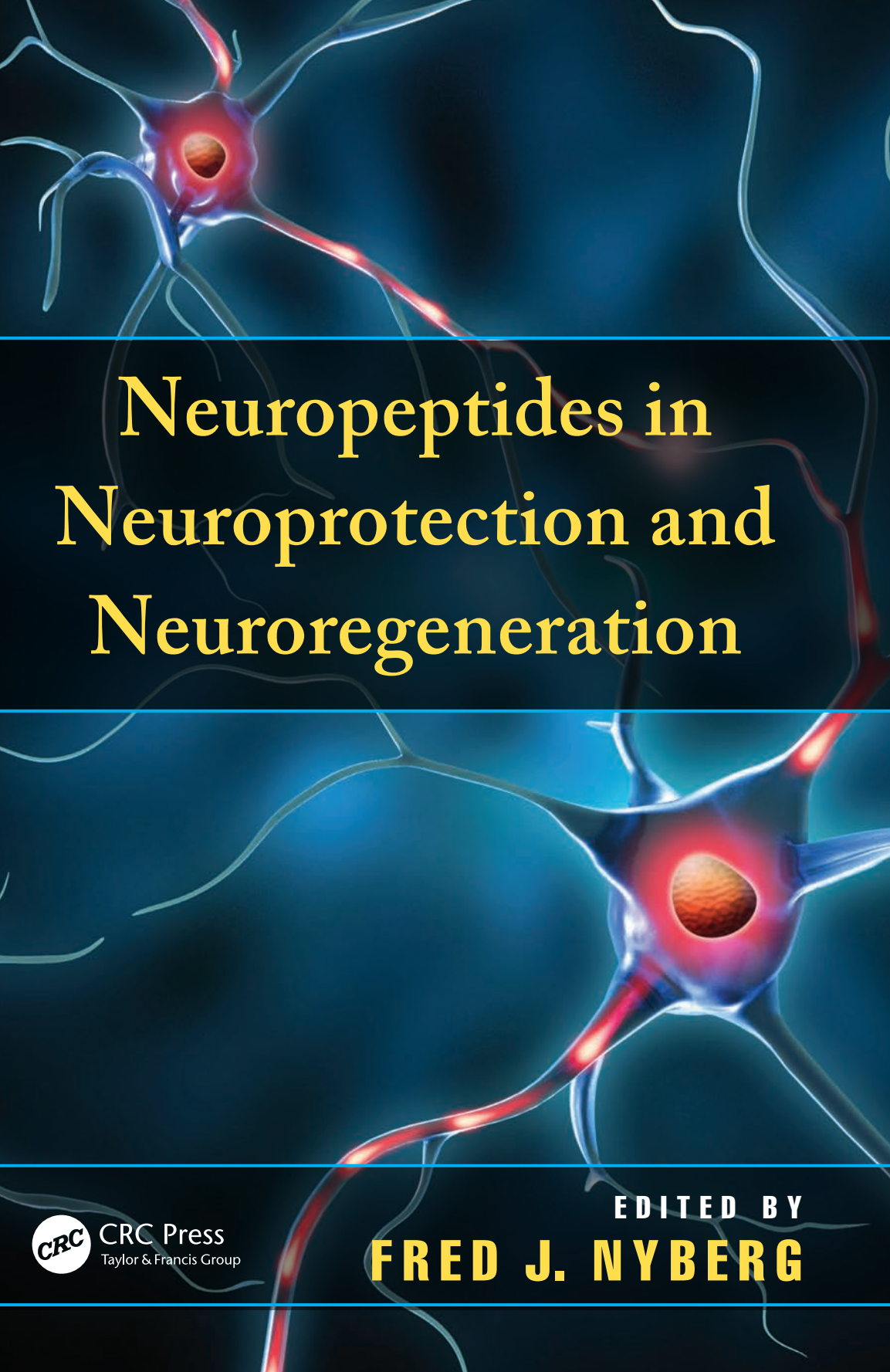
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Preface

Over the past four decades, the number of peptides identified as neurotransmitters or neuromodulators in the central and peripheral nervous systems has significantly increased. These compounds, known as neuropeptides, have been recognized for playing an important role in the communication between cells in a variety of neuronal networks. Although a considerable number of neuropeptides have been characterized so far, their quantity is limited compared to the number of precursor proteins that are actually found to be expressed in the cells of the nervous system. Numerous studies have confirmed that neuropeptides are involved in a number of biological activities. These include modulation of brain reward, pain processing, and immune response, as well as neuroendocrine regulations, control of neurovegetative functions, and trophic effects.

In recent years, it has become evident that the role of neuropeptides as fast-acting neurotransmitters is challenged by the observation that many of them may act as growth factors by stimulating cell proliferation and slow-acting mitogenesis. It has thus been demonstrated that a number of neuroactive peptides, such as pituitary adenylate cyclase–activating polypeptide (PACAP), adrenocorticotrophin (ACTH), opioid peptides, somatostatin, and substance P, may modulate proliferation and cell viability. Some peptides act as stimulatory factors on brain circuits involved in cognition, whereas others may act as inhibitory factors. Accordingly, many neuropeptide systems appear as important targets for neuroprotective drugs and drugs that promote neuroregeneration. The recently discovered nuclear protein or polypeptide prothymosin alpha (ProTα), which inhibits neuronal necrosis, suggested to be of clinical use for stroke, is also a neuroprotective agent of interest in this regard. This volume aims to describe recent aspects on the impact of neuropeptides on processes involved in neuroprotection and neuroregeneration. It is by no means exhaustive, but intends to highlight some examples of neuropeptides, neuroactive polypeptides, and growth factors that typify the actual area of research.

This book begins with chapters describing important features of the endogenous neuropeptide systems with regard to their formation, receptor signaling, and inactivation. However, unlike many books dealing with this topic, it also includes chapters focused on the design and development of peptide-like drugs (peptidomimetics). It further includes a contribution highlighting neuropeptide circuits as targets of cognitive enhancers. Most of the authors who have contributed to this volume belong to the core of international top scientists in their particular area of research.

Finally, it is interesting to note that it has been possible to get contributors to this volume who are such a good representation of geographical districts around the world.

