

Dean's Lecture

Neurotrophins, Growth-Factor-Regulated Genes and the Control of Energy Balance

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Abstract

Neurotrophic growth factors are proteins that control neuronal differentiation and survival, and consequently play important roles in the developing and adult stages of the nervous system. Study of the genes that are regulated by these growth factors has provided insight into the proteins that are critical to the maturation of the nervous system, suggesting that select neurotrophins may play a role in the control of body homeostasis by the brain and peripheral nervous system. Our understanding of the mechanisms of action of neurotrophic growth factors has increased through experimental manipulation of cultured neurons and neuronal cell lines. In particular, the PC12 pheochromocytoma cell line, which displays many properties of adrenal chromaffin cells and undergoes differentiation into sympathetic neuron-like cells when treated with nerve growth factor, has been extensively investigated to identify components of neurotrophin signaling pathways as well as the genes that they regulate. VGF was one of the first neurotrophin-regulated clones identified in NGF-treated PC12 cells. Subsequent studies indicate that the *vgf* gene is regulated *in vivo* in the nervous system by neurotrophins, by electrical activity, in response to injury or seizure, and by feeding and the circadian clock. The *vgf* gene encodes a polypeptide rich in paired basic amino acids; this polypeptide is differentially processed in neuronal and neuroendocrine cells and is released via the regulated secretory pathway. Generation and analysis of knockout mice that fail to synthesize VGF indicate that this protein plays a critical, non-redundant role in the regulation of energy homeostasis, providing a possible link between neurotrophin function in the nervous system and the peripheral control of feeding and metabolic activity. Future experiments should clarify the sites and mechanisms of action of this neurotrophin-regulated neuronal and neuroendocrine protein.

Key Words: Neurotrophin, VGF, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), leptin, hypothalamus, obesity, diabetes, PC12.

Glossary

AGRP agouti-related polypeptide
Akt protein kinase B (non-acronymic)
 α -MSH alpha-melanocyte-stimulating hormone
ARC arcuate nucleus
BDNF brain-derived neurotrophic factor
B-raf member of Raf protein family
CART cocaine- and amphetamine-regulated transcript
CNTF ciliary neurotrophic factor

CREB cAMP-response element binding protein
Crk SH2/SH3 adaptor protein related to the crk oncogene of avian sarcoma virus
C3G Crk SH3-binding guanine nucleotide-releasing protein
DMV dorsal motor nucleus of the vagus
DRG dorsal root ganglion
Gab1 Grb2-associated binder-1
Grb-2 growth factor receptor-bound protein-2
GTG gold thioglucose
LH lateral hypothalamus
LHA lateral hypothalamic area
LTP long-term potentiation
MAPK mitogen-activated protein kinase
MC4-R melanocortin 4 receptor
MEK mitogen-activated protein kinase
MKP-1 MAP-kinase phosphatase-1
MSG monosodium glutamate
Nf κ B nuclear factor kappa B

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Adapted from a Dean's Lecture presentation to the Department of Medicine, Mount Sinai School of Medicine, New York, NY on June 6, 2001 and updated as of July 23, 2002.

NGF	nerve growth factor
NGFI-A	NGF Inducible-A
NILE	NGF-Inducible Large External Glycoprotein; L ₁ — same protein in human and mice
NPY	neuropeptide Y
NTS	nucleus tractus solitarius
NT-3, NT-4/5, NT-6 and NT-7	neurotrophin-3, -4/5, -6, -7
PC12	pheochromocytoma 12 cell line
PC-1, PC-2	prohormone convertase-1, -2
PCR	polymerase chain reaction
PI-3 K	phosphatidylinositol-3 kinase
PKC	protein kinase C
PLC- γ	phospholipase C gamma
Pomc	pro-opiomelanocortin
PNS	peripheral nervous system
p75NTR	p75 neurotrophin receptor
PVN	paraventricular nucleus
Raf	cellular homologue of murine sarcoma virus 3611 transforming protein
Rap-1	small guanosine triphosphatase (GTPase) of the Ras family
Ras	cellular homologue of murine sarcoma virus transforming protein
Rsk	ribosomal S6 kinase
SHC	Src homology region 2 (SH2)-containing protein
SH-PTP2	Src homology protein tyrosine phosphatase 2
Shp-2	Src homology-2 domain phosphatase
SNT	suc-associated neurotrophin-induced tyrosine-phosphorylated target (suc is yeast p13suc1, which binds the p34cdc2/cdk2 kinase complex)
SOS	son of sevenless; guanine nucleotide exchange factor that activates Ras
Src	cellular homologue of Rous sarcoma virus transforming protein
trkA, trkB and trkC	tropomyosin-related kinase A, B, and C
VGF	protein; a polypeptide secreted by neurons and endocrine cells (non-acronymic)
<i>vgf</i>	gene (when talking about multiple species)
<i>Vgf</i>	mouse gene
<i>VGF</i>	human gene
VMN	ventromedial nucleus

