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OPIOID PEPTIDE PROCESSING AND RECEPTOR SELECTIVITY

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INTRODUCTION

Since the discovery of the enkephalins in 1975, an increasing number of larger opioid peptides, which contain the sequence of either met-enkephalin or leu-enkephalin at their N-terminus, have been isolated. All these opioid peptides belong to one of three peptide families, each deriving from a distinct precursor molecule: Proopiomelanorcortin (POMC), the common precursor for β-endorphin, ACTH, and additional MSH-containing peptides; proenkephalin A, the common precursor for met- and leu-enkephalin and several larger enkephalin-containing peptides (e.g. peptide E, peptide F) and proenkephalin B (prodynorphin), another precursor for leu-enkephalin and for larger opioid peptides (e.g. the dynorphins, neo-endorphins and leumorphin). The structures of these precursor molecules of several species have been determined using recombinant DNA techniques [reviewed by Numa (1)].

The precise proteolytic processing of POMC has been analyzed in pituitary cells and described in recent reviews (2–5). Similarly, the processing of proenkephalin A in the bovine adrenal medulla has been extensively reviewed recently (6, 7). Less is known about the processing of proenkephalin B [prodynorphin, summarized in (8)]. Moreover, the proteolytic fragmentation of all three opioid peptide precursors within the central nervous system is still poorly understood.

In addition to the multiplicity of opioid peptides, there is pharmacological evidence for multiple opioid receptors. Martin et al (9) classified opioid receptors in terms of their effects in whole animals as being morphine-like (μ), ketocyclazocine-like (κ), and N-allylnormetazocine-like (σ). The pharmacological characterization of opioid receptors by the use of isolated tissues such as

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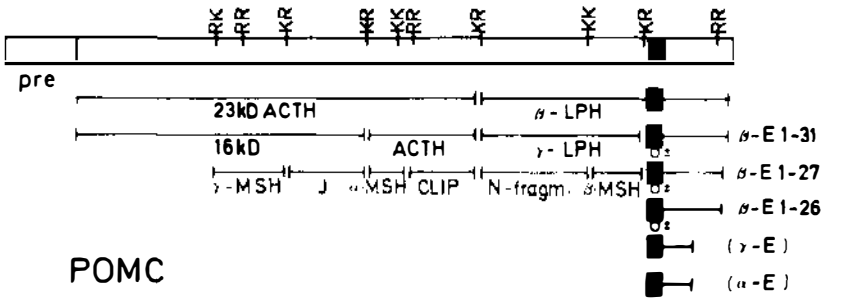

