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Review

Neuroendocrine regulatory peptide-1 and -2: Novel bioactive peptides processed from VGF

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Abstract. Neuroendocrine regulatory peptides (NERP)-1 and NERP-2 are derived from distinct regions of VGF, a neurosecretory protein that was originally identified as a product of a nerve growth factor-responsive gene in rat PC12 cells. The amino acid length of human NERP-1 is 26, and that of rat NERP-1 is 25. Human and rat NERP-2 are both 38 amino acid peptides. NERPs colocalize with vasopressin in the storage granules of the paraventricular and supraoptic nuclei in the hypothalamus of both rats and humans. Administration of NERPs suppresses hypertonic saline- or angiotensin II-induced vasopressin release from the hypothalamus and pituitary. Thus, VGF is a precursor of multiple bioactive peptides with diverse neuroendocrine functions, and NERPs are novel hypothalamic peptides involved in the control of body fluid homeostasis by regulating vasopressin release.

Keywords. Neuroendocrine regulatory peptide, vasopressin, hypothalamus, VGF, processing, feeding regulation.

Introduction

The identification of new bioactive peptides is an important step towards elucidation of novel biological systems in the body and the development of innovative drugs. A large array of peptide hormones and neuropeptides function as cell-cell signaling molecules and mediate a variety of physiological processes. The hypothalamus, which occupies the ventral half of the diencephalon and lies immediately above the pituitary gland, functions as an essential interface between endocrine, autonomic, and somatomotor systems [1]. It regulates the cardiovascular system, thermoregulatory responses, and the abdominal viscera, as well as defensive-aggressive behavior, feeding behaviors, and sexual and maternal behaviors. Many neuropeptides serve as signaling molecules in hypothalamic control mechanisms linking the brain and peripheral organs. The majority of peptide receptors are G protein-coupled receptors (GPCRs), but the ligands for these GPCRs have not all been identified. An endogenous ligand screen using cell lines that artificially express orphan GPCRs, together with genetic engineering techniques, has expanded our understanding of novel cell-cell signaling systems in the hypothalamus. Some hypothalamic neuropeptides, such as orexins [2], ghrelin [3], and neuropeptide W [4] have been identified as ligands for orphan GPCRs. However, although an increasing number of mammalian genomes have been sequenced, the discovery of new bioactive peptides has not increased at

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the expected pace, mostly due to the lack of *in vivo* data on bioactive peptides.

Bioactive peptides are cleaved from their precursor proteins via limited cleavage and often must undergo post-translational modifications to become biologically active [5, 6]. Recently, a comprehensive analysis of all secretory peptides produced by human medullary thyroid carcinoma TT cells identified 230 peptides [7]. Some derive from calcitonin, a peptide functioning in skeletal conservation and fertilization [8–10], others from calcitonin gene-related peptide (CGRP), a potent dilator of blood vessels [11]. Still others derive from chromogranin, secretogranin, or other known peptide hormone precursors [12]. Only 10% of the 230 peptides were derived from proteins annotated as non-secretory peptides in the public database. Nineteen peptides with C-terminal amidation, the post-translational modification most frequently observed in bioactive peptides [13], were identified, with 15 corresponding to entire or partial sequences of calcitonin and CGRP [7]. Of particular note were two novel amidated peptides with monoisotopic masses of 2677.4 and 4062.2 and shorter fragments with masses of 2521.4 and 3405.2. We designated these neuroendocrine regulatory peptides (NERP)-1 and -2 on the basis of their localization and physiological roles described here. NERPs are derived from distinct regions of the neurosecretory protein VGF. In this review, we describe distribution and biological functions of NERPs, and discuss the role of VGF as a precursor of multiple bioactive peptides with diverse neuroendocrine functions.

Structures of VGF and NERPs

VGF protein is a neurosecretory protein originally identified as a product of the nerve growth factor-responsive gene *Vgf* in rat pheochromocytoma PC12 cells [14, 15]. This name is based on the selection of this clone from plate V of a nerve Growth Factor-induced PC12 cell cDNA library [14]. The *Vgf* gene is highly conserved from zebrafish to humans and encodes a 617-amino acid protein in rats, and a 615-amino acid protein in humans [15]. The human and mouse *Vgf* genes map to chromosome 7q22 and chromosome 5, respectively [16, 17]. Two introns interrupt the region encoding the 5' untranslated sequence of *Vgf*, and the entire VGF protein is encoded by exon 3 [16]. During rat development, VGF mRNA is expressed in the neural crest cells fated to become enteric ganglia at embryonic day (E)11.5 and in discrete regions of the brain and the primordia of the dorsal root, cranial, and sympathetic ganglia at E13.5 [18]. Thus, VGF expression appears to occur in the peripheral nervous system as maturing neurons cluster to form ganglia. In adult rats, VGF mRNA was detected in subsets of endocrine cells in the anterior and posterior pituitary glands, adrenal medulla, pancreas, and gastrointestinal tract [19]. VGF mRNA is also expressed in the brain, spinal cord, dorsal root ganglia, sympathetic ganglia, and enteric nervous system, being particularly abundant in the hypothalamus, especially in the preoptic, periventricular, paraventricular (PVN), supraoptic (SON), suprachiasmatic, and arcuate nuclei [19–22].