With the current advances in neuropeptide research, this volume presents a timely book that underlines an important aspect of interest to a diverse group of people, from scientists and medical practitioners to basic clinical investigators and students.

I am grateful to all my colleagues who have contributed such excellent chapters to this volume. As editor, I would like to point out that all these chapters were submitted within a time period of 15 months and therefore they present the most recent knowledge in the respective fields covered within the volume.

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1 Neuropeptide Systems—Some Basic Concepts

Tomas Hökfelt, Zhi-Qing David Xu, Tiejun Shi, Csaba Adori, Kang Zheng, Swapnali Barde, and Mingdong Zhang

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1.1 NEUROPEPTIDE RESEARCH—THE BEGINNINGS

Early in the 1970s, an intense research on neuropeptides was initiated, fueled by the following seminal discoveries: (1) most of the hypothalamic-releasing and -inhibiting hormones were shown to be peptides by Roger Guillemin and Andrew Schally's groups; (2) substance P, though discovered in 1931 by Ulf von Euler and John Gaddum, was chemically identified as an 11-amino acid peptide by Susan Leeman and coworkers only in 1971; (3) the first endogenous ligands for the morphine receptors were identified as two pentapeptides, leucine- and methionine-enkephalin

by John Hughes, Hans Kosterlitz, and coworkers; (4) the gut peptide cholecystokinin (CCK), discovered by Viktor Mutt and Erik Jorpes, and the vasodilatory peptide vasoactive intestinal polypeptide (VIP), isolated by Sami Said and Viktor Mutt, were found in the brain; and last but not least (5) David de Wied and coworkers' pioneering behavioral work, showing interesting central effects of peripherally administered peptides like vasopressin and oxytocin, opening up the idea that these posterior pituitary hormones can bypass the blood–brain barrier (BBB) and act on receptors in the brain, further corroborated by Abba Kastin and coworkers. Subsequently, the rate of discovery of neuropeptides accelerated, and during the first decade of the third millennium new members of the family have been added. Thus, we now know of several hundred neuropeptides, many of them belonging to chemically, or functionally, distinct families. Clearly, neuropeptides have emerged by far as the largest group of messenger molecules in the nervous system, as recently reviewed by Burbach (2010) (Table 1.1).

1.2 NEUROPEPTIDES—TRANSMITTERS OR TROPHIC FACTORS, OR BOTH?

Neuropeptides were initially considered as transmitter-like substances, in any case in our laboratory, perhaps with a slow onset and certainly with a long duration of action. As discussed later in this chapter, it was at that time unclear on which type of receptor neuropeptides act. However, as time passed by, more and more reports described trophic, regenerative, neuroprotective, and developmental effects. One important peptide in this context was alpha-melanocyte-stimulating hormone (α-MSH) that, in addition to many other effects, influenced nerve regeneration and was neuroprotective (Strand 1999). Other important neuropeptides in this category are VIP (Brenneman 2007; Gozes et al. 1999; Waschek 1995), pituitary adenylyl cyclase-activating peptide (PACAP) (Brenneman 2007; Gozes et al. 1999; Waschek 1995) (see Chapter 11 by Dr. Eiden in this book), and neuropeptide Y (NPY) (Hansel et al. 2001; Hökfelt et al. 2008; Xapelli et al. 2006). In this chapter, as an example for the aforementioned activities, we will describe some of galanin's actions, both as a transmitter and a trophic factor. It should be mentioned that, surprisingly, there are also reports indicating the contrary, for example, neurotoxic actions of somatostatin (Gaumann et al. 1990; Mollenholt et al. 1988) and dynorphin (Gaumann et al. 1990; Skilling et al. 1992; Stewart and Issac 1989) (see Chapter 6 by Dr. Hauser in this book). Taken together, neuropeptides display a wide range of biological effects, making it difficult to arrange them into a single category. Having said this, equally interesting, classic transmitters have neurotrophic effects (Schwartz 1992).

1.3 HOW NEUROPEPTIDES HAVE BEEN DISCOVERED

The methods of discovery have developed over time, as a consequence of the breathtaking generation of new tools during the past 50 years. The aforementioned hypothalamic-releasing and -inhibiting factors as well as most gut hormones were extracted from huge amounts of tissues from appropriate organs/tissues, and the purity was monitored by bioassays. Tatemoto and Mutt (1980) realized

TABLE 1.1 (from Burbach 2010)
Neuropeptide Gene Families: Classical Neuropeptides

1. Opioid Peptide Family

Prepro enkephalin: Leu-enkephalin, met-enkephalin, amidorphin, adrenorphin, peptide B, peptide E, peptide F, BAM22P

Proopiomelanocortin (POMC): α-melanocyte-stimulating hormone (α-MSH), γ-melanocyte-stimulating hormone (γ-MSH), β-melanocyte-stimulating hormone (β-MSH), adrenocorticotropic hormone (ACTH), β-endorphin, α-endorphin, γ-endorphin, β-lipoprotein (β-LPH), γ-lipoprotein (γ-LPH), corticotropin-like intermediate peptide (CLIP)

Prepro dynorphin: Dynorphin A, dynorphin B, α-neo-endorphin, β-neo-endorphin, dynorphin-32, leu-morphin

Prepro nociceptin, prepro-orphanin: Nociceptin (orphanin FQ), neuropeptide 1, neuropeptide 2

2. Vasopressin/Oxytocin Gene Family

Prepro vasopressin-neurophysin II: Vasopressin (VP), neurophysin II (NP II), C-terminal glycopeptide CPP

Prepro oxytocin-neurophysin I: Oxytocin (OT), neurophysin I (NP 1)

3. CCK/Gastrin Gene Family

Prepro gastrin: Gastrin-34, gastrin-17, gastrin-4

Prepro cholecystokinin (CCK): CCK-8, CCK-33, CCK-58

4. Somatostatin Gene Family

Prepro somatostatin (SST): SS-12, SS-14, SS-28, antrin

Prepro cortistatin: Cortistatin-29, cortistatin-17

5. F- and Y-Amide Family

Prepro neuropeptide RF: QRF-amide (neuropeptide RF-amide, gonadotropin inhibitory hormone [GnIH], p518, RF-related peptide-2), RF-related peptide-1, RF-related peptide-3, neuropeptide VF