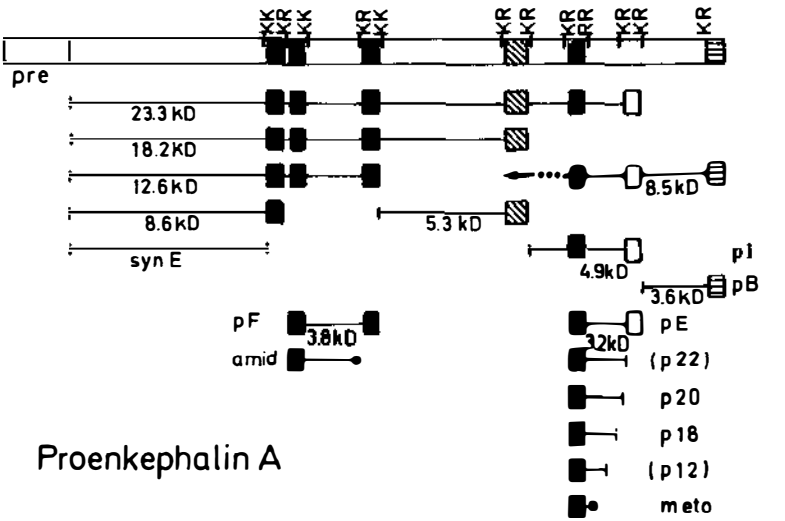

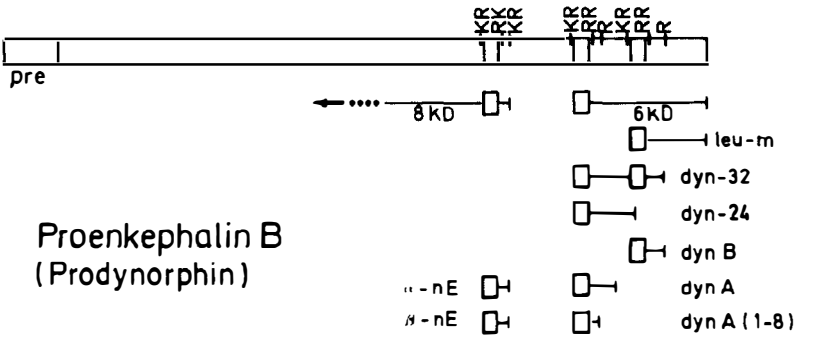

the guinea-pig ileum and the vasa deferentia of the mouse, rat, and rabbit has led to the observation that, in addition to interacting with the μ- and κ-types of opioid receptors, the opioids can interact with δ- (10) and ϵ- receptors [(11); reviewed in (12)]. In this review an attempt is made to summarize the literature describing the various products of processing of the three opioid precursors and their selectivity for the different classes of opioid receptors.

THE PROOPIOMELANOCORTIN (POMC) SYSTEM

Processing Products

The structure of bovine POMC, together with its major processing products, is shown schematically in Figure 1 (13). Each of the peptides in the precursor molecule is bounded by pairs of basic amino acid residues, which represent the sites of proteolytic processing (14). The major source of POMC production is the pituitary. Studies of biosynthesis have revealed that the processing of POMC in the intermediate lobe of rats is different from that in the anterior lobe (3, 5). In general, POMC undergoes more cleavages and its products more posttranslational modifications in the intermediate lobe than in the anterior pituitary. There is little or no β-lipotropin in the intermediate pituitary. β-Endorphin is posttranslationally modified by α-N-acetylation which leads to a complete loss of its activity at opioid receptors. In addition, it is shortened at its C-terminus by 4 or 5 amino acids. Thus, N-α-acetyl-β-endorphin 1–27, and N-acetyl-β-endorphin 1–26 are major end products in the processing of POMC in the intermediate pituitary of rats (2, 3). In contrast, β-lipotropin is a major POMC end product in the anterior pituitary. Its concentrations are about twofold higher than those of β-endorphin 1–31, which is not posttranslationally modified. Differential processing in the anterior and intermediate pituitary lobes also exists for the ACTH domain of POMC. Whereas ACTH 1–39 is the major product in the anterior lobe, this peptide undergoes further proteolysis

Figure 1 Opioid peptide precursors and processing products. Data are derived from the following references: POMC (1–3, 5, 13, 24), proenkephalin A (1, 6, 7, 41, 47–60), proenkephalin B (1, 86–95, 99). The presence of modifications in the nonlipotropin portion of POMC has been omitted for simplicity. Upward marks indicate cleavage at basic amino acids (K = lysine, R = arginine). Abbreviations are: POMC = proopiomelanocortin; ACTH = corticotropin; LPH = lipotropin; MSH = melanotropin; CLIP = corticotropin-like intermediate lobe peptide; J = joining peptide; α, β, γ-E = α, β, γ-endorphin; kD = kilodaltons; syn E = synenkephalin; pB, E, F, I = peptide B, E, F, I; p22, 20, 18, 12 = BAM-22P, -20P, -18P, -12P; amid = amidorphin; meto = metorphamide; dyn = dynorphin; leu-m = leumorphin; nE = neoendorphin; pre = signal sequence. ■ = met-enkephalin, □ = leu-enkephalin; ▤ = met-enk-arg-phe, ▨ = met-enk-arg-gly-leu; ● = amido group, ○ = N-α-acetyl-residue; ← indicates that the N-terminus of the peptide is not precisely defined.

and posttranslational processing in the intermediate pituitary. After acetylation and amidation, the major end product of POMC is α-MSH. In addition to acetylation and amidation, many posttranslational modifications occur in the non-β-lipotropin portion of POMC that are not discussed here (4, 5).

Processing of POMC in the brain appears to be similar to that found in the intermediate pituitary. In general, immunoreactive material of a molecular size similar to that of β-endorphin and α-MSH is the predominant POMC product in the brains of rats and humans (15, 16). However, N-acetylation of β-endorphin seems much less pronounced in the brain than in the intermediate pituitary. Some authors (17, 18) were not able to detect N-acetyl-β-endorphin and α-MSH in rat brain. Other groups found N-acetylated variants in several brain regions of rats, although to a varying degree (19–21). In some brain regions the acetylated forms of β-endorphin and α-MSH were the predominant POMC products (3, 21). Moreover, cleavage at the carboxyl terminus of β-endorphin appears to occur in the brain. This is indicated by the detection of acetylated and nonacetylated forms of β-endorphin 1–26 and β-endorphin 1–27 (3).