Human NERP-1 consists of 26 amino acid residues, while NERP-2 has 38 amino acid residues (Fig. 1A). The primary sequences of human and rat NERP-1 differ by four amino acids, and those of human and rat NERP-2 differ by only one amino acid. NERP-1 also exists in a short version with a single residue N-terminal deletion, while NERP-2 also exists in a six-residue N-terminal deleted form. Rat NERP-1 is 25 amino acids long and lacks the arginine residue at the N-terminus of human NERP-1. NERP-1 is derived from amino acids 281–306 of human VGF and amino acids 285–309 of rat VGF, while NERP-2 derives from amino acids 310–347 of human VGF and amino acids 313–350 of rat VGF. NERPs are novel peptides with no significant homology to any previously described peptides.

VGF protein in humans, chimpanzees, rats, and mice [14, 16, 17] have several paired, basic amino acid residues that represent potential cleavage sites for protein convertases of the kexin/subtilisin-like serine proteinase family [23]. Indeed, human and rat NERP-1 derive from processing of VGF at a typical dibasic cleavage site, ^{306}AGRR↓Q (human) or ^{309}AGRR↓Q (rat) (Fig. 1B). In contrast, human and rat NERP-2 are cleaved at a non-typical site following a single arginine within the ^{347}GGR↓G (human) or ^{350}GGR↓G (rat) sequence.

NERPs suppress vasopressin release

Radioimmunoassays (RIAs) using antibodies raised against the C-terminal regions of rat NERP-1 or NERP-2 combined with HPLC showed that both peptides are highly abundant in the rat hypothalamus [7]. The immunoreactive NERP-1 and NERP-2 contents in the whole rat brain were 6.06 ± 0.27 and 4.00 ± 0.17 fmol/mg wet weight, respectively. The whole hypothalamus contained 14.3 ± 1.1 fmol NERP-1/mg wet weight and 12.6 ± 1.5 fmol NERP-2/mg wet weight. Cell bodies with strong immunostaining of NERPs were observed in the magnocellular neurons of the SON and PVN [7], which produce the antidiuretic neuropeptide vasopressin and the reproduc-