Prepro neuropeptide FF (NPFF): Neuropeptide FF, neuropeptide AF, neuropeptide SF

Prepro neuropeptide Y (NPY): C-flanking peptide of NPY (CPON)

Prepro pancreatic polypeptide Y (PPY): PPY

Prepro peptide YY (PYY): PYY, PYY-(3-36)

Prepro prolactin-releasing peptide (PrLH): PrRP-31, PrRP-20

6. Calcitonin Gene Family

Prepro calcitonin: Calcitonin, katacalcin

Prepro CGRP-α: Calcitonin gene-related peptide I (α-CGRP)

Prepro CGRP-β: Calcitonin gene-related peptide II (β-CGRP)

Prepro islet amyloid polypeptide (IAPP): Amylin: IAPP (amylin, amyloid polypeptide)

Prepro adrenomedullin: Adrenomedullin, AM, PAMP

Prepro adrenomedullin-2: Adrenomedullin-2, intermedin-long (IMDL), intermedin-short (IMDS)

7. Natriuretic Factor Gene Family

Preproatrial natriuretic factor: Atrial natriuretic factor (natriuretic peptide A, ANF, ANP, natriodilatine, cardiodilatine-related peptide)

Prepro brain natriuretic factor: Brain natriuretic factor (natriuretic peptide B, BNF, BNP)

Prepro natriuretic peptide precursor C: C-type natriuretic peptide (CNP-23), CNP-29, CNP-53

(*Continued*)

TABLE 1.1 (CONTINUED)
Neuropeptide Gene Families: Classical Neuropeptides

8. Bombesin-Like Peptide Gene Family

Prepro gastrin-releasing peptide 1 (GRP-1): GRP-27, GRP-14, GRP-10 (neuromedin C)
Prepro gastrin-releasing peptide 2 (GRP-2): GRP-27, GRP-14, GRP-10 (neuromedin C)
Prepro gastrin-releasing peptide 3 (GRP-3): GRP-27, GRP-14, GRP-10 (neuromedin C)
Prepro neuromedin B1: Neuromedin B (ranatensin-like peptide, RLP)
Prepro neuromedin B2: Neuromedin B (ranatensin-like peptide, RLP)

9. Endothelin Gene Family

Prepro endothelin 1 (PPET1): Endothelin 1 (ET-1)
Prepro endothelin 2 (PPET2): Endothelin 2 (ET-2)
Prepro endothelin 3 (PPET3): Endothelin 3 (ET-3)

10. Glucagon/Secretin Gene Family

Prepro glucagon: Glicentin, glicentin-related polypeptide (GRPP), oxyntomodulin (OXY) (OXM), glucagon, glucagon-like peptide 1 (GLP-1), glucagon-like peptide 1(7-37) [GLP1 (7-37)], glucagon-like peptide 1(7-36) [GLP-1(7-36)], glucagon-like peptide 2 (GLP-2)
Prepro secretin (SCT): Secretin
Prepro vasoactive intestinal peptide (VIP-1): VIP, PHM-27/PHI-27, PHV-42
Prepro vasoactive intestinal peptide (VIP-2): VIP, PHM-27/PHI-27, PHV-42
Prepro pituitary adenylcyclase-activated peptide: PACAP-38, PACAP-27, prolactin-relating peptide (PRP)-48
Prepro growth hormone-releasing hormone (GHRH): GHRH (somatoliberin, GRF, somatocrinin, somatorelin, sermorelin)
Prepro gastric inhibitory peptide (GIP): GIP (gastric inhibitory peptide, glucose-dependent insulinotropic polypeptide)

11. CRH-Related Gene Family

Prepro corticotropin-releasing hormone (CRH): CRH
Prepro urocortin-I: UNC I
Prepro urocortin-II: UNC II, stresscopin-related peptide
Prepro urocortin-III: UNC III, stresscopin
Prepro urotensin-2, isoform a: Urotensin-2
Prepro urotensin-2, isoform b: Urotensin-2
Prepro urotensin-2B: Urotensin-2-related peptide, urotensin-2B

12. Kinin and Tensin Gene Family

α-Prepro tachykinin A (α-PPTA): Substance P, neurokinin A (NKA, substance K, neuromedin L), neuropeptide K, neuropeptide γ
β-Prepro tachykinin A (β-PPTA): Substance P, neuropeptide K, neurokinin A
γ-Prepro tachykinin A (γ-PPTA): Substance P, neurokinin A, neuropeptide γ
δ-Prepro tachykinin A (δ-PPTA): Substance P, neuropeptide K, neurokinin A
Prepro tachykinin B (PPTB), isoform 1: Neuromedin K, neurokinin B
Prepro tachykinin B (PPTB), isoform 2: Neuromedin K, neurokinin B

13. Neuromedins

Prepro neuromedin S: Neuromedin S (NMS)
Prepro neuromedin U, multiple isoforms Neuromedin U: (NMU)

TABLE 1.1 (CONTINUED)
Neuropeptide Gene Families: Classical Neuropeptides

14. Tensins and Kinins

Kininogen-1 precursor, isoform 1: Bradykinin, kallidin, LMW-K-kinin, HMW-K-kinin
Kininogen-1 precursor, isoform 2: Bradykinin, kallidin, LMW-K-kinin, HMW-K-kinin
Angiotensinogen preprotein: Angiotensin I, angiotensin II, angiotensin (1-7)
Prepro neurotensin: Neurotensin (NT), neuromedin N

15. Motilin Gene Family

Prepro motilin isoform 1: Motilin, motilin-associated peptide
Prepro motilin isoform 2: Motilin, motilin-associated peptide
Prepro ghrelin isoform 1: Ghrelin, obestatin
Prepro ghrelin isoform 2: Ghrelin, obestatin
Prepro ghrelin isoform 3: Obestatin
Prepro ghrelin isoform 4: Obestatin
Prepro ghrelin isoform 5: Obestatin

16. Galanin Gene Family

Prepro galanin: Galanin, galanin message-associated peptide (GMAP)
Galanin-like peptide precursor: Galanin-like peptide (GALP)

