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Review

Behavioral pharmacology of neuropeptides related to melanocortins and the neurohypophyseal hormones

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Abstract

Neuropeptides are peptides which affect the nervous system. They are derived from large precursor molecules. These are converted to neurohormones, neuropeptides of the ''first generation'', which can be further converted to neuropeptides of the ''second generation''. This review is a brief survey of the nervous system effects of neuropeptides derived from pro-opiomelanocortin (POMC) and the neurohypophyseal hormones. Processing of these molecules results in neuropeptides of the first and second generation which have similar, different, more selective or even opposite effects. Among those are effects on learning and memory processes, grooming, stretching and yawning, social, sexual and rewarded behavior, aging and nerve regeneration, thermoregulation, pain, sensitivity to seizures, and cardiovascular control. Results of animal studies as well as those of clinical studies suggest that these neuropeptides may be beneficial in aging, neuropathy, memory disturbances and schizophrenia. Most of these nervous system effects in animal studies were found before receptors in the nervous system for the various neuropeptides were detected. G-protein-coupled receptors for the neuropeptides of the ''first generation'', i.e., melanocortin receptors, opioid receptors, and neurohypophyseal hormone receptors have been found, in contrast to the receptors for neuropeptides of the ''second generation'', although there are indications that G-protein coupled receptors for these may be present in the brain.

Keywords: Neuropeptide; Melanocortin; Pro-opiomelanocortin (POMC); Neurohypophyseal hormone; Behaviour; Regeneration; Aging; Cardiovascular control; Thermoregulation

1. Introduction

The term neuropeptide denotes peptides which affect the nervous system. Neuropeptides are formed from large precursor molecules which are mainly produced in nerve cell bodies. The precursor protein is cleaved by proteolytic enzymes to generate neurohormones, neuropeptides of the "first generation". A great variety of neuropeptide precursors are formed in the nervous system. Over 50 genes that encode these precursor molecules have been identified (Burbach, 1986a). One neuron expresses one or two of these, while sometimes different cells use the same gene to produce different precursor molecules like calcitonin and calcitonin gene-related peptide (CGRP). Thus, the conversion of precursor proteins into neuropeptides of the first generation is a cell specific phenomenon. An example is pro-opiomelanocortin (POMC), which is the precursor molecule of adrenocorticotropic hormone (ACTH), various melanocyte stimulating hormones (MSHs), and endorphins in the pituitary gland, the brain and other tissues. The processing depends on the tissue. Thus, the corticotrophs in the pituitary form ACTH and β-endorphin predominantly, whereas the melanotrophs produce α-, β- and γ-MSH rather than ACTH and several endorphins (γ- and α-endorphin). These neuropeptides can undergo chemical modification, such as acetylation, oxidation, etc., which results in profound changes in their biological activity. They can be further converted extracellularly into neuropeptide metabolites, so-called neuropeptides of the "second generation".

Receptors have been found in the brain for all of the neuropeptides of the first generation. However, as yet no receptors have been cloned for the neuropeptides of the second generation. Binding sites for a few of these have been found as well as evidence for G-protein coupled receptors in the brain. Neuropeptides modulate the classi-

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cal neurotransmitter activity rather than act as a neurotransmitter, although vasopressin (VP) acts as both (Joëls and Urban, 1984). They may affect the expression of the genetic information, the synthesis of enzymes or transmitter precursors, interfere with electrical activity in the axon and the transport system, in the nerve terminal by interaction with the synthesis and metabolism of neurotransmitters, release and re-uptake of neurotransmitters, membrane fluidity, pre- and postsynaptic receptors and the cascade of events following receptor activation.

2. POMC-derived neuropeptides

2.1. Melanocortins

Effects of ACTH/MSH neuropeptides, the so-called melanocortins on the brain which are independent of the adrenal cortex are already known for more than three decades (De Wied and Jolles, 1982). The demonstration of melanocortin immunoreactivity in central nervous system (CNS) neurons (Watson et al., 1978) indicated the presence of a melanocortin system in the brain. The subsequent demonstration that the precursor POMC is expressed in the brain (Gee et al., 1983) further underscored the importance of the melanocortin system in nervous system function.