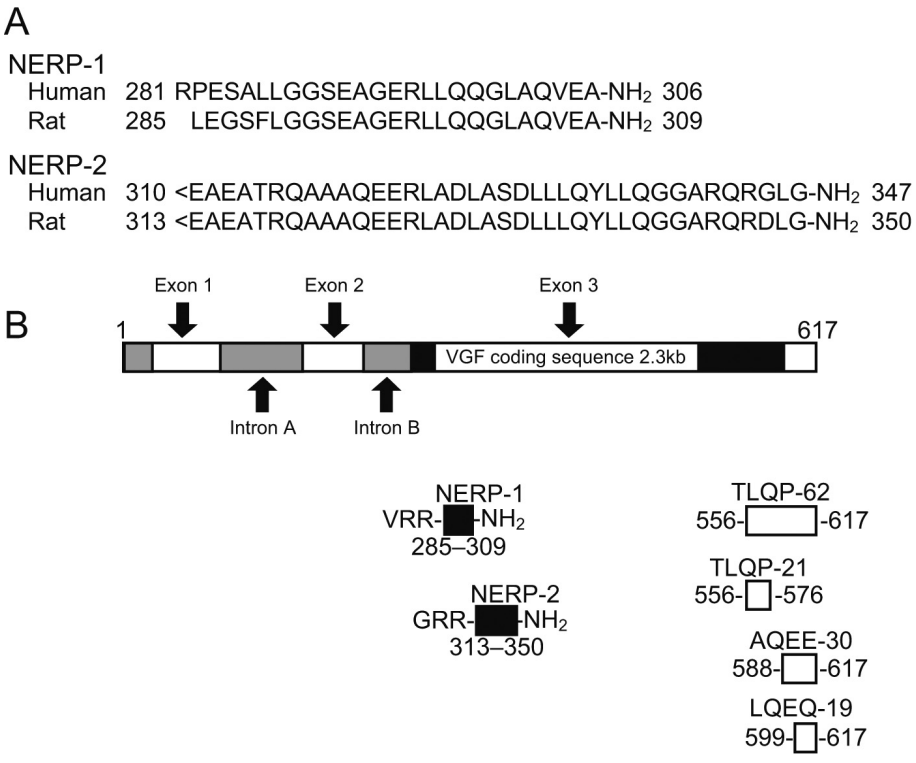

Figure 1. *(A)* Sequence alignments of human and rat NERPs. <**E**, pyroglutamate. *(B)* Schematic diagram of peptides derived from VGF. The numbers indicate the positions of amino acid residues in rat VGF protein. The closed boxes represent NERP-1 and NERP-2.

tive neuropeptide oxytocin [24]. Vasopressin is a 9-amino acid peptide that stimulates water reabsorption in the kidney. NERP immunoreactivity and VGF mRNA frequently colocalized with vasopressin, but rarely with oxytocin [7, 25]. Immunogold electron microscopy revealed the colocalization of NERPs with vasopressin in storage granules. In addition, immunoreactive signals for NERPs and vasopressin are colocalized in magnocellular neurons of the human SON and PVN. NERP-1 and NERP-2 both circulate in human plasma, with respective plasma concentrations of 3.5 ± 1.0 and 2.0 ± 0.4 fmol/ml. Although the vasopressin level in the plasma of normal human subjects increased during osmotic stimulus by salt-loading, the plasma levels of NERP-1 and -2 did not change.

VGF mRNA levels in both the PVN and SON were upregulated upon water deprivation in rats (PVN, 153.0 ± 13.6 %; SON, 161.9 ± 12.4 %; % of controls), concomitant with the upregulation of vasopressin mRNA levels. VGF mRNA levels in the PVN and SON of salt-loaded rats also increased with vasopressin mRNA level [26]. These *in vivo* and immunocytochemical observations suggest that NERPs may be involved in the central control of body fluid balance. An intracerebroventricular (icv) injection of hypertonic NaCl or angiotensin II (AII) increased plasma vasopressin levels in rats. This stimulation was suppressed in a dose-dependent manner by icv injection of NERP-1 before injection of vasopressin secretagogues. Similar but weaker effects were observed with NERP-2. Neither nonamidated NERP-1 (NERP-1-Gly) nor nonamidated NERP-2 (NERP-2-Gly) suppressed vasopressin secretion. The increase in plasma vasopressin concentrations caused by water deprivation in rats was also suppressed by icv-administered NERP-1 or NERP-2. Furthermore, immunoneutralization by icv administration of anti-NERP-1 IgG or anti-NERP-2 IgG reversed plasma vasopressin suppression induced by acute water loading, suggesting that NERPs function as endogenous peptides that regulate vasopressin secretion. Also, *in vitro* experiments using rat hypothalamic explants demonstrated that NERP-1 reversibly suppressed basal and AII-induced vasopressin secretion. NERP-2 had a similar effect, but NERP-1-Gly and NERP-2-Gly were not able to suppress vasopressin secretion. Vasopressin-producing magnocellular neurosecretory cells send their axons to the posterior pituitary, from which vasopressin is secreted into the circulatory system [24, 27]. NERPs also suppressed vasopressin secretion from the posterior pituitary in rats. Thus, NERPs may be potent endogenous suppressors of vasopressin secretion.

The neurons that produce vasopressin and oxytocin in the SON and PVN have characteristic electrophysiological properties and firing patterns [28]. Vasopressin neurons increase their firing rate both after admin-

