. The presence of specific NPFF receptors in the rat vascular wall might be hypothesized to explain these catecholamine-independent blood pressure effects, but this requires experimental confirmation. The dense NPFF innervation of brain microvasculature that we observed suggests that this peptide could directly influence local microcirculation and may serve as an impetus to define a larger role for NPFF in the control of vascular tone.

Comparison of NPFF distribution in the human and rat forebrain

Our comparison of NPFF localization in the human and rat forebrain reveals that this peptide represents a widely distributed neurochemical system. In general, its organization is similar in both species. First, a dense plexus of NPFF fibers was found to innervate an ependymal layer of the wall of cerebral ventricles in both the human and the rat brain. Moreover, a high density of NPFF immunoreactivity in specific hypothalamic nuclei was observed in both species. For example, numerous NPFF fibers surrounding Fx within the BST, dense plexi of NPFF fibers in perinuclear zone dorsal to the SO, or a high number of NPFF neurons in some medial hypothalamic nuclei were characteristic for both species. Also, NPFF fibers contacting the VP neuronal population in the SCh were similarly located, and a comparable density was found in both human and rat. At the same time, coexpression of NPFF and VP in magnocellular neurosecretory neurons within the human Pa was observed much less frequently compared with the rat Pa. The most prominent differences in this regard were seen between the human and the rat SO. We did not observe significant NPFF immunoreactivity within the human SO, whereas numerous NPFF cells and fibers were found in the rat SO. Moreover, in the rat SO, many large VP neurosecretory cells also expressed NPFF. In most human hypothalamic nuclei, NPFF cells were distributed seemingly randomly, whereas they were usually clustered in the rat hypothalamus, especially in the caudal part, where such clusters were identified at boundaries of the nuclei proper. Finally, considerable NPFF innervation of the vasculature was a general phenomenon for both human and rat forebrain nuclei.

In summary, our study provides novel data concerning the distribution of NPFF in the human brain and its relationship to an important neuropeptide, VP, in the context of autonomic and neuroendocrine regulation. In particular, our observations on the NPFF innervation of the ependyma and cerebral vasculature suggest a new role for this peptide as a neurohumoral and neurovascular regulator. We report a distribution of NPFF in the human forebrain similar to that observed in the rat forebrain, but we also highlight important differences in this regard between the two species. These comparative observations may be particularly salient in extrapolating data on NPFF to the human situation from studies in the rat, in which multiple physiological functions for NPFF have been identified.

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LITERATURE CITED


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