17. GnRH Gene Family

Prepro gonadotropin-releasing hormone 1: GnRH (LHRH, gonadoliberin)
Prepro gonadotropin-releasing hormone 2, isoform-a: GnRH2 (LHRH II, gonadoliberin II)
Prepro gonadotropin-releasing hormone 2, isoform-b: GnRH2 (LHRH II, gonadoliberin II)
Prepro gonadotropin-releasing hormone, isoform-c: GnRH2 (LHRH II, gonadoliberin II)

18. Neuropeptide B/W Gene Family

Prepro neuropeptide B, PPL7: Neuropeptide B-23 (peptide L7), neuropeptide B-29
Prepro neuropeptide W, PPL8: Neuropeptide W-23 (peptide L8), neuropeptide W-30
Prepro neuropeptide S: Neuropeptide S

19. Insulin/Relaxins

Prepro relaxin-1: Relaxin-1
Prepro relaxin-2, isoform 1: Relaxin-2
Prepro relaxin-2, isoform 2: Relaxin-2
Prepro relaxin-3: Relaxin-3

20. No-Family Neuropeptides

Prepro thyrotropin-releasing hormone: TRH (thyroliberin)
Prepro parathyroid hormone-like hormone, isoform CRA_a: PTHrP (1-36), PTHrP (38-94), PTHrP (107-139) (osteostatin)
Prepro parathyroid hormone-like hormone, isoform CRA_b
Prepro melanin-concentrating hormone: MCH, neuropeptide Glu-Ile (NEI), neuropeptide Gly-Glu (NGE)
Prepro hypocretin: Hypocretin-1 (orexin A), hypocretin-2 (orexin B)
Prepro cocaine- and amphetamine-regulated transcript (CART): CART (1-39), CART (42-89)

(*Continued*)

TABLE 1.1 (CONTINUED)
Neuropeptide Gene Families: Classical Neuropeptides

Agouti-related protein precursor isoform 1: AGRP
Agouti-related protein precursor isoform 2: AGRP
Prolactin precursor: Prolactin (PRL)
Prepro apelin: Apelin-13, apelin-17, apelin-36 (APJ ligand, AGTRL1 ligand)
Metastasis-suppressor KISS 1: Metastin (kisspeptin-54), (Golgi transport 1 homolog A, golt1a), kisspeptin-14, kisspeptin-13, kisspeptin-10
Diazepam-binding inhibitor isoform 1: Diazepam-binding inhibitory peptide
Diazepam-binding inhibitor isoform 2: Diazepam-binding inhibitory peptide
Diazepam-binding inhibitor isoform 3: Diazepam-binding inhibitory peptide
Prokineticin-I precursor: Prokineticin-1, (PK1) endocrine gland-derived EGF (EGVEGF)
Prokineticin-2 precursor isoform 1: Prokineticin-2 (PK2)
Prokineticin-2 precursor isoform 2: Prokineticin-2 (PK2)

21. Cerebellins

Cerebellin-1 precursor: Cerebellin-1 (Cbln1)
Cerebellin-2 precursor: Cerebellin-2 (Cbln2)
Cerebellin-3 precursor: Cerebellin-3 (Cbln3)
Cerebellin-4 precursor: Cerebellin-4 (Cbln4, cerebellin-like glycoprotein-1)

22. Granins

Chromogranin A precursor: Chromogranin A, β-granin, vasostatin
Chromogranin B precursor: Chromogranin B (secretogranin I), CCB peptide, GAWK peptide
Secretogranin II precursor, chromogranin C precursor: Secretogranin II (chromogranin C), EM66, secretoneurin
Secretogranin III precursor: Secretogranin III
Secretory granule neuroendocrine precursor: Secretory granule neuroendocrine protein-1 (7B2, secretogranin 5)
VGF nerve growth factor inducible protein precursor: VGF (NGF-inducible protein, neurosecretory protein), TLPQ-62, TLPQ-21, AQEE-30, LQEQ-19

23. Neuroexophilins

Neuroexophilin-1 precursor: Neuroexophilin-1
Neuroexophilin-2 precursor: Neuroexophilin-2
Neuroexophilin-3 precursor: Neuroexophilin-3
Neuroexophilin-4 precursor: Neuroexophilin-4

24. Adipose Neuropeptides

Prepro-leptin: Leptin (obesin)
Adiponectin precursor: Adiponectin (Acpr30, adipocyte complement-related protein, adipocyte, C1Q and collagen domain containing)
Visfatin precursor: Visfatin-1 [pre-B cell colony enhancing factor-1 (PBEF1), nicotinamide phosphoribosyltransferase]
Resistin precursor: Resistin (cysteine-rich secreted protein FIZZ3, adipose tissue-specific secretory factor, cysteine-rich secreted protein A12-α-like 2, ADSF, Xcp4)
Resistin- δ2 precursor: Resistin-δ2
Resistin-like molecule α precursor: Resistin-like molecule α (found in inflammatory zone 1; FIZZ1, hypoxia-induced mitogenic factor, Xcp2)

TABLE 1.1 (CONTINUED)
Neuropeptide Gene Families: Classical Neuropeptides

Resistin-like β precursor: Resistin-like molecule β (cysteine-rich secreted protein FIZZ2, colon and small intestine-specific cysteine-rich protein, cysteine-rich secreted protein A12-α-like 1, colon carcinoma-related gene protein, Xcp3)
Resistin-like molecule γ precursor: Resistin-like molecule γ (cysteine-rich secreted protein FIZZ3, Xcp1)
Nucleobindin-2: Nesfatin-1
Beacon precursor: Beacon

25. Insulin Family

Prepro insulin: Insulin
IGF-1 precursor: IGF-1 (somatomedin C)
IGF-2 precursor (multiple precursors): IGF-2 (somatomedin A)

that C-terminal amidation is a hall mark for many neuropeptides. They, through a series of studies, were able to isolate, purely on this chemical principle, that is, without bioassay, several new neuropeptides, including peptide histidine isoleucine (PHI), peptide tyrosine tyrosine (PYY), NPY, pancreastatin, and galanin. In Italy, Erspamer and coworkers isolated a large number of bioactive peptides from frog skin and discovered that many of them had mammalian counterparts with similar structures and pharmacological activities (Ersparmer 1984).