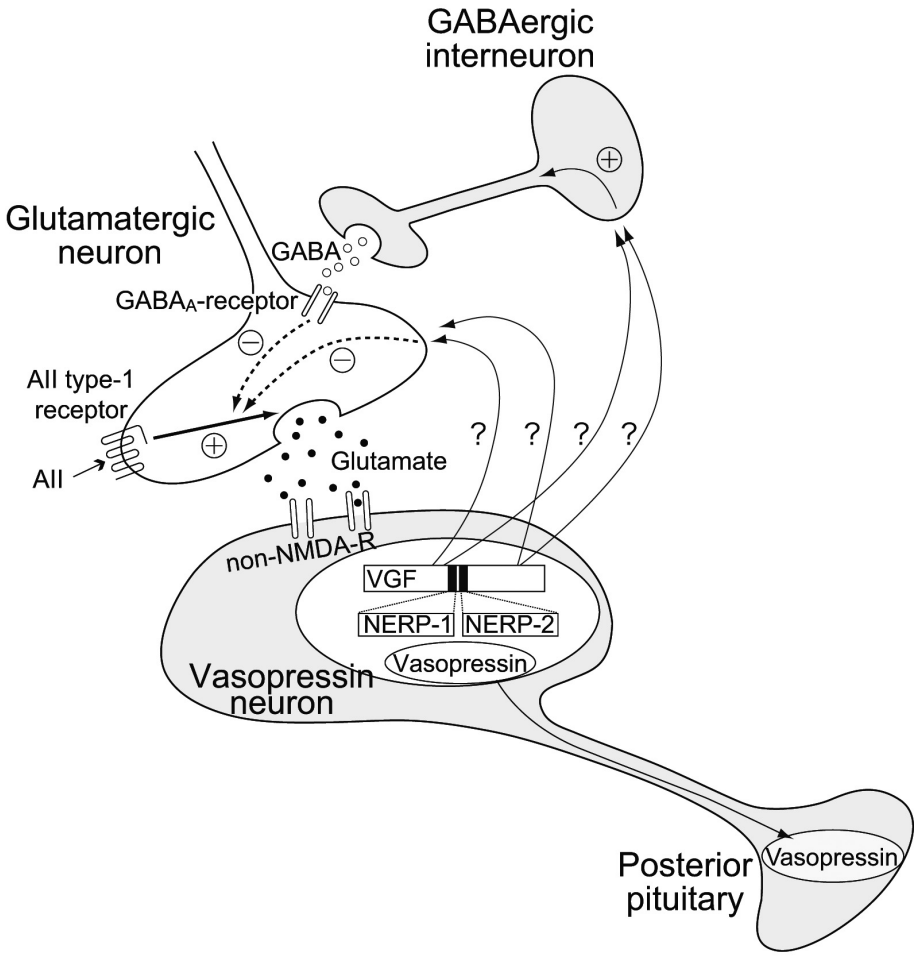

Figure 2. Proposed mechanisms by which NERPs regulate vasopressin secretion. Vasopressin is synthesized in the PVN and SON and transported to the posterior pituitary from where vasopressin is secreted into the circulation. Vasopressin secretion is regulated by excitatory glutamatergic input and inhibitory GABAergic input. AII acts on the AII type-1 receptor at the presynaptic terminal of glutamatergic neurons and increases spontaneous glutamate release, thereby activating the non-NMDA receptor. The GABA-evoked $GABA_A$ receptor-mediated response suppresses excitation of glutamatergic neurons. NERPs localize in the vasopressin neurons and suppress AII-induced vasopressin release. NERPs may suppress presynaptic release of glutamate or stimulate GABA release. **non-NMDA-R**, non-N-methyl-D-aspartate receptor.

istration of hyperosmotic saline and non-osmotic stimuli such as hemorrhaging [29, 30]. The major neural signals that regulate vasopressin neurons are presynaptic release of glutamate and γ-aminobutyric acid (GABA). Whole-cell patch clamp recording of the excitatory postsynaptic currents in the SON suggests that AII increases presynaptic glutamate release [31]. Hypertonic saline also stimulates vasopressin release through activation of non-N-methyl-D-aspartate (NMDA) receptor expressed on the anteroventral third ventricular region [32]. Although cell-surface receptors and target proteins of NERPs have not yet been identified, the action of NERPs in suppression of AII- and NaCl-induced vasopressin release suggests that they presynaptically inhibit glutamatergic inputs or enhance GABAergic inputs to vasopressin neurons (Fig. 2). Further investigation using whole-cell patch clamp recordings of PVN or SON slice preparations to examine the effect of NERPs on synaptic inputs to vasopressin neurons should elucidate the mechanisms by which NERPs modulate vasopressin release.

Phenotype of *Vgf*$^{-/-}$ mice and the activities of other VGF-derived peptides

Vgf$^{-/-}$ mice showed a significant lack of body weight gain at postnatal day 3 [16]. After weaning, the body weight of *Vgf*$^{-/-}$ mice was 50–70% that of wild-type littermates. Whereas *Vgf*$^{-/-}$ mice showed increased oxygen consumption compared to wild-type and *Vgf*$^{+/-}$ littermates, they had normal core body temperature and sympathetic tone and low circulating thyroid hormone levels [16]. Energy homeostasis is regulated by distinct hypothalamic regions: i) the arcuate nucleus, which contains populations of neurons expressing the orexigenic factors neuropeptide Y (NPY) and agouti-related protein (AgRP) [33, 34], and the anorexigenic factors pro-opiomelanocortin (POMC) (from which α-melanocyte stimulating hormone derives) and cocaine-amphetamine regulated transcript [35]; ii) the lateral hypothalamus, which contains the orexigenic peptides orexin-A and -B [2] (also known as hypocretin-1 and -2 [36]) and melanin-concentrating hormone [37]; and iii) the PVN, which contains the anorexigenic corticotropin-releasing hormone [38]. The mRNA level of