Introduction

NEUROTROPHINS ARE PROTEINS that are required for neuronal differentiation and survival. Pioneering work by Rita Levi-Montalcini, Stanley Cohen and Viktor Hamburger in the 1950s and early 1960s identified nerve growth factor (NGF) and demonstrated that this protein was required for sympathetic neuron survival both *in vivo* and *in vitro* (1, 2). The later purification and characterization of brain-derived neurotrophic factor (BDNF) (3), a close relative of NGF, allowed the cloning of additional family members based on sequence homology and polymerase chain reaction (PCR) amplification. Six members of the neurotrophin family have been identified in vertebrates, namely NGF, BDNF, NT-3, NT-4/5, NT-6 and NT-7. These polypeptides bind, with different affinities, to the Trk family of receptor tyrosine kinases (TrkA, TrkB and TrkC) and with similar affinities to the p75 neurotrophin receptor (p75NTR), a member of the tumor necrosis factor receptor family, activating complex intracellular signaling pathways, some of which are shown in Fig. 1. Target tissues secrete specific neurotrophins, and these proteins initiate programs of cellular differentiation and support the survival of neuronal subpopulations that express the appropriate receptors and signaling pathways.

Much of our current knowledge of neurotrophin receptor signaling is based on experimental manipulation of PC12 pheochromocytoma cells, a neural-crest-derived line that expresses both TrkA and p75NTR, and responds to NGF by ceasing cell division and differentiating from adrenal chromaffin-like cells (Fig. 2A) into sympathetic neuron-like cells (Fig. 2B) (4). In addition to their use in understanding the transduction of NGF signals via the Akt and PI3 kinases that are critical to cell survival, and the activation of the MAPK and PLC- γ cascades that trigger neural differentiation (5, 6), PC12 cells have been employed to identify the nuclear target genes of these signaling pathways. Synthesis of a number of proteins is rapidly induced in PC12 cells by NGF treatment; these proteins include the transcription factors NGFI-A and Fos, and the dual-specificity tyrosine phosphatase MAPK phosphatase-1 (MKP-1). Long-term NGF treatment leads to increased synthesis of proteins that function in the differentiated neuron, such as the cytoskeletal protein peripherin and the cell adhesion molecule NGF-Inducible Large External Glycoprotein (NILE or L₁).