Using the tools of molecular biology, Rosenfeld, Amara, and coworkers in their work on the calcitonin gene discovered another putative peptide in the precursor molecule (Amara et al. 1982; Rosenfeld et al. 1983). They deduced the amino acid sequence, synthesized the peptide, raised antibodies, and were able to demonstrate that this peptide, called calcitonin gene-related peptide (CGRP), was produced in a tissue-specific way, that is, in neurons as compared with the biosynthesis of calcitonin in the thyroid gland. CGRP has subsequently become a highly studied peptide.

Based on receptor deorphanization and reverse pharmacology (Civelli 2005), the neuropeptide nociceptin/orphanin FQ (N/OFQ) was identified as the transmitter for the orphan G-protein-coupled receptor (GPCR) and opioid receptor-like 1 (ORL-1) receptor (Meunier et al. 1995; Reinscheid et al. 1995). This approach has generated further interesting peptides. Among other fairly recently discovered neuropeptides, hypocretin/orexin and its receptors may be mentioned (de Lecea et al. 1998; Sakurai et al. 1998).

1.4 HOW NEUROPEPTIDES ARE MONITORED

Early works on peptides were mainly based on immunological principles. Thus, quantitative biochemical evaluations on peptide distribution and levels in various tissues were obtained using radioimmunoassay techniques originally described by Berson et al. (1956). At the histochemical level, the same antibodies can often be used to demonstrate the cellular and subcellular distribution of peptides using immunohistochemical techniques, originally developed by Coons et al. (1942) and

then complemented by more sensitive techniques, for example, tyramide signal amplification (Adams 1992). Subsequently, molecular biological approaches were introduced. Thus, by monitoring mRNA levels for various peptides using Northern blot analysis and solution hybridization, as well as *in situ* hybridization, using both radioactively labeled and nonradioactive probes, levels and distribution/cellular localization of transcripts can be demonstrated. Advanced molecular biological/genetic approaches are now used to further visualize localization of neuropeptides and their receptors (and other molecules). They include the use of various reporter gene strategies, that is, genetically modified mice; for example, replacement of a seven transmembrane receptor by an active receptor-enhanced green fluorescent protein fusion (knock-in mice) (Scherrer et al. 2006) or introduction of marked bacterial artificial chromosomes (BAC mice) (Heintz 2001).

Release of peptides in the peripheral and central nervous system can be monitored using push–pull systems as well as microdialysis developed by Ungerstedt and coworkers (Zetterstrom et al. 1983). Another approach was pioneered by Duggan and his group—the microprobe technique (Duggan 1990). A micropipette coated with antibodies to, for example, substance P is inserted into the dorsal horn of the spinal cord, and the peripheral nerve of the corresponding segment is stimulated. Substance P released from primary afferents in the dorsal horn then binds to the antibody-coated micropipette. Subsequently, the micropipette is pulled out and dipped into radiolabeled substance P, which binds only to those areas of the pipette where no endogenous substance P is occupying the antibody-binding sites. In this way, quantitative information with a high spatial resolution can be obtained.

Another approach, the "FMRFamide tagging" method, based on molecular biological/genetic tools, that can measure peptide secretion on a millisecond timescale, was developed by Whim and Moss (2001). Here, the neuropeptide FMRFamide is added as an electrophysiological "Tag" to the DNA that codes for the peptide of interest, for example NPY. The FMRFamide-tagged neuropeptide prohormone is then expressed together with the FMRFamide receptor, a unique neuropeptide ionotropic receptor, which forms a sodium-permeable channel (Lingueglia et al. 1995). Triggering exocytosis from a single transfected cell results in cosecretion of both FMRFamide and NPY. The released FMRFamide then activates FMRFamide receptors, resulting in an influx of sodium ions that is detected as a rapid inward current, which reflects the cosecretion of NPY—the peptide of interest.

1.5 WHAT MAKES NEUROPEPTIDES UNIQUE AS MESSENGER MOLECULES?

Neuropeptides represent a type of chemical messengers, which, in several aspects, are different from acetylcholine (ACh), catecholamines, serotonin, and amino acids, which are sometimes collectively referred to as classic transmitters. The following is a summary of some of the main characteristics of neuropeptide signaling (Figure 1.1):

1. Neuropeptides are ribosomally synthesized as large precursor molecules (molecular weight ~25,000) in soma/dendrites and stored in large dense core vesicles (LDCVs, diameter ~1.000Å). The bioactive peptide is then

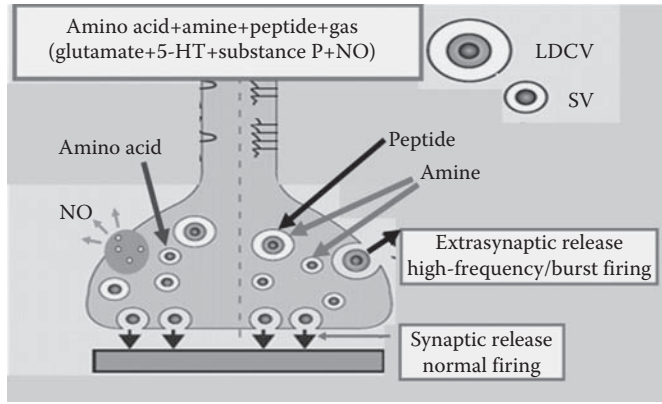

FIGURE 1.1 (See color insert) The one neuron multiple transmitters concept. This nerve ending stores four different types of messenger molecules, partly in different compartments: Peptides in large dense core vesicles (LDCVs, diameter ~1,000Å), amines in LDCVs and synaptic vesicles (SVs, diameter ~500Å), and glutamate in SVs. Nitric oxide (NO) is not stored but generated "upon demand" by NO synthase and diffuses through the membrane. Neuropeptides are released extrasynaptically in response to burst firing/high firing frequency, amino acids mainly into the synapse, and amines presumably in both ways. (From Hökfelt 1991; Lundberg 1996; Merighi 2002.)

excised by convertases. In contrast, classic transmitters are mainly stored in synaptic vesicles (SVs) (diameter 500Å), although amines also are present in LDCVs.