Shorter cleavage products of β-endorphin such as α-endorphin (β-endorphin 1–16) and γ-endorphin (β-endorphin 1–17) have been isolated from pituitary tissue. These peptides do not, however, seem to be true processing products of POMC in the pituitary as indicated by pulse-labelling studies (2). Similarly, met-enkephalin is not a processing product of POMC in the pituitary (2).

γ-Endorphin and α-endorphin and their des-tyrosine forms have been found in brain extracts (22). It is still unclear whether these peptides might be extraction artifacts or degradation products resulting from the extracellular proteolysis of β-endorphin.

POMC has recently been biosynthesized in the hypothalamus of the rat (23). Liotta et al found some differences in the processing of POMC in brain as compared to that in the pituitary. Whereas β-lipotropin is first cleaved from its precursor to yield a biosynthetic intermediate termed 23 kD ACTH (24) in both pituitary lobes, at least part of POMC in the hypothalamus appears to undergo a primary cleavage that removes all or part of the N-terminal fragment. This indicates an altered order of processing (23). As in the intermediate pituitary, hypothalamic POMC was processed into molecules of sizes similar to those of β-endorphin and α-MSH. In contrast to the peptides of the intermediate pituitary, these peptides were physicochemically similar to des-acetyl α-MSH and authentic rat β-endorphin 1–31, indicating that no major acetylation of POMC products occurs in the hypothalamus.

Receptor Selectivity

Although β-endorphin was discovered almost 10 years ago, its receptor selectivity characteristics have not yet been fully clarified. In guinea-pig brain β-endorphin shows a slight binding selectivity for μ- over δ-receptor sites, with

negligible affinity for κ-sites (25). It exhibits no opioid activity on the rabbit vas deferens, a preparation proposed to have κ-opioid receptors only (12, 26–28). In the mouse vas deferens, however, β-endorphin shows no selectivity for μ- over κ- or δ-receptors as revealed in receptor inactivation experiments using a site-directed alkylating agent (29). It is possible, however, that β-endorphin interacts with ε-receptors in the mouse vas deferens. Moreover, there is increasing evidence that, in addition to μ- and δ-sites, β-endorphin interacts with a distinct population of binding sites in brain [putative ε-sites that differ from classical μ-, δ-, and κ-sites (30–33a)]. ε-Receptors occur in high abundance in the rat vas deferens (11). In this preparation, β-endorphin 1–27 is slightly more potent than its parent peptide β-endorphin 1–31. Further C-terminally shortened cleavage products, such as γ-endorphin (β-endorphin 1–17), α-endorphin (β-endorphin 1–16), and met-enkephalin (β-endorphin 1–5) are considerably less potent than β-endorphin 1–31 in the rat vas deferens (11). Although part of this potency difference may be attributed to the preferential inactivation of the shorter peptides by enzymatic degradation (33b), there appears to exist an intrinsically high potency of β-endorphin 1–31 and its processing product β-endorphin 1–27 on this preparation. Structure-activity studies revealed that the activation of the ε-receptors in the rat vas deferens requires large β-endorphin sequences (at least β-endorphin 1–21) (11, 33b, 34).

Similar observations have been made in binding studies using ^{3}H-β-endorphin as ligand. Met-enkephalin, α-, and γ-endorphin had considerably lower binding affinities than β-endorphin 1–31 and β-endorphin 1–27 (32). Thus, a C-terminally extended long peptide chain in β-endorphin as compared to met-enkephalin appears to increase the selectivity and potency for putative ε-receptors, and increases the resistance to degrading enzymes. On the other hand, modification of the N-terminus by acetylation virtually abolishes binding and opioid activity in the various preparations (3, 31).

Of a wide variety of endogenous opioid peptides tested, β-endorphin has been shown to be the most potent peptide in inducing analgesia after intracerebroventricular injection in mice (35). Interestingly, the complete carboxyterminal end of β-endorphin is necessary for full analgesic potency. Thus, the processing product β-endorphin 1–27 is much less analgesically active than β-endorphin 1–31 (3, 36). Furthermore, β-endorphin 1–27 even antagonizes the analgesic effect of β-endorphin 1–31 when coinjected into the brains of mice (36). Therefore, it has been suggested that the C-terminal processing of the parent peptide may produce a physiologically important antagonist of β-endorphin (36). However, no evidence of an antagonist action of β-endorphin 1–27 or other β-endorphin fragments was found in isolated tissue preparations (34). Moreover, the dramatic loss of analgesic activity of the C-terminally shortened peptide as compared to β-endorphin 1–31 was not reflected by similar changes in the affinity to either μ-, δ-, or ε-receptors (11) in