Studies on the conversion of melanocortins by brain peptidase (Burbach et al., 1986) revealed that ACTH-(1–39) is cleaved to ACTH-(1–16) and ACTH-(17–39) by an endopeptidase similarly as has been found in the rat intermediate lobe where α-MSH is formed from ACTH-(1–39). The sequence ACTH-(1–16) carries all nervous system activities sofar found with the ACTH/MSH neuropeptides (Greven and De Wied, 1980). ACTH-(1–16) is further converted to ACTH-(1–13) and acetylated to form α-MSH. α-MSH and desacetyl-α-MSH are two endogenous forms of MSH in the brain (Burbach et al., 1986). α-MSH is rather stable, whereas the desacetyl compound is converted to α-MSH-(3–13), -(4–13) and -(5–13) (Burbach et al., 1986) by aminopeptidase activity. Only α-MSH 11–13 is a common peptide generated from the two forms of α-MSH. ACTH-(4–10) can be formed in particular from desacetyl-α-MSH or ACTH-(1–16). ACTH-(1–16)NH_2 might generate ACTH-(7–16)NH_2 as found when ACTH-(1–16)NH_2 was incubated at pH 7.4 and 8.5 in synaptosomal membranes. No evidence was found for the generation of ACTH-(4–7).

It was only late in the eighties, that binding sites for melanocortins were detected (Tatro, 1990; Tatro and Reichlin, 1987). The cloning of the melanocyte MSH (MC_1) receptor and the adrenal melanocortin MC_2 receptor was soon followed by the cloning of the melanocortin MC_3, melanocortin MC_4 and melanocortin MC_5 receptor (see for review, Adan and Gispen, 1997). Various of these receptors are expressed in the brain, which further underscores the significance of the melanocortins in nervous system effects. α- and β-MSH as well as the very potent agonist NDP-MSH possess high affinity for melanocortin MC_3 and melanocortin MC_4 receptors. The melanocortin MC_3 and melanocortin MC_4 receptors are widely expressed in the brain and probably the melanocortin MC_5 receptor as well. The third MSH peptide known is γ-MSH, which occurs in various forms (γ_1, γ_2, γ_3). γ_2-MSH has selectivity for the melanocortin MC_3 receptor (Roselli-Refuss et al., 1993). Some of the neuropeptides of the second generation have affinity for either of the MC receptors, but of insufficient magnitude to explain their CNS effects. The availability of cell lines which express the melanocortin MC_3 and melanocortin MC_4 receptors, led to the development of selective agonists and antagonists, which helped to determine which receptor is involved in given behaviors as induced by neuropeptides of the first generation derived from POMC (Adan and Gispen, 1997). An important antagonist with selectivity for the melanocortin MC_4 receptor appeared to be SHU 9119 (Ac-cyclo-[NCe^4,Asp^5, D-$Nal(2)^7$,Lys^{10}]α-$MSH_{4-10}(NH_2)$) (Hruby et al., 1995). Few antagonists were found with higher affinity for the melanocortin MC_4 receptor, such as [D-Arg^8]ACTH-(4–10) (Adan and Gispen, 1997) and HSO14 (cyclic[Ac-Lys^{11},D-Nal^{14}, Cys^{18},Asp^{22}-NH_2]-β-MSH-(11–22)) (Schiöth et al., 1998).

During three decades, numerous studies have been performed with ACTH/MSH neuropeptides to determine the neuroactive and neutrotrophic loci for the various nervous system effects. This was done prior to the time that receptors had been characterized. It was not easy to indicate with reasonable certainty, the specific loci for the many nervous system effects that had been discovered since the first studies on avoidance behavior (for review, see De Wied and Jolles, 1982).

ACTH-(4–7) and ACTH-(7–16) appeared to have specificity for avoidance behavior. Structure activity studies to determine the locus of action in the ACTH/MSH peptides on learning and memory revealed that ACTH-(4–7) was the smallest fragment to be fully active with additional activity in ACTH-(7–10) and ACTH-(11–13). None of these fragments possess affinity for either of the known melanocortin receptors. Amino acid substitution by a D-amino enantiomer in position 7 in ACTH-(1–10) or ACTH-(4–10) resulted in reversal of the effect on active avoidance behavior but not of that on passive avoidance behavior. Whereas α- and β-MSH affected active and passive avoidance behavior in the same way as ACTH-(1–24) or ACTH-(4–7), γ_2-MSH, which has a slightly different amino acid sequence, possessed an opposite effect; it appeared to facilitate extinction of active avoidance behavior (Van Ree et al., 1981). Although [D-Phe^7]ACTH-(4–10) has affinity for the melanocortin MC_3 and melanocortin MC_4 receptor and γ-MSH is selective for the melanocortin MC_3 receptor, none of the melanocortin receptors is activated by or bind the ACTH-(4–9) analogue Met(O)-Glu-His-Phe-D-Lys-Phe-OH (Org 2766). This is a peptide with

potent effects on avoidance behaviour, whereas it lacks all intrinsic endocrine activities. In low doses it facilitates, while in high doses it attenuates passive avoidance behavior. It has been concluded that the influence of ACTH/MSH neuropeptides on learning is not mediated by the a receptor of the melanocortin receptor family (Adan and Gispen, 1997). The attenuating effect on passive avoidance behavior of high doses of Org 2766 is present in the C-terminal 7–9 tripeptide Phe-D-Lys-Phe and is blocked by naltrexone (Fekete and De Wied, 1982). This suggests that this influence of Org 2766 is mediated by an opioid receptor.