2. Neuropeptides can be released both from nerve endings and soma/dendrites, in fact similar to some classic transmitters. After release, they are mainly degraded by extracellular peptidases.
3. Each released molecule has to be replaced by new synthesis (=increased transcript levels) and is then intraaxonally transported to nerve endings, or to dendrites. Thus, there is no reuptake mechanism, neither at the cell membrane nor at the membrane of the storage vesicles.
4. Neuropeptides are not the main messengers but coexist with other molecules, classic transmitters (e.g., ACh, dopamine, serotonin, γ-aminobutyric acid (GABA), glutamate), nitric oxide, and others.
5. Neuropeptides are, when compared with classic transmitters, active at very low concentrations, in the nanomolar range.

1.6 PLASTICITY OF NEUROPEPTIDE EXPRESSION

It has been demonstrated in several systems that the expression of genes for peptides and peptide receptors is highly dynamic. Thus, there are marked changes under various experimental conditions, for example, in response to stimuli, such as nerve injury, electrical activity, and pharmacological manipulations. One reason for this is that, as mentioned earlier, every neuropeptide molecule released has to be replaced by ribosomal synthesis associated with a rapid increase in transcript

levels. However, induction, even *de novo* synthesis of neuropeptides, may occur in response to nerve injury. A highly uneconomic system, one may think, perhaps due to an ancient origin of peptide signaling. Subsequently more efficient working mechanisms, such as synapses, have evolved, allowing rapid transmission in the mammalian nervous system.

Plasticity and expression of neuropeptides occur in many systems, for example, in primary sensory neurons (Hökfelt et al. 1994) and sympathetic neurons (Zigmond and Sun 1997). The former contain, under normal circumstances, high levels of CGRP, substance P, and somatostatin, and low levels of galanin. VIP and NPY can hardly be detected. After axotomy (transection of the sciatic nerve), there is appearance of VIP (mainly in small neurons) and NPY (mainly in large neurons), and upregulation of galanin (in small and large neurons) in the corresponding dorsal root ganglia (L4, L5). In contrast, substance P and CGRP are downregulated. Because the latter two peptides have an excitatory function in the dorsal horn, and NPY and galanin mainly are inhibitory, these regulations are thought to cause attenuation of dorsal horn excitability, perhaps to suppress pain. VIP (Waschek 1995), galanin (Hobson et al. 2008), and NPY (Hansel et al. 2001) can also have trophic effects and promote survival and regeneration. This reaction pattern is in agreement with the general view that the synthetic machinery of a neuron after injury is converted from transmitter synthesis to production of molecules of importance for survival and recovery. Interestingly, the expression of neuropeptide receptors also changes after nerve injury.

1.7 NEUROPEPTIDE RECEPTORS

For quite some time, the question of peptide receptors was open, in spite of extensive evidence for the presence of neuropeptide-binding sites based on experiments, both with isolated membranes and ligand-binding autoradiography (Kuhar et al. 1991). Could it be that the peptides act on specific sites on major transmitter receptor proteins, such as the benzodiazepines/diazepam-binding inhibitory peptide on the GABA receptor? This idea gained some credibility, when it was realized that neuropeptides are costored and coreleased with classic transmitters, such as monoamines and GABA (see aforementioned). Nakanishi and coworkers gave a clear answer to that question by cloning the first neuropeptide receptor, a substance K receptor (Masu et al. 1987). This receptor turned out to belong to the 7-transmembrane (7-TM) GPCR family. Subsequent work showed that virtually all other, several hundreds or so, neuropeptide receptors also are members of this family. However, there is at least one exception: the peptide Phe-Met-Arg-Phe-NH_2 (FMRFamide), which induces a fast excitatory depolarizing response via direct activation of an amiloride-sensitive sodium channel (Green et al. 1994; Lingueglia et al. 1995). Needless to say, the multiplicity of receptors offers unique and important openings for the development of specific agonists and antagonists, which are potential candidates for treatment of various diseases.

The cloning of the peptide receptors allowed mapping at the message level with *in situ* hybridization and, after production of antisera, defining the exact localization and trafficking of the receptor protein, using immunohistochemistry.

An early, impressive example was the dramatic internalization of the substance P (NK1) receptor in second-order spinal dorsal horn neurons after peripheral nerve stimulation (Mantyh et al. 1995).

1.8 NEUROPEPTIDE DRUGS AND PHARMACOLOGY

Our knowledge of the functional roles of classic transmitters has to a large extent been based on the large extent on the availability of a huge number of drugs influencing transmitter mechanisms. It has taken a long time to generate this type of tools in the peptide field, and still there is a long way to go. Peptide drugs can be classified into at least three categories, antagonists, agonists, and peptidase inhibitors, the latter preventing peptide breakdown and thus strengthening peptidergic transmission. Peptide conversion, an interesting mechanism, is a potential pathway for modulating G-protein signaling (Nyberg and Hallberg 2007). Also, early on, antisense methodology and, more recently, methods based on microRNA, siRNA, and viral approaches have been successfully used.

Following modified peptide antagonists (often D-amino acid substitutions), nonpeptide substances were developed for most neuropeptides, mostly within the framework of the pharmaceutical industry. By penetrating the BBB, they raise hope of treating various brain disorders. Perhaps, the most spectacular progress apparently was the report that an NK1 (substance P) receptor antagonist has clinical efficacy in major depression, virtually without side effects (Kramer et al. 1998). This could not be confirmed in the phase 3 trial, but a recent study gives new hope for NK1 antagonists in the treatment of depression (Ratti et al. 2011). Nevertheless, there are now a number of peptide antagonists close to the clinic, for example, an orexin antagonist in phase 3 trials as a novel type of sleeping pill (Brisbare-Roch et al. 2007) and CGRP antagonists as a new-generation antimigraine medicine (Diener et al. 2011).

1.9 WHY ARE NEUROPEPTIDE RECEPTORS ATTRACTIVE TARGETS FOR DRUG DEVELOPMENT?

Just the sheer number of neuropeptides and the correspondingly large number of 7-TM GPCRs make them interesting, also because more than half of all drugs prescribed today are acting via this type of receptor. Moreover, their wide distribution in discrete systems, many directly associated with diseases, such as neuropathic pain and depression, provides a basis for drug development. An important aspect is that neuropeptide systems are prone to species variations (Bowers 1994). Thus, targets based on animal experiments may not be valid for design of drugs for treatment of human diseases. And, the concept of cotransmission presents some advantages, as well as problems, when considering novel pharmacological treatment strategies for disease-involving peptides. Here are some aspects:

1. Neuropeptides are comparatively large molecules and have difficulties in passing through the BBB, reaching receptors in brain and spinal cord only to a limited extent. Thus, small, BBB-penetrating, nonpeptide molecules

have been developed. Even if they are efficacious in animal experiments, it has been difficult to perform clinical tests in several cases, as such compounds are associated with serious side effects in humans, not rarely liver toxicity.