isolated tissues or in its binding properties to rat brain membranes (32). Thus, the functional significance of the C-terminal processing of β-endorphin in terms of altered opioid activity is not yet clear. Recent investigations, however, indicated that β-endorphin 1–31 might possess a second biologically active sequence at its C-terminus which activates the complement-binding system (37). β-Endorphin 1–27 is inactive in this system. Moreover, β-endorphin has been demonstrated to bind to lymphocytes through its C-terminal fragment, (38) and there is increasing evidence that the C-terminal portion of β-endorphin may influence the immune system by a nonopioid mechanism (39). Moreover, a dipeptide that is probably released by C-terminal processing of β-endorphin 1–31 (glycyl-glutamine) has recently been isolated from pituitary tissue and shown to possess properties characteristic of neurotransmitters (40).

In summary, β-endorphin 1–31 and β-endorphin 1–27 are processing end products in the pituitary and also in the brain. These peptides interact preferentially with μ-, δ-, and ε-, but not κ-opioid receptors. N-acetylation of these peptides in the intermediate pituitary (and possibly also in the brain) abolishes the opioid activities of these peptides and may be regarded as a physiological inactivation mechanism. C-terminal processing may, however, have a regulatory function in abolishing interactions of β-endorphin 1–31 with the immune system.

THE PROENKEPHALIN A SYSTEM

Processing Products

The structure of bovine proenkephalin A together with its major processing products is given in Figure 1 (41). Assuming that paired basic amino acids are processing signals, the structure of proenkephalin A suggests potential cleavage into four copies of met-enkephalin and one copy each of leu-enkephalin, the heptapeptide met-enkephalin-arg-phe (met-enk-arg-phe), and the octapeptide met-enk-arg-gly-leu. In addition, however, a wide variety of larger enkephalin-containing peptides have been isolated from bovine adrenal medulla (6, 7). The same copies of enkephalin and extended peptides are contained in human proenkephalin A (1, 42) and in rat (43–45). However, in *Xenopus laevis* proenkephalin A contains only met-enkephalin and extended sequences, but no leu-enkephalin (46).

In the bovine adrenal medulla proenkephalin A processing appears to occur predominantly at the C-terminus, since the majority of isolated enkephalin-containing precursor peptides possess the N-terminus of proenkephalin A [23.3 kD (47), 18.2 kD (48), 12.6 kD, 8.6 kD (49)]. In contrast, only two peptides that contain the intact C-terminus of proenkephalin A [peptide B (3.6 kD) (50) and an 8.5-kD peptide (51)] have also been described. In addition, several

enkephalin-containing peptides have been isolated that are derived from the mid-portions of the precursor. One is 5.3 kD (52); another, peptide I, is 4.9 kD (50).

There is a series of peptides that contain the sequence of met-enkephalin at its N-terminus and thus exhibit opioid activity. One such example is peptide F (53), a 3.8-kD peptide containing two met-enkephalin sequences, one at its N- and another at its C-terminus. A carboxy-terminally amidated peptide comprising the first 26 amino acids of peptide F has recently been isolated and named amidorphin (54). Peptide E is a 3.2-kD peptide possessing the sequence of met-enkephalin at its N-terminus and that of leu-enkephalin at its C-terminus (55). Several carboxy-terminally shortened peptides of peptide E have been isolated: BAM-22P (56), BAM-20P (56), BAM-18P (57), BAM-12P (58), and a carboxy-terminally amidated octapeptide corresponding to the first 8 amino acids of peptide E. This last peptide has been named metorphamide or adrenorphin (59, 60). Most of these peptides appear to be processed by cleavage at pairs of basic amino acids. Exceptions are BAM-22P and BAM-12P. It has therefore been claimed that these peptides are products of nonspecific proteolysis (7).

The generation of metorphamide (59) involves proteolytic cleavage at a single arginine amino acid—a cleavage site that exists in several peptide precursors (4). Furthermore, the C-terminal glycine residue is converted into an amino group by a specific amidating enzyme (61, 62). Similar mechanisms exist for the conversion of peptide F (1–27) into amidorphin (54).