POMC in *Vgf*$^{-/-}$ mice was downregulated, while that of NPY and AgRP was upregulated [39]. Additional phenotypes of *Vgf*$^{-/-}$ mice include delay of puberty, abnormal sexual behavior, small reproductive end organs, and shortening of the circadian rhythm. These reproductive abnormalities are thought to be associated with the suppression of gonadotropin-releasing hormone release from the hypothalamus and of sex hormones, follicle stimulating hormone, and lutenizing hormone from the pituitary [16]. The phenotypes of *Vgf*$^{-/-}$ mice suggest that VGF is an endogenous modulator of neuropeptide release in the hypothalamus.

Several types of VGF-derived peptides have been detected in the rat brain, bovine pituitary, and human cerebrospinal fluid [40–48]. Immunoblotting of PC12 cells or rat brain extracts using an antibody raised against the C-terminal region of VGF showed the presence of peptides with molecular weights of approximately 20 kDa (VGF20/NAPP-129), 10 kDa (VGF10/TLQP-62), and 2 kDa (VGF2/LQEQ-19 and AQEE-30) (Fig. 1B) [42, 44]. These VGF-derived peptides are detected in dense core secretory granules of neuronal and neuroendocrine cells [42, 44].

We demonstrated that NERP-2 colocalized with orexins, but not the melanin-concentrating hormone, in the lateral hypothalamus of rats. Icv administration of NERP-2 induced Fos, a marker of neuronal activation, in the orexin neurons. Icv administration to rats of NERP-2, but not NERP-1, enhanced food intake in an orexin-dependent manner (paper in submission).

These peptides possess multiple biological activities, functioning in synaptic plasticity, antidepression, penile erection, autonomic activation, and increases in energy expenditure [43, 44, 47, 48]. Icv administration of TLQP-21 increased energy expenditure and prevented diet-induced obesity in mice on a high fat diet [46] and decreased food intake in Siberian hamsters [49]. VGF-derived peptides are enriched in the secretory granules which are preferentially secreted upon cell membrane depolarization [43]. The storage of multiple species of VGF-derived peptides in secretory granules and widespread expression of VGF throughout the central and peripheral nervous systems and endocrine tissues are similar to the patterns seen for the chromogranin-secretogranin family [50]. Chromogranins and secretogranins, collectively known as "granins", are a unique group of acidic, soluble secretory proteins with molecular weights ranging from 21 to 67.5 kDa [50]. Granin-derived polypeptides regulate the secretion of other peptides in autocrine, paracrine, and endocrine fashions. The degree of similarity in the expression pattern and characteristic protein sequences of VGF with proteins from the chromogranin-secretogranin family suggests the potential involvement of NERPs in the regulation of peptide release.

Concluding remarks and future perspectives

In conclusion, VGF is synthesized exclusively in neuronal and neuroendocrine cells, and VGF-derived peptides appear to regulate the release of peptides or hormones via the regulated secretory pathway. NERPs are novel bioactive peptides involved in body fluid homeostasis that appear to modulate the actions and secretion of other neuropeptides in an autocrine, paracrine, or endocrine fashion. We demonstrated that NERP-2, but not NERP-1, stimulated feeding behavior in rats and mice. We also found that NERP-producing cells are widely distributed in the endocrine and neuroendocrine cells in systemic organs. Further studies of NERPs and their receptors will pave the way for elucidating unknown extracellular signaling mechanisms as well as understanding the roles of NERPs not only in body fluid homeostasis, but also in the regulation of various physiological phenomena.

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1 Richard, B. S. (1995) Anatomical Substrates of Hypothalamic Integration. In: The Rat Nervous System. 2nd edition, pp.353–376 Paxinos, G. (ed.), Academic Press, San Diego, California.
2 Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R. M., Tanaka, H., Williams, S. C., Richardson, J. A., Kozlowski, G. P., Wilson, S., Arch, J. R., Buckingham, R. E., Haynes, A. C., Carr, S. A., McNulty, D. E., Liu, W. S., Terrett, J. A., Elshourbagy, N. A., Bergsma, D. J. and Yanagisawa, M. (1998) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 92, 573–585.
3 Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H. and Kangawa, K. (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402, 656–660.
4 Tanaka, H., Yoshida, T., Miyamoto, N., Motoike, T., Kurosu, H., Shibata, K., Yamanaka, A., Williams, S. C., Richardson, J. A., Tsujino, N., Garry, M. G., Lerner, M. R., King, D. S., O'Dowd, B. F., Sakurai, T. and Yanagisawa, M. (2003) Characterization of a family of endogenous neuropeptide ligands for the G protein-coupled receptors GPR7 and GPR8. Proc. Natl. Acad. Sci. USA. 100, 6251–6256.
5 Zhou, A., Webb, G., Zhu, X. and Steiner, D. F. (1999) Proteolytic processing in the secretory pathway. J. Biol. Chem. 274, 20745–20748.