2. Using antagonists, it may not be sufficient to block one receptor to obtain the expected effect, if several transmitters are released from the same nerve ending. For example, early animal work on substance P antagonists on pain pathways clearly indicated that an antagonist would be analgesic. However, when tested in clinical trials, no such effect was observed (Hill 2000). Perhaps release of costored, excitatory transmitters, for example, glutamate, CGRP, and others, from central sensory nerve endings conveyed the pain message even if NK1 receptors were blocked.
3. Intervention with antagonists may be preferred, because
   a. Antagonists will only affect deranged (upregulated) systems; peptide transmission is mostly silent under normal conditions, which should result in less side effects.
   b. Agonists will act on receptors in the entire body, resulting in more adverse reactions. A good example is morphine, which, in addition to its well-established and unsurpassed antinociception, can produce serious side effects.

1.10 NEUROPEPTIDE SYSTEMS AS TARGETS FOR TREATMENT OF NEUROPATHIC PAINS

Targeting neuropeptide receptors for disease treatment represents a promising venue, but can be full of pitfalls. As an example, here we focus on neuropathic pain, which still is of major concern, as no efficacious and safe pharmacological treatment is available (Costigan et al. 2009) (see Chapter 8 by Dr. Ueda in this book) (Figure 1.2). A research focus in this field has been on the manifold and often dramatic phenotypic neurochemical changes occurring in dorsal root gangila (DRGs) and spinal cord in various types of rodent neuropathic pain models (Costigan et al. 2002; Hökfelt et al. 1994; Xiao et al. 2002). One such molecule is the neuropeptide galanin (Tatemoto et al. 1983), which is strongly upregulated in lumbar L4 and L5 DRGs after transection of the sciatic nerve (Hökfelt et al. 1987; Villar et al. 1989). In a long series of studies, it has become clear that this upregulation serves at least two purposes, to modulate pain signaling and to enhance regeneration.

1.11 GALANIN AS A PAIN TRANSMITTER

It has been proposed that galanin after nerve injury, via the GalR1 receptor, serves as an endogenous analgesic, attenuating the excitatory tone at the spinal level (Xu et al. 2008). Galanin also has a pronociceptive effect, exerted via the GalR2 receptor, stimulation of phospholipase C (PLC) β3, increase of intracellular Ca^{2+}, and release of excitatory transmitters, for example, glutamate and CCK (Liu et al. 2001) (Figure 1.3). However, a GalR2 antagonist does not distinctly attenuate pain (Shi et al. 2012, unpublished data). One reason may be that in neuropathic pain,

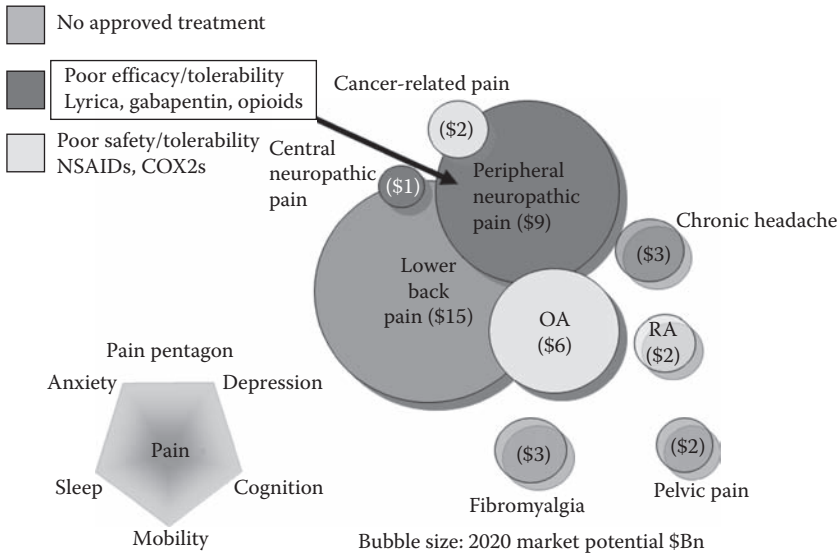

FIGURE 1.2 (See color insert) Schematic presentation of various pain states and their estimated values in the market. Dark grey indicates that available drugs have poor efficacy, as is the case for those presently used to treat peripheral neuropathic pain. The market for neuropathic pain drugs is large, here estimated to be 9 billion USD in 2020. NSAID, nonsteroidal anti-inflammatory drugs; COX, cyclooxygenase; OA, osteoarthritis; RA, rheumatoid arthritis. (Courtesy of Dr Andy, Dray, AstraZeneca, Montreal.)

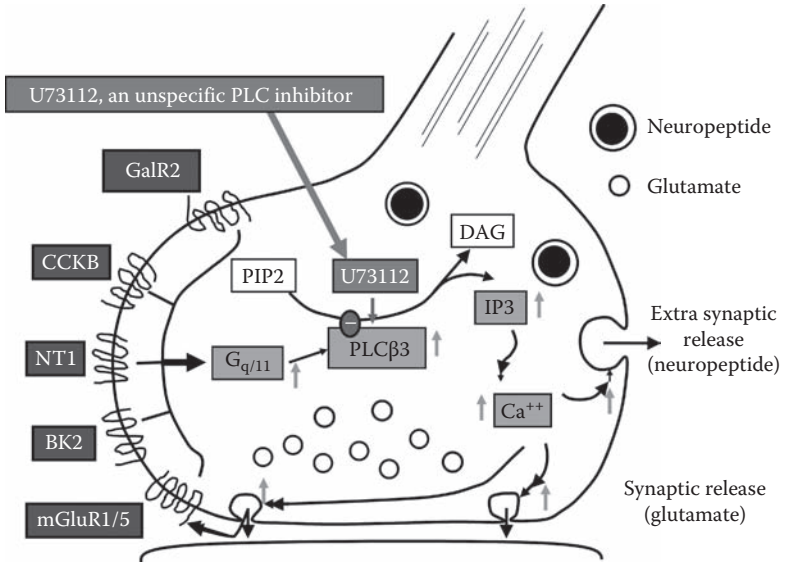

FIGURE 1.3 Schematic drawing proposing that one and the same nerve ending (or neuron) has five different receptors, all acting via $G_{q/11}$ G-protein, PLCβ3, IP3, and increased intracellular Ca^{2+}, resulting in release of glutamate and neuropeptides and eventually pain. DAG, diacylglycerol.