There is strong evidence that proenkephalin A in bovine brain is similar or identical to that found in the adrenal medulla. Liston et al (63) isolated a 10-kD peptide from bovine caudate nucleus, the sequence of which is identical to residues 1–70 of adrenal medullary proenkephalin A. This peptide contains no opioid sequences and has been termed synenkephalin. Moreover, using antibodies against peptides from the adrenal medulla, immunoreactive peptides with the chromatographic properties of met-enk-arg-phe (64, 65), met-enk-arg-gly-leu (65), BAM-12P (66), BAM-22P (51, 67), peptide F (67), amidorphin (54), metorphamide (59), and BAM-18P (57) have been found in bovine brain. Moreover, proenkephalin A mRNA in bovine adrenal medulla and in various bovine brain areas has been shown to have the same size of about 1400 nucleotides (68, 69).

On the other hand, there is increasing evidence that processing of proenkephalin A in the adrenal medulla is different from that in the bovine brain. In the adrenal medulla the initial cleaving occurs near the carboxyl-termini, liberating large intermediates of 8.6–23.3 kD with intact N-termini (Figure 1). In the supraoptic nucleus, however, initial processing steps appear to involve the removal of the N-terminal fragment (70). The high-molecular-weight enkephalin-containing peptides are predominant in the supraoptic nucleus,

whereas the neurohypophysis almost exclusively contains free enkephalins. This indicates an almost complete processing of proenkephalin A along the hypothalamo-neurohypophyseal pathway (70).

In general, the processing of proenkephalin A appears to be more complete in the brain than in the adrenal medulla. Thus, the bovine adrenal medulla contains high amounts of high-molecular-weight enkephalin-containing peptides and relatively low amounts of free enkephalins (7). In the brain, however, free enkephalins are the predominant processing products of proenkephalin A.

In addition, peptides of higher molecular weight reacting with antibodies against BAM-22P, peptide F, met-enk-arg-phe, and met-enk-arg-gly-leu are more abundant in the adrenal medulla than in bovine hypothalamus or striatum (65, 67). In particular, in human brain tissue very low amounts of larger peptides reacting with antibodies directed against BAM-22P, BAM-12P, met-enk-arg-phe, and met-enk-arg-gly-leu have been found (71). This indicates that neuronal proenkephalin A is processed very rapidly in the brain and that the processing intermediates do not accumulate to any significant extent.

However, species differences may exist, since substantial amounts of proenkephalin A precursor and high-molecular-weight intermediates have been found in striatal tissue of rats and guinea pigs (72). Moreover, putative processing products of higher molecular weight appear to be present in some rat brain areas when antibodies directed against met-enk-arg-phe (73), or met-enk-arg-gly-leu (74) have been used.

The distribution of proenkephalin A–derived peptides in rats has been extensively investigated by immunohistochemical studies. An anatomical description of the widespread distribution of the proenkephalin A system in the brain is beyond the scope of this chapter and the reader is referred to a recent review by Watson et al (75).

An important problem is whether or not leu-enkephalin, present in a single copy in proenkephalin A, derives from this precursor or from proenkephalin B (prodynorphin), in which it is present in three copies.

The ratio of leu-enkephalin to met-enk-arg-phe or to met-enk-arg-gly-leu, putative processing end products also present as single copies in proenkephalin A, has been found to be about 1 : 1 in many areas of the rat brain (76, 77). This indicates that leu-enkephalin might predominantly arise from the processing of proenkephalin A with no major contribution of leu-enkephalin by proenkephalin B (prodynorphin). However, in certain regions, such as the substantia nigra, leu-enkephalin is derived from proenkephalin B (77). Thus, leu-enkephalin in the brain can be derived from either proenkephalin A or B, indicating regional differences in the processing rates of the precursor molecules.

As compared with met-enk-arg-phe the levels of metorphamide (adrenorphin = met-enk-arg-arg-val-NH2) were very low in the rat brain (78, 79). In

addition, there was no correlation between the distributional patterns of metorphamide (adrenorphin) and met-enk-arg-phe, implying that the amidated peptide is generated from proenkephalin A in a way distinct from that by which met-enk-arg-phe is formed in each region of the brain.

Interestingly, the highest concentration of the amidated octapeptide has been found in the olfactory bulb where other opioid peptides have been found in negligible amounts (78). This unique distributional pattern suggests that this peptide might have specialized physiological functions distinct from those of other opioid peptides.

A major processing product of proenkephalin A in the rat brain appears to be BAM-18P. This peptide has been shown to occur in higher concentrations in the hypothalamus than metorphamide (adrenorphin), BAM-12P, BAM-22P, and peptide E (57).