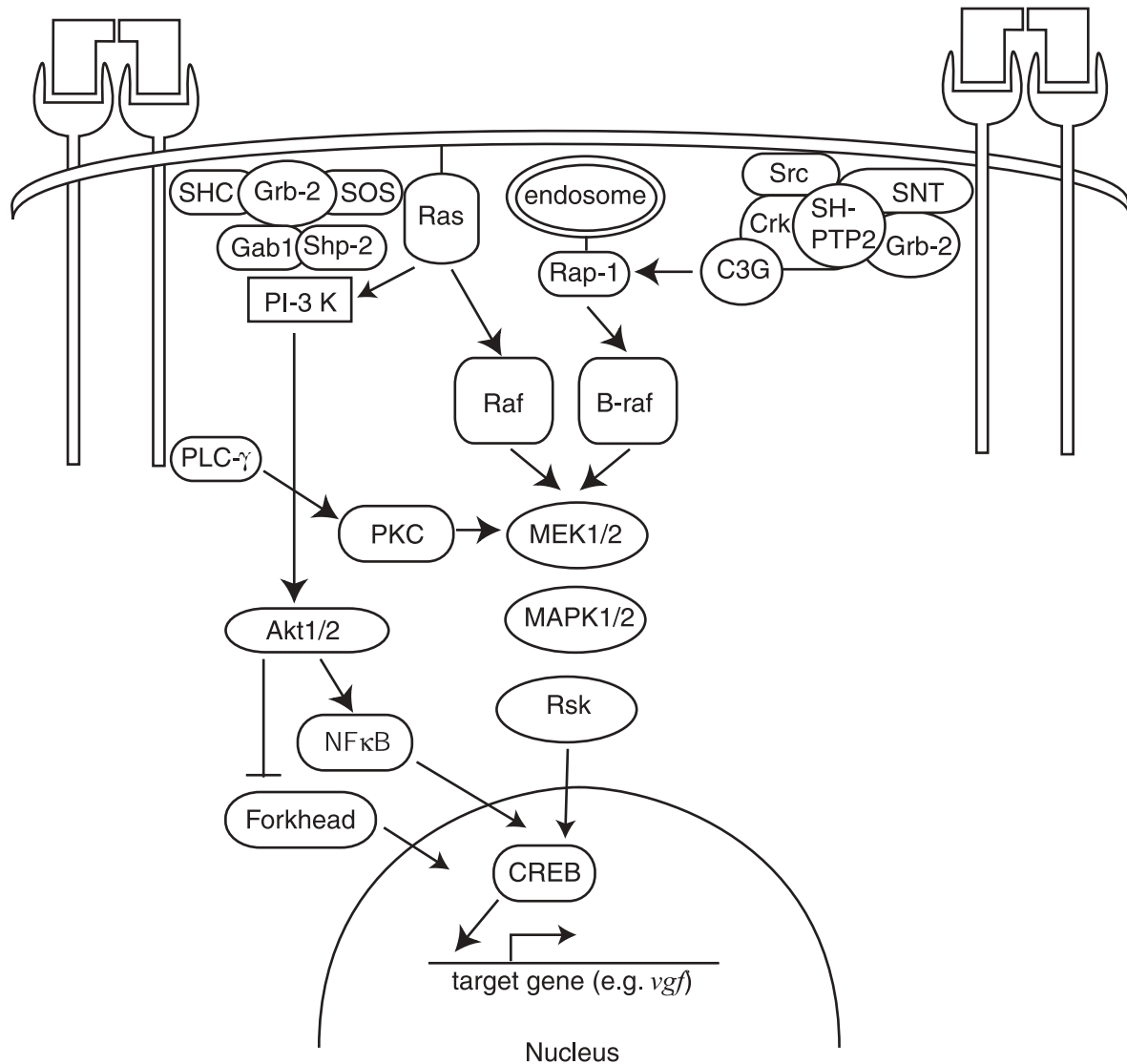


Fig. 1. Signaling cascades that are activated by neurotrophins. Neurotrophic growth factors bind as dimers to their cognate tyrosine kinase receptors, stimulating receptor autophosphorylation and generating specific phosphotyrosine sites that subsequently interact with adaptor protein complexes and activate intracellular signaling pathways.

Our work over the past several years has focused on characterizing the expression, regulation and function of neurotrophin-inducible polypeptides, with recent emphasis on a protein called VGF (non-acronymic) (7, 8). Studies of lean, hypermetabolic knockout mice that are deficient in VGF intriguingly suggest that this protein is a key regulator of energy balance. Neurotrophins may therefore play a modulatory role in the complex circuits that control energy balance in the brain, specifically in the hypothalamus, and throughout the remainder of the peripheral and central nervous systems. In support of this hypothesis, reduced BDNF synthesis in haplo-insufficient mice (9) and targeted deletion of the neurotrophin-regulated molecule

VGF (10), each lead to profound alterations in energy homeostasis. Moreover, systemic administration of BDNF has been shown to improve blood glucose control and regulate energy expenditure in obese mice (11–16), and BDNF gene expression has been shown to be induced in calorically restricted mice (17). Finally, ciliary neurotrophic factor (CNTF) has been recognized to have robust, leptin-independent effects in reducing body weight (18, 19), and administration of recombinant human BDNF to patients with diabetic polyneuropathy resulted in increased gut motility as a side effect (20). Using VGF as a prototype for neurotrophin-regulated gene products that function as intermediates, linking the nervous system