6 Clynen, E., Baggerman, G., Veelaert, D., Cerstiaens, A., van der Horst, D., Harthoorn, L., Derua, R., Waelkens, E., De Loof, A., and Schoofs, L. (2001) Peptidomics of the pars intercerebralis-corpus cardiacum complex of the migratory locust, Locusta migratoria. Eur. J. Biochem. 268, 1929–1939.
7 Yamaguchi, H., Sasaki, K., Satomi, Y., Shimbara, T., Kageyama, H., Mondal, M. S., Toshinai, K., Date, Y., González, L. J., Shioda, S., Takao, T., Nakazato, M. and Minamino, N. (2007) Peptidomic identification and biological validation of neuroendocrine regulatory peptide-1 and -2. J. Biol. Chem. 282, 26354–26360.
8 Zaidi, M., Moonga, B. S. and Abe, E. (2002) Calcitonin and bone formation: a knockout full of surprises. J. Clin. Invest. 110, 1769–1771.
9 Ginessi, L., Silvestroni, L., Fabbri, A., Moretti, C., Panerai, A. E., Bonifacio, V. and Fraioli, F. (1984) Salmon calcitonin inhibits human sperm motility *in vitro*. Biochem. Biophys. Res. Commun. 125, 199–204.
10 Fraser, L. R., Pondel, M. D. and Vinson, G. P. (2001) Calcitonin, angiotensin II and FPP significantly modulate mouse sperm function. Mol. Hum. Reprod. 7, 245–253.
11 Jansen-Olesen, I., Mortensen, A. and Edvinsson, L. (1996) Calcitonin gene-related peptide is released from capsaicin-sensitive nerve fibers and induces vasodilatation of human cerebral arteries concomitant with activation of adenylyl cyclase. Cephalalgia 16, 310–316.
12 Sasaki, K., Satomi, Y., Yamaguchi, H., Takao, T., Nakazato, M. and Minamino, N. (2007) Peptidomic identification of novel neuroendocrine regulatory peptide-1 and -2. Proc. 4th Int. Peptide Symp., 1–2.
13 Eipper, B. A., Stoffers, D. A. and Mains, R. E. (1992) The biosynthesis of neuropeptides: Peptide alpha-Amidation. Ann. Rev. Neurosci. 15, 57–85.
14 Possenti, R., Eldridge, J. D., Paterson, B. M., Grasso, A., and Levi, A. (1989) A protein induced by NGF in PC12 cells is stored in secretory vesicles and released through the regulated pathway. EMBO J. 8, 2217–2223.
15 Levi, A., Eldridge, J. D. and Paterson, B. M. (1985) Molecular cloning of a gene sequence regulated by nerve growth factor. Science 229, 393–395.
16 Hahm, S., Mizuno, T. M., Wu, T. J., Wisor, J. P., Priest, C. A., Kozak, C. A., Boozer, C. N., Peng, B., McEvoy, R. C., Good, P., Kelley, K. A., Takahashi, J. S., Pintar, J. E., Roberts, J. L., Mobbs, C. V. and Salton, S. R. (1999) Targeted deletion of the *vgf* gene indicates that the encoded secretory peptide precursor plays a novel role in the regulation of energy balance. Neuron 23, 537–548.
17 Canu, N., Possenti, R., Ricco A. S., Rocchi, M. and Levi, A. (1997) Cloning, structural organization analysis, and chromosomal assignment of the human gene for the neurosecretory protein VGF. Genomics 45, 443–446.
18 Snyder, S. E., Pintar, J. E. and Salton S. R. (1998) Developmental expression of VGF mRNA in the prenatal and postnatal rat. J. Comp. Neurol. 394, 64–90.
19 Snyder, S. E. and Salton, S. R. (1998) Expression of VGF mRNA in the adult rat central nervous system. J. Comp. Neurol. 394, 91–105.
20 Salton, S. R., Fischberg, D. J. and Dong, K. W. (1991) Structure of the gene encoding VGF, a nervous system-specific mRNA that is rapidly and selectively induced by nerve growth factor in PC12 cells. Mol. Cell Biol. 11, 2335–2349
21 Snyder, S. E., Cheng, H. W., Murray, K. D., Isackson, P. J., McNeill, T. H. and Salton, S. R. (1998) The messenger RNA encoding VGF, a neuronal peptide precursor, is rapidly regulated in the rat central nervous system by neuronal activity, seizure and lesion. Neuroscience 82, 7–19.
22 Wisor, J. P. and Takahashi, J. S. (1997) Regulation of the *vgf* gene in the golden hamster suprachiasmatic nucleus by light and by the circadian clock. J. Comp. Neurol. 378, 229–238.
23 Steiner, D. F. (1998) The proprotein convertases. Curr. Opin. Chem. Biol. 2, 31–39.