Receptor Selectivity

As revealed by binding studies, met-enkephalin and leu-enkephalin possess a high selectivity for δ binding sites in guinea-pig brain (10, 25) and mouse brain (28, 80). The heptapeptide met-enk-arg-phe and the octapeptide met-enk-arg-gly-leu have comparable affinities for μ- and δ- opioid receptors with lower affinities for κ-sites [reviewed in (12)]. The larger opioid peptides derived from the peptide E domain of proenkephalin A (peptide E, BAM-22P, BAM-12P) show high affinity and selectivity for μ-sites; in addition, they have high affinities for κ-sites—about 40–50% of the affinities for the μ-sites. In contrast, the affinity for δ-sites is low (28, 80, 81). The binding selectivity of metorphamide (59) is similar to that of the above peptides, indicating that the site selectivity does not markedly change when peptide E is processed to metorphamide. In contrast, peptide F exhibits an about 100-fold lower affinity for μ-sites than the peptides of the peptide E domain. Moreover, peptide F does not have any preferential affinity for any of the three types of binding sites tested (28, 80, 81).

In general, this binding selectivity is confirmed by the pharmacological selectivity of the opioid peptides in various bioassays. The "μ-selective" pattern of pharmacological activity of the peptide E–derived peptides is indicated by the high potency of this peptide in the guinea-pig ileum compared to the low potency of these peptides in the mouse vas deferens (55, 56, 59, 81, 82).

The interaction of the peptide E–derived peptides with functional κ-receptors is indicated by their high activity on the rabbit vas deferens—a preparation that has been shown to exclusively contain opioid κ-receptors (27, 28, 59, 82). Also, peptide E, BAM-22P, and BAM-12P appear to interact with κ-opioid receptors in the mouse vas deferens as revealed in experiments using a site-directed alkylating agent (29).

In contrast, the smaller opioid products of proenkephalin A (met-enkephalin, leu-enkephalin, met-enk-arg-phe, and met-enk-arg-gly-leu) exhibit a clear selectivity for δ-opioid receptors in the mouse vas deferens (29, 83). The octapeptide met-enk-arg-gly-leu appears also to interact with κ-opioid receptors as evidenced in the mouse vas deferens rendered cross-tolerant to κ-opioid receptor agonists (84).

Peptide F is not active on the rabbit vas deferens, and exhibits a low potency on the guinea pig ileum and mouse vas deferens (27, 55, 82), indicating that it does not have any particular affinity at μ-, κ, and δ-opioid receptors.

However, peptide F and the larger peptides of the peptide E domain (peptide E, BAM-22P) show a substantial potency on the rat vas deferens, indicating that they interact with putative ε-receptors (82, 85). Moreover, these peptides elicit a pronounced analgesia after injection into the mouse brain (34). It is noteworthy that the relative potencies of the above peptides on the rat vas deferens, i.e. on putative ε-receptors correlate well with their analgesic potencies in mice (82). This suggests that the ε-receptor may play a role in analgesia.

In conclusion, the enkephalins are not the ultimate products of proenkephalin A in many areas of the brain. A wide variety of larger carboxy-terminally extended peptides possessing different receptor selectivities have been identified. The peptides of the peptide E domain are potent agonists at μ-, κ-, and ε-receptors, whereas peptide F exhibits substantial potency at ε-receptors only. Complete processing of proenkephalin A into the enkephalins, the heptapeptide, and the octapeptide, is associated with a change in the receptor selectivity towards δ-opioid receptors.

THE PROENKEPHALIN B (PRODYNORPHIN) SYSTEM

Processing Products

The structure of porcine proenkephalin B (prodynorphin) has been deduced from cloned DNA sequences complementary to hypothalamic mRNA (86). It contains three leu-enke-phalin sequences each flanked by pairs of basic amino acids (Figure 1). If, however, lys-arg pairs were the only processing signals, proenkephalin B would be cleaved into three larger opioid peptides: β-neoendorphin (87), dynorphin A (88, 89), and leumorphin (90). However, several other peptides derived from proenkephalin B have been identified, indicating that enzymes with more complex specifications are involved. The pro-lys bond at the carboxy-terminus of β-neoendorphin is often resistant to proteolysis, and the larger decapeptide [α-neoendorphin, (91)] is a major processing product. Moreover, cleavage at dibasic residues is not obligatory but may also occur where single arginines exist. Thus, the formation of dynorphin B [rimorphin, (92, 93)] results from a cleavage between threonine-13 and arginine-14 of leumorphin. By a similar cleavage, dynorphin A (1–8)

(94, 95) is formed from dynorphin A. This type of cleavage appears to occur frequently, and the smaller peptides are thus more abundant than the larger ones in many areas of the brain (96–98). In addition, several larger peptides have been identified as putative processing products: A 4-kD peptide containing dynorphin A at the N-terminus and dynorphin B at the C-terminal end has been isolated [dynorphin 32 (92)]. Dynorphin 24 contains dynorphin A with a C-terminal extension of lys-arg and the sequence of leu-enkephalin (92). There is also evidence for a 6-kD peptide comprising dynorphin A and leumorphin (99). In addition, 8-kD peptides representing N-terminally extended forms of α- and/or β-neoendorphin have been found in the adenohypophysis of rats (100, 101).