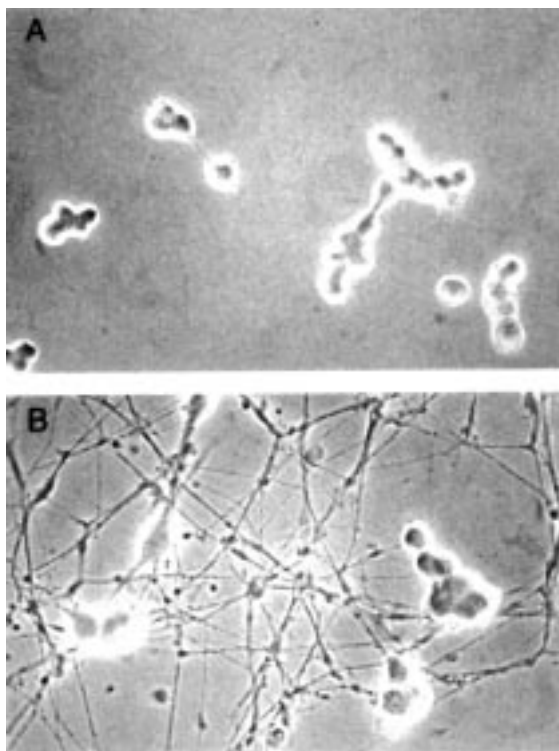


Fig. 2. The PC12 pheochromocytoma cell line. PC12 cells grown in serum-containing medium display many properties of adrenal chromaffin cells (Panel A). Treatment with NGF for two weeks leads to the cessation of cell division, intense neurite outgrowth, and the induction of gene expression and a program of neuronal differentiation that models the sympathetic neuron (Panel B).

with peripheral tissues, I would like to discuss our current understanding of the regulation, expression and function of this secreted neuronal and neuroendocrine polypeptide.

Cloning and Characterization of the Neurotrophin-Inducible Gene Product VGF

VGF was independently identified as VGF8a (7), NGF33.1 (21, 22) and $\alpha 2$ (23) on the basis of its rapid induction by NGF in rat PC12 pheochromocytoma cells. Among gene products that are regulated with immediate-early or delayed-early kinetics, VGF is unusual in that it is induced robustly and relatively selectively by neurotrophic growth factors (7, 22, 24), is synthesized exclusively in neuronal and neuroendocrine cells (22, 25–29), and is transported in dense core vesicles with its processed peptides released via the regulated secretory pathway (30, 31). In addition, the *vgf* coding and promoter sequences are highly homologous from mouse to human. VGF mRNA has been detected in the rat by *in situ* hybridization as

early as embryonic day 11.5 (E11.5) in what appear to be migrating neural crest cells fated to become enteric ganglia (28). The earliest expression of VGF therefore appears to occur in the peripheral nervous system (PNS) as maturing neurons cluster to form ganglia. In later stages of embryogenesis, VGF mRNA is also expressed in regions of the brain, pituitary, adrenal medulla and myenteric plexus.

After birth, VGF is widely expressed throughout the rodent brain (25, 27–29, 32–34), and immunocytochemical studies indicate that the highest concentration of VGF is found in the medial hypothalamus (25, 27). Following colchicine blockade of axonal transport, VGF-immunoreactive cells can be identified as strongly stained in neurosecretory regions of the hypothalamus, including the arcuate, paraventricular and supraoptic nuclei, and in the suprachiasmatic nucleus (25, 27). In addition to abundant expression in the brain, VGF mRNA has been detected in the adult rat spinal cord, and in the peripheral nervous system, where VGF polypeptide and mRNA are found in dorsal root and sympathetic ganglia (26, 28). VGF is also expressed by endocrine cells in the pituitary, adrenal medulla and gastrointestinal tract, and in pancreatic β cells (35), indicating that peripherally synthesized VGF may function by reaching target tissues via the systemic circulation.