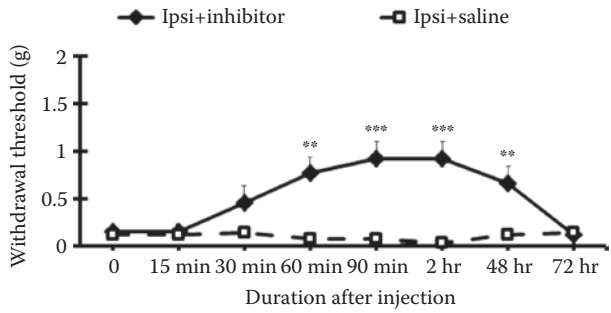

FIGURE 1.4 The unspecific PLCβ inhibitor U73112, administered as one dose (30 mg/kg), 14 days after performing unilateral SNI, causes a long-lasting, ~48 hours, increase in pain threshold. (From Shi, T.J., et al., *Proc. Natl. Acad. Sci. U S A,* 105, 20004–20008, 2008. With permission.)

many other receptors, which converge on PLCβ3, are activated (Figure 1.3). Thus, blocking one of them may not be sufficient to achieve pain relief. When pain is induced after various types of peripheral nerve injuries, several dramatic changes occur in rat DRGs, including (i) increased number of CCK mRNA-positive DRG neuron profiles (up from a few to 30%) (Verge et al. 1993); (ii) increase in CCK subtype B (2) receptor mRNA-positive DRG neuron profiles (up by 20-fold) (Zhang et al. 1993); and (iii) increased bradykinin subtype 1 receptor (BK1) (up by 8-fold) and BK 2 (up by 1.5-fold) binding (Petcu et al. 2008). All these changes would appear to be pronociceptive. One approach is therefore to test more than one antagonist, for example, a GalR2 plus a BK1 antagonist. Such experiments are ongoing in our laboratory.

PLCβ, in particular the PLCβ3, has been shown to be expressed in rodent DRG neurons and associated with pain and itch (Han et al. 2006; Joseph et al. 2007; Shi et al. 2008). We have administered an unspecific PLCβ3 inhibitor, U73112, to mice exposed to spared nerve injury, a neuropathic pain model (Decosterd and Woolf 2000), and observed a dramatic antinociceptive effect, the pain threshold being elevated for ~48 hours (Shi et al. 2008) (Figure 1.4). To our knowledge, this is a uniquely long-lasting effect on neuropathic pain. As PLCβ3 is expressed in human dorsal root ganglia, both at the protein (Shi et al. 2008) and transcript (Barde and Hökfelt 2012, unpublished data) levels, inhibition of PLC may represent a novel and powerful treatment strategy for neuropathic pain.

1.12 GALANIN AS A TROPHIC MOLECULE

The first evidence that galanin also could have trophic functions was presented by Wynick and coworkers. Thus, it was noted that in galanin knockout mice, there was a clear reduction in the number of DRG neurons (down by 13%) (Holmes et al. 2000). Here, GalR2 seems to play a critical role, as genetic deletion of this receptor results in a similar reduction in the percentage of DRG neurons (Hobson et al. 2006; Shi et al. 2006). Wynick and coworkers also showed that galanin is involved in regeneration. Thus, following a crush injury, sciatic nerve regeneration is reduced

by 35% in galanin (Holmes et al. 2000) and GalR2 (Hobson et al. 2006) knockout mice, deficits that can be rescued by administration of galanin and the GalR2 agonist galanin(2-11) (Mahoney et al. 2003). Galanin has survival effects also in the brain, for example, in galanin knockout mice a third of the cholinergic forebrain neurons are lost (O'Meara et al. 2000).

Several studies have demonstrated a neuroprotective role of galanin. For example, galanin knockout mice show a higher loss of pyramidal neurons in the hippocampus than wild-type mice after peripheral injection of kainic acid, and galanin or galanin(2-11) counteracts cell death induced by glutamate in hippocampal cultures (Elliott-Hunt et al. 2004; Pirondi et al. 2005).

1.13 CONCLUDING REMARKS

Serious research on neuropeptides has been in progress for some four decades and remarkable strides have been made. Today, we encounter a wealth of neuropeptides and corresponding receptors in animal experiments associated with both normal brain function and many pathologies. They include the topic of this book, neuroprotection and neuroregeneration. Clinical trials targeting neuropeptide mechanisms have been initiated and, even if not reaching prescription drugs, there are many promising candidates that could take the decisive step into the clinic in the near future.

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2 Neuropeptide Biosynthesis

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2.1 INTRODUCTION

Neuropeptide biosynthesis takes place in the regulated secretory pathway of neurons. Prepro-neuropeptides are cotranslationally inserted into the endoplasmic reticulum (ER), where the signal sequence that targeted them to the ER is removed by the signal peptidase, producing a proneuropeptide. Proneuropeptides are further modified in the rough endoplasmic reticulum by protein folding, formation of disulfide bonds, and glycosylation of asparagines. The proneuropeptides move to the Golgi apparatus and are posttranslationally modified by addition of carbohydrate groups to serine and threonine residues and sulfates to tyrosine residues. In the trans-Golgi network (TGN), they are sorted into large dense core synaptic vesicles. In the Golgi and in these vesicles, they are cleaved in a specific order by endoproteases, exoproteases, and, finally, in some cases, further modified by carboxyl-terminal amidation, *N*-octylation, amino-terminal acetylation, and conversion of amino-terminal glutamines to pyroglutamate. The "finished" products are stored in synaptic vesicles and transported to the synaptic cleft until they are released by an appropriate stimulus. Once released, they are degraded by extracellular proteases.