Proenkephalin B–derived peptides are most abundant in the neural lobe of the rat pituitary. They are colocalized with vasopressin in neurons originating in the magnocellular nuclei of the hypothalamus (102, 103).

Several products of proenkephalin B have been found in the posterior pituitary including the 6-kD peptide, dynorphin 32, dynorphin A, dynorphin A(1–8), α-neoendorphin, β-neoendorphin, and dynorphin B (97–99, 104).

There is also some evidence that leu-enkephalin is a processing product of proenkephalin B in the rat posterior pituitary. Osmotic stimuli such as salt-loading concomitantly decrease dynorphin A and leu-enkephalin levels in the posterior pituitary but have no effect upon met-enkephalin levels (105, 106).

Part of the leu-enkephalin in the rat posterior/intermediate pituitary, but not in the brain, can undergo N-acetylation (107). N-acetyl-leu-enkephalin is devoid of opioid activity. Dynorphin A and its high-molecular-weight forms, however, are not acetylated. On the other hand, a minor portion of dynorphin A (1–8) appears to be α-N-acetylated, suggesting that N-acetylation may occur during the late stages of proenkephalin B processing (104).

In contrast to the processing of proenkephalin B in the posterior pituitary, differential processing of proenkephalin B appears to occur in the anterior lobe. A putative end product in this lobe is a 6-kD dynorphin peptide containing the C-terminal part of proenkephalin B with the sequence of dynorphin A at its N-terminus. This peptide shows opioid activity (97, 99, 101). There is no evidence for the further processing of the 6-kD peptide into dynorphin A, leumorphin, or dynorphin B in the adenohypophysis of rats. On the other hand, α-neoendorphin and β-neoendorphin are processing products in this lobe. In addition, large N-terminally extended (non-opioid) forms of α- and β-neoendorphin have been found with a molecular size of about 8 kD (100, 101).

The distribution of the various proenkephalin B–derived peptides has been extensively studied in many areas of rat (96–98, 100, 108) and human (71, 100, 109, 110) brain. These studies strongly suggest that proenkephalin B is differentially processed in the various brain areas (96–98, 108). Whereas dynorphin A and dynorphin A (1–8) occur in about equal amounts in the posterior

pituitary, dynorphin A (1–8) is the predominant peptide in the brain. The ratio of dynorphin A(1–8) to dynorphin A is particularly high in the striatum and the midbrain (96, 98, 111).

Similarly, the molar ratio of α-neoendorphin to β-neoendorphin differs in various brain regions (112). In two thirds of 100 microdissected brain areas, α-neoendorphin is more abundant than β-neoendorphin (113). This indicates regional differences in the activity of enzymes that remove the C-terminal lysine of α-neoendorphin. The best candidates for such enzymes are post-proline cleaving enzymes, which exist in the brain (114).

Although dynorphin B is abundant within the rat brain, the presence of leumorphin in brain is still a matter of controversy (97, 98, 115). Suda et al used an efficient extraction method, and reported the existence of substantial amounts of leumorphin in human striatum (110).

A striatonigral pathway in rat brain containing very dense collections of fibers and terminals reacting with antibodies directed to dynorphin A (1–13) has been identified (116). The cell bodies of these fibers are located in the head of the caudate nucleus (117). The substantia nigra has one of the highest concentrations of dynorphins and neoendorphins within the central nervous system of rat (77) and man (109). Deafferentation of the globus pallidus results in a concomitant decrease in the levels of leu-enkephalin and dynorphin B, but not of met-enk-arg-gly-leu (77). This experiment provides evidence for processing of proenkephalin B into leu-enkephalin and for a distinct anatomical location of the proenkephalin A and proenkephalin B systems in the brain. On the other hand, there is also evidence for colocalization of both systems such as in medullary neurons of the rat (118).

Leu-enkephalin, which can derive from proenkephalin A and proenkephalin B, may undergo further posttranslational modifications. Indeed, a tyr-o-sulfated form of leu-enkephalin has been isolated from brain that is devoid of opioid activity (119).