Since VGF expression is induced by neurotrophins *in vitro*, in PC12 cells (7, 22, 23) and in cortical neurons (24), VGF and neurotrophin receptor distributions have been compared to offer insight into potential mechanisms of VGF regulation *in vivo*. Localization of VGF, trkA, trkB and trkC mRNAs by *in situ* hybridization during rat development indicates overlap in trk and VGF mRNA expression. Although no trk mRNA is exclusively co-localized with VGF mRNA, the expression patterns in the brain, spinal cord and peripheral nervous system of mRNAs encoding the trkB catalytic isoform and VGF were found to be the most similar (36). In agreement with these studies, a recent investigation demonstrated that robust ectopic expression of VGF in embryonic cortex can be triggered *in vivo* by locally injected BDNF (37). In addition, since growth factors have been implicated not only in neuronal development but also in the response of the nervous system to injury and regulation of synaptic plasticity, regulation of VGF expression has been examined in the brain during critical developmental periods, and following seizure or lesioning. Of considerable interest, VGF mRNA levels are regulated

by electrical activity in the developing visual system, by seizure and long-term potentiation (LTP) in the hippocampus, and following injury in the cerebral cortex and striatum, paradigms that lead to neurotrophin induction, synaptic remodeling and axonal sprouting.

Targeted Ablation of VGF Affects Energy Balance

Based on the regulatory studies described above, the phenotype of the VGF-mutant mouse, in which the entire VGF protein coding sequence was deleted by homologous recombination, was somewhat unanticipated (10). At birth, homozygous VGF-deficient mice are indistinguishable from wild-type or heterozygous littermates. Thus, the former appear to have no major defects in the patterning or wiring of their nervous system. However, VGF-mutant mice fail to gain weight efficiently, and as adults they are lean (with 50–70% of the body weight of wild-type mice), hypermetabolic, hyperactive, and infertile (10). How is body weight regulated, and what role might VGF play in the control over appetite and metabolic activity?

Several regions within the brain have classically been associated with the control of food intake and energy output, including the caudal brain stem and most notably the hypothalamus, where lesions lead to profound alterations in feeding behavior, body weight and fat storage. The state of peripheral fat stores is relayed to the brain via circulating hormones such as leptin, an adipocyte-synthesized protein which transduces its signal through transport and binding to the leptin-receptor system in the hypothalamus (38, 39) (Fig. 3). Characterization of leptin-responsive hypothalamic neurons suggests that subsets of these cells contain orexigenic neuropeptides such as neuropeptide Y (NPY) and agouti-related polypeptide (AGRP), which stimulate feeding, and anorexigenic neuropeptides such as alpha-melanocyte stimulating hormone (α -MSH) and cocaine- and amphetamine-regulated transcript (CART), which decrease feeding. Balancing the output of these orexigenic and anorexigenic circuits allows food consumption to be controlled. The satiety-inducing melanocortin pathways in the hypothalamus decrease food intake through the interplay of two peptides that compete for binding to the melanocortin 4 receptor (MC4-R), α -MSH and its antagonist AGRP. Effects of these leptin-responsive circuits on energy balance are

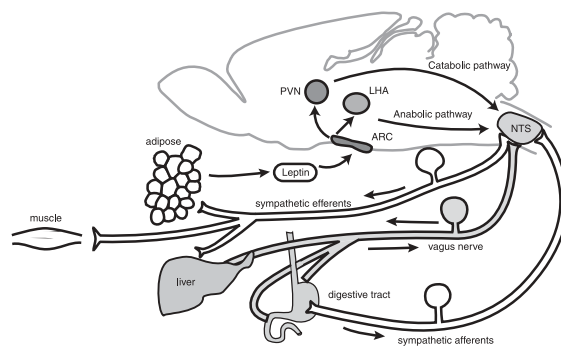


Fig. 3. Schematic diagram of the circuits that control energy balance. Leptin is synthesized by adipose tissue and binds receptors in the hypothalamus, relaying the state of peripheral fat stores to the brain. Feeding and metabolic rate are sensed and controlled in large part by the autonomic nervous system, which is interconnected with the hypothalamus and brain stem (including the NTS), and relays afferent and efferent signals to fat, muscle, liver, and the digestive tract. Catabolic pathways decrease feeding and stimulate peripheral metabolism, a response to the satiety signals from arcuate-derived neuropeptides α -MSH and CART. Anabolic signals increase feeding and decrease peripheral metabolism, leading to energy storage, primarily in response to arcuate neuropeptides such as NPY and AGRP.

mediated by projections to other areas of the hypothalamus, and to the brainstem, spinal cord and cortex (38).