24 Yamashita, H., Inenaga, K., Kawata, M. and Sano, Y. (1983) Phasically firing neurons in the supraoptic nucleus of the rat hypothalamus: immunocytochemical and electrophysiological studies. Neurosci. Lett. 37, 87–92.
25 Okumura, H., Tanaka, M., Kanemasa, K., Ban, Y., Inouye, S. T. and Ibata, Y. (1995) *In situ* hybridization histochemistry of vgf mRNA in the rat suprachiasmatic nucleus: co-localization with vasopressin/neurophysin and VIP/PHI. Neurosci. Lett. 189, 181a–184a.
26 Mahata, S. K., Mahata, M., Fischer-Colbrie, R. and Winkler, H. (1993) *In situ* hybridization: mRNA levels of secretogranin II, VGF and peptidylglycine alpha-amidating monooxygenase in brain of salt-loaded rats. Histochemistry 99, 287–293.
27 Caldwell, H. K., Lee, H. J., Macbeth, A. H. and Scott Young III, W. (2008) Vasopressin: behavioral roles of an "original" neuropeptide. Prog. Neurobiol. 84, 1–24.
28 Stern, J. E. and Armstrong, W. E. (1995) Electrophysiological differences between oxytocin and vasopressin neurons recorded from female *in vitro*. J. Physiol. 488, 701–708.
29 Swaminathan, S. (1980) Osmoreceptors or sordium receptors: an investigation into ADH release in the rhesus monkey. J. Physiol. 307, 71–83.
30 Frithiof, R., Eriksson, S., Bayard, F., Svenson, T. and Rundgren, M. (2007) Intravenous hypertonic NaCl acts via cerebral sodium-sensitive and angiotensinergic mechanisms to improve cardiac function in haemorrhaged conscious sheep. J. Physiol. 583, 1129–1143.
31 Ozaki, Y., Soya, A., Nakamura, J., Matsumoto, T. and Ueta, Y. (2004) Potentiation by angiotensin II of spontaneous excitatory postsynaptic currents in rat supraoptic magnocellular neurones. J. Neuroendocrinol. 16, 871–879.
32 Yamaguchi K, Yamada T. (2006) Involvement of anteroventral third ventricularAMPA/kainate receptors in both hyperosmotic and hypovolemic AVP secretion in conscious rats. Brain. Res. Bull. 71, 183–192.
33 Shutter, J. R., Graham, M., Kinsey, A. C., Scully, S., Lüthy, R. and Stark, K. L. (1997) Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice. Genes. Dev. 11, 593–602.
34 Luquet, S., Perez, F. A., Hnasko, T. S. and Palmiter, R. D. (2005) NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. Science 310, 683–685.
35 Kristensen, P., Judge, M. E., Thim, L., Ribel, U., Christjansen, K. N., Wulff, B. S., Clausen, J. T., Jensen, P. B., Madsen, O. D., Vrang, N., Larsen, P. J. and Hastrup, S. (1998) Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature 393, 72–76.
36 de Lecea, L., Kilduff, T. S., Peyron, C., Gao, X., Foye, P. E., Danielson, P. E., Fukuhara, C., Battenberg, E. L., Gautvik, V. T., Bartlett, F. S. 2nd., Frankel, W. N., van den Pol, A. N., Bloom, F. E., Gautvik, K. M. and Sutcliffe, J. G. (1998) The hypocretin: hypothalamus-specific peptides with neuroexcitatory activity. Proc. Natl. Acad. Sci. USA. 95, 322–327.
37 Qu, D., Ludwig, D. S., Gammeltoft, S., Piper, M., Pelleymounter, M. A., Cullen, M. J., Mathes, W. F., Przypek, R., Kanarek, R. and Maratos-Flier, E. (1996) A role for melanin-concentrating hormone in the central regulation of feeding behaviour. Nature 380, 243–247.
38 Krahn, D. D., Gosnell, B. A., Levine, A. S. and Morley, J. E. (1988) Behavioral effects of corticotropin-releasing factor: localization and characterization of central effects. Brain Res. 443, 63–69.
39 Hahm, S., Fekete, C., Mizuno, T. M., Windsor, J., Yan, H., Boozer, C. N., Lee, C., Elmquist, J. K., Lechan, R. M., Mobbs, C. V. and Salton, S. R. (2002) VGF is required for obesity induced by diet, gold thioglucose treatment, and agouti and is differentially regulated in pro-opiomelanocortin- and neuropeptide Y-containing arcuate neurons in response to fasting. J. Neurosci. 22, 6929–6938.
40 Levi, A., Ferri, G. L., Watson, E., Possenti, R. and Salton, S. R. (2004) Processing, distribution, and function of VGF, a neuro-