Receptor Selectivity

With the exception of leu-enkephalin, all opioid peptides derived from proenkephalin B have been shown to exhibit a pronounced selectivity for κ-opioid receptors (25, 28, 29, 81, 82, 120–123). In binding experiments, dynorphin A appears to have the highest affinity and highest selectivity for κ-binding sites, whereas its fragment dynorphin A (1–8) has a lower affinity and a somewhat lower selectivity for κ-sites in guinea-pig membranes (25). All these peptides are active in the rabbit vas deferens, a preparation that has been proposed to contain exclusively functional κ-receptors (26, 27, 81, 122, 124, 125).

Dynorphin A, α-neoendorphin, and dynorphin B are selective κ agonists in the guinea-pig ileum, a tissue containing μ- and κ-opioid receptors. Dynorphin A (1–8) and leumorphin appear to interact with κ- and μ-opioid receptors as

assessed by differing sensitivities of the peptides to antagonism by naloxone (123). Similarly, in the mouse vas deferens, dynorphin A, dynorphin B, and leumorphin are selective κ agonists, whereas dynorphin A(1–8) and β-neoendorphin are distinctly less selective for κ-receptors and appear to interact also at δ-receptors as revealed in alkylation experiments (29) and in cross-tolerance studies (83). In the rat vas deferens, which contains a high abundance of ϵ-receptors, however, all proenkephalin B–derived peptides with the exception of leu-enkephalin are virtually inactive (85, 122).

The structural determinants for κ-selectivity appear to be an arginine residue at position 7 and another basic amino acid at position 10 or 11 (8). Dynorphin A, dynorphin B, and α-neoendorphin fulfill these criteria, but not dynorphin A(1–8) or β-neoendorphin, thus explaining their distinctly lower κ activity. Hence, if dynorphin A or α-neoendorphin were processed into dynorphin A(1–8) or β-neoendorphin, and further to leu-enkephalin, there would be a stepwise decrease in the selectivity for κ- and an increase in the selectivity for δ-opioid receptors.

In contrast to the POMC product β-endorphin and the majority of the proenkephalin A–derived peptides, the proenkephalin B–derived peptides have only weak analgesic properties, if any, when injected into the brain (34, 126). It is likely, however, that κ-opioid receptors are involved in mediating antinociception at the level of the spinal cord [reviewed in (82)].

In conclusion, the proteolysis of proenkephalin B generates a set of opioid peptides that exhibit selectivity for κ-opioid receptors. Dynorphin A is the most potent and selective peptide. Its further processing into dynorphin A(1–8) and further into leu-enkephalin occurs differentially in the various areas of the brain and this is associated with a change in the receptor selectivity towards δ-opioid receptors.

CONCLUDING REMARKS

This article reviews data evidence that posttranslational proteolysis of opioid peptide precursors occurs differently in various regions of the brain and pituitary and that the processing products can differ markedly in their selectivity for the various types of opioid receptors. In general, no simple allocation can be made between the opioid peptides of a precursor family and their preference for a certain class of receptors. Thus, processing of POMC yields peptides that interact with μ-, δ-, and ϵ-receptors; proenkephalin A–derived peptides interact with μ-, δ-, κ-, and ϵ-receptors; and the peptides derived from proenkephalin B interact preferentially with κ- and δ-opioid receptors. Some peptides exhibit a high selectivity for a certain receptor type, such as dynorphin A for κ-receptors or the enkephalins for the δ-receptors. Although some peptides have a preference for μ-receptors (β-endorphin and the peptides derived from peptide E),

their selectivity is insufficient to accept them as prime candidates for the endogenous ligands of the μ-receptor. The only compound that has exclusive selectivity for the μ-type of receptors is thought to be of plant origin, namely morphine. Recent evidence suggests that such a compound is present in animals (127).

The formation from a single precursor of opioid peptides with presumably different biological activities offers a level at which physiological regulation may take place. Thus, specific processing enzymes may play a key role in regulating the biological activities of the opioid peptide system. It is important to remember that the opioid receptors are still poorly defined on the basis of relatively crude techniques and that the biological functions mediated by the various opioid receptor types are not understood. There is a clear need to define opioid receptors structurally, and it is likely that their structure, as well as the structures of the enzymes involved in opioid precursor processing, will be elucidated by recombinant DNA techniques in the near future. This will allow the regulation of the processing of the precursors and the interaction of the products with the opioid receptors to be studied at a molecular level. Finally, the design of specific inhibitors of the processing enzymes may provide further insight into the physiological role of opioid peptide processing and may offer a basis for the development of new opioids.

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