Based on the functional studies described above, one might anticipate that VGF would be synthesized in abundance in the brainstem and hypothalamus, and that expression of the *vgf* gene would be regulated in these regions in response to feeding and/or fasting. Several studies have demonstrated high levels of VGF mRNA and polypeptide expression in the hypothalamus (25, 27–29, 33, 34) and an increase in VGF mRNA levels in the hypothalamic arcuate nucleus (ARC) in response to fasting (10). VGF expression is also regulated in neurons of both the dorsal motor nucleus of the vagus (DMV) and nucleus tractus solitarius (NTS) (40, 41), which provide vagal afferent and efferent fibers that mediate gastric visceral sensitivity and secretory, trophic and motor regulation of the stomach. VGF is therefore appropriately situated to exert a functional influence on the neural pathways that regulate feeding and metabolism.

How might VGF control energy homeostasis? Relative resistance to environmentally induced or genetically induced obesity is a useful indicator of a functional role in the control of energy balance. VGF-mutant mice fail to develop diet-induced obesity that normally results from consumption of high-fat, high-carbohydrate food. In addition, VGF deficiency blocks

the development of obesity which results from toxic injury to the hypothalamus. VGF ablation therefore protects mice against two independent environmental causes of obesity.

Does VGF deficiency also block the genetically induced obesity that is found in several well-studied strains of mice, including *ob/ob* mice (a strain which is profoundly obese due to a failure to synthesize the adipose hormone leptin), and *A^{y/a}* (a strain which develops maturity onset obesity due to ectopic expression of the melanocortin receptor-antagonist Agouti)? Mice with mutations in the leptin (*ob/ob*) or leptin-receptor (*db/db*) genes develop early onset obesity that is associated with reduced metabolic rate, increased food intake, diabetes, and decreased fertility (42), while mice with defective signaling in the melanocortin pathway develop a later onset obesity syndrome that is associated with hyperphagia, hyperinsulinemia and hyperglycemia (43, 44). Interbreeding of these obese mice with other strains that harbor additional genetic mutations, and analysis of the phenotypes of the resulting offspring, has been an extremely useful technique to better define the molecular components of the leptin and melanocortin pathways. Interestingly, interbreeding of *A^{y/a}* with VGF-mutant mice completely blocks the development of obesity, hyperglycemia, and hyperinsulinemia in double-mutant mice (Fig. 4, panels A and B). On the other hand, targeted ablation of the *Vgf* gene in *ob/ob* mice does reduce their weight, plasma glucose and food intake in comparison to control *ob/ob* mice, but does not decrease percentage of body fat or increase core body temperature (as a marker of metabolic activity) (Fig. 4, panels C and D). Genetic analysis therefore very strongly suggests that VGF does function in the melanocortin pathway, either in the hypothalamus itself or perhaps in autonomic outflow pathways that ultimately innervate peripheral metabolic tissues (Fig. 5).

Indirect experimental evidence suggests that VGF may somehow regulate peripheral sympathetic tone. VGF mRNA is detected at the earliest stages of development in sensory and sympathetic neurons of the embryonic peripheral nervous system. Administration of monosodium glutamate (MSG) to neonatal mice generally causes maturity-onset obesity, through toxic damage to the hypothalamus and sympathetic nervous system, and gold thioglucose (GTG) treatment injures glucose-sensitive hypothalamic neurons in adult mice, similarly leading to obesity. VGF-mutant mice become