nal and endocrine peptide precursor. Cell. Mol. Neurobiol. 24, 517–533.
41 Liu, J. W., Andrews, P. C., Mershon, J. L., Yan, C., Allen, D. L. and Ben-Jonathan, N. (1994) Peptide V: a VGF-derived neuropeptide purified from bovine posterior pituitary. Endocrinology 135, 2742–2748.
42 Trani, E., Giorgi, A., Canu, N., Amadoro, G., Rinaldi, A. M., Halban, P. A., Ferri, G. L., Possenti, R., Shininà, M. E. and Levi, A. (2002) Isolation and characterization of VGF peptides in rat brain. Role of PC1/3 and PC2 in the maturation of VGF precursor. J. Neurochem. 81, 565–574.
43 Alder, J., Thakker-Varia, S., Bangasser, D. A., Kuroiwa, M., Plummer, M. R., Shors, T. J. and Black, I. B. (2003) Brain-derived neurotrophic factor-induced gene expression reveals novel actions of VGF in hippocampal synaptic plasticity. J. Neurosci. 23, 10800–10808.
44 Succu, S., Cocco, C., Mascia, M. S., Melis, T., Melis, M. R., Possenti, R., Levi, A., Ferri, G. L. and Argiolas, A. (2004) Pro-VGF-derived peptides induce penile erection in male rats: possible involvement of oxytocin. Eur. J. Neurosci. 20, 3035–3040.
45 Rüetschi, U., Zetterberg, H., Podust, V. N., Gottfries, J., Li, S., Hviid Simonsen, A., McGuire, J., Karlsson, M., Rymo, L., Davies, H., Minthon, L. and Blennow, K. (2005) Identification of CSF biomarkers for frontotemporal dementia using SELDI-TOF. Exp. Neurol. 196, 273–281.
46 Bartolomucci, A., La Corte, G., Possenti, R., Locatelli, V., Rigamonti, A. E., Torsello, A., Bresciani, E., Bulgarelli, I., Rizzi, R., Pavone, F., D'Amato, F. R., Severini, C., Mignogna, G., Giorgi, A., Shininà, M. E., Elia, G., Brancia, C., Ferri, G. L., Conti, R., Ciani, B., Pascucci, T., Dell'Omo, G., Muller, E. E., Levi, A. and Moles, A. (2006) TLQP-21, a VGF-derived peptide, increases energy expenditure and prevents the early phase of diet-induced obesity. Proc. Natl. Acad. Sci. USA. 103, 14584–14589.
47 Thakker-Varia, S., Krol, J. J., Nettleton, J., Bilimoria, P. M., Bangasser, D. A., Shors, T. J., Black, I. B. and Alder, J. (2007) The neuropeptide VGF produces antidepressant-like behavioral effects and enhances proliferation in the hippocampus. J. Neurosci. 27, 12156–12167.
48 Hunsberger, J. G., Newton, S. S., Bennett, A. H., Duman, C. H., Russell, D. S., Salton, S. R. and Duman, R. S. (2007) Antidepressant actions of the exercise-regulated gene VGF. Nat. Med. 13, 1476–1482.
49 Jethwa, P. H., Warner, A., Nilaweera, K. N., Brameld, J. M., Keyte, J. W., Carter, W. G., Bolton, N., Bruggraber, M., Morgan, P. J., Barrett, P. and Ebling, F. J. (2007) VGF-derived peptide, TLQP-21, regulates food intake and body weight in Siberian hamsters. Endocrinology 148, 4044–4055.
50 Taupenot, L., Harper, K. L. and O'Connor, D. T. (2003) The chromogranin- secretogranin family. N. Engl. J. Med. 348, 1134–1149.