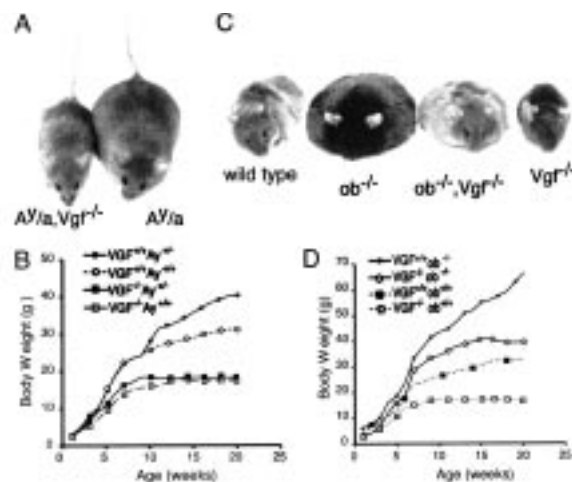


Fig. 4. Interbreeding of lean VGF-mutant mice with obese *A^{y/a}* Agouti or *ob/ob* mice indicates that targeted deletion of *Vgf* selectively blocks obesity and weight gain in Agouti mice, but has less effect on leptin-deficient *ob/ob* mice. *A^{y/a}* and double-mutant *A^{y/a}*, *Vgf^{-/-}* mice are shown in panel A and weights of representative mice from these crosses are indicated in panel B. Wild type, *Vgf^{-/-}*, *ob/ob*, and double-mutant *ob/ob*, *Vgf^{-/-}* mice are shown in panel C and representative weights of mice with these genotypes are indicated in panel D.

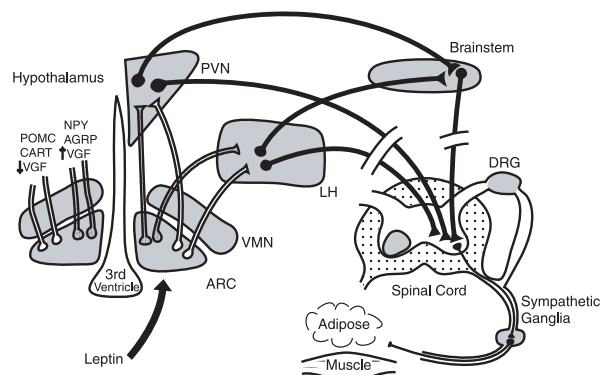


Fig. 5. Schematic diagram of hypothesized sites of VGF action in the nervous system. VGF mRNA and polypeptide levels are regulated by fasting, in the arcuate nucleus (ARC) of the hypothalamus. Based on lesion and genetic studies, pathways in black that project from the paraventricular nucleus (PVN) and lateral hypothalamus (LH) directly to the spinal cord, or to the brain stem, and ultimately relay signals to the periphery via the sympathetic nervous system, have been implicated as critical sites of VGF action in the regulation of energy output.

obese and hyperglycemic following MSG treatment, but not following GTG treatment, an indication that intact sympathetic outflow tracts are likely to be essential for the lean phenotype, and implying that VGF might be involved in the control of sympathetic activity.

Mechanism(s) of Action and Future Directions

Analyses of VGF-mutant mice suggest that this protein might be a target for anti-obesity pharmaceutical intervention. How might VGF function? In rodents and humans, the highly conserved VGF polypeptide contains at least 10 potential sites for proteolytic cleavage, suggesting that one or more bioactive peptides might result from enzymatic processing prior to secretion. In fact, the C-terminal-30-amino-acid VGF-derived peptide has been isolated from bovine posterior pituitary, and several studies have identified differential tissue- and cell-specific VGF processing. Recent studies have identified VGF peptides in cerebrospinal fluid (45) and brain (46), and work underway suggests that VGF-C-terminal-derived peptides have biological activity in the gut, uterus and brain. Characterization of putative cell surface VGF receptors is obviously dependent on the initial identification of one or more bioactive fragments of VGF, and the additional detection of these peptides *in vivo*. Studies that better define the function of predicted and identified VGF peptides are currently being pursued, utilizing intra-cerebroventricular (icv) injection approaches, peripheral tissue models and transgenic mice. Alternatively, since VGF is secreted from neuronal and endocrine cells, this protein may play an indirect role, perhaps by regulating cleavage, stability, and/or secretion of other peptide hormones or neuropeptides. In fact, targeted deletion of the enzyme carboxypeptidase E results in obesity and diabetes, an indirect result of aberrant peptide processing, while another polypeptide, 7B2, has been shown to function as a specific regulator of PC-2 convertase activity in secretory granules (of note, PC-1 mutations cause obesity in humans).

Summary

Ongoing studies suggest that neurotrophins and neurotrophin-regulated gene products play a global role not just during development, but also in the adult nervous system. Understanding how these proteins contribute to processes that are controlled in part by the nervous system, such as energy homeostasis, may provide insight into and suggest new treatment modalities for obesity and anorexia.

Acknowledgments

Supported by NIH grants AG10676 and DK 57702, and NSF grant IBN-9986657.

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