Another early finding with ACTH/MSH peptides were effects on stretching and yawning and grooming (Ferrari, 1958). It was found after intracranial administration only (Spruijt et al., 1992). Adan et al. (1994) recently showed that excessive grooming is mediated by the melanocortin MC_4 receptor. Results of binding studies, and of receptor activation studies by measuring cAMP and the use of antagonists for the melanocortin MC_4, and the melanocortin MC_3 receptor (SHU 9119) nicely showed this to be the case. Structure activity studies had shown that the sequence ACTH-(5–13) is needed to induce excessive grooming (Gispen et al., 1975). The sequence 4–13, which has a similar effect, activated the melanocortin MC_3 receptor in low doses and the melanocortin MC_4 receptor submaximally at higher doses. ACTH-(1–10) and ACTH-(4–10) had minor or no activity in this respect while D-Phe^{7}ACTH-(4–10) which in contrast to ACTH-(4–10) also induces excessive grooming (50–60% of ACTH-(1–24)), was able to bind and activate the melanocortin MC_4 receptor. Another argument for this assumption is that γ-MSH, which selectively activates the melanocortin MC_3 receptor, does not induce grooming. Additional proof for the hypothesis that excessive grooming is mediated by the melanocortin MC_4 receptor was obtained when more selective antagonists were used. Vergoni et al. (1998), who used the more selective melanocortin MC_4 antagonist HSO14, found inhibition of α-MSH-induced excessive grooming but not of penile erection which is found in grooming rats. HSO14 also blocked stretching and yawning. Novelty induced grooming was also blocked by SHU 9119, suggesting that this response to novelty in the rat is mediated by the melanocortin system. The order of potency of NDP-MSH, α-MSH and ACTH-(4–13) on excessive grooming correlates with that for the activation of the melanocortin MC_4 receptor in vitro (Adan, 1999).

It has also been known for a long time that melanocortins enhance the maturation of the nervous system (see for review, Strand et al., 1994). Using the rat sciatic nerve crush model it was found that α-MSH accelerated the recovery of sensory and motor function and nerve conduction velocity (Bijlsma et al., 1981). This effect resided in the 6–10 moiety of ACTH. However, Org 2766 is also active, whereas γ-MSH and [D-Phe^7]ACTH-(4–10) are not, suggesting that at least two receptors are involved (Gispen et al., 1987). The melanocortin MC_4 receptor is expressed in the spinal cord (see Adan, 1999), and, thus, might be involved. Interestingly, an Org 2766 receptor has been characterized on rat Schwann cells (Dyer et al., 1995). The activation causes the releases of an unidentified neurotrophic factor. Surprisingly, the Org 2766 binding is displaced by α-MSH. However, the effects of the two peptides on primary cultures of rat spinal cord cells do not overlap completely (Hol et al., 1993) suggesting that they act via separate but related receptors. Expression of melanocortin MC receptors on spinal cord neurons provides a mechanism for melanocortins to stimulate neurite outgrowth of these neurons. Plantinga et al. (1995) showed that a melanocortin MC receptor antagonist decreased spontaneous recovery after a sciatic nerve crush. This antagonist was also able to block α-MSH induced excessive grooming. This indicated, that recovery from nerve damage is mediated by melanocortin MC_4 receptors. This was recently confirmed with the selective melanocortin MC_4 antagonist [D-Arg^8]ACTH-(4–10) in an in vitro system (Adan, 1999).

Org 2766 appeared to be 1000 times more potent than ACTH-(4–10) on passive and active avoidance behavior. It is also orally active (Greven and De Wied, 1980). In the 6-OH-dopamine induced lesion of the nucleus accumbens paradigm, it was also 1000 times more potent than ACTH-(4–10) in facilitating regeneration and orally active (Wolterink et al., 1990). In the sciatic nerve crush regeneration, it was markedly more active than ACTH-(4–10) (Van der Zee et al., 1991) but not orally active. It was also rather potent on social behavior, but in this paradigm it acted in a way opposite to ACTH-(4–10) which reduced social behavior in rats (Niesink and Van Ree, 1984). It was as active as ACTH-(4–10) in reducing cognitive defects and social behavior in old rats (Spruijt, 1992) and in protecting against pilocarpine-induced epilepsy (Croiset and De Wied, 1992). It failed to induce grooming, stretching and yawning following i.c.v. administration (Spruijt et al., 1992). Accordingly, it appeared to be a selective compound for a number of nervous system effects, but not for all. Thus, selectivity found in one test cannot be generated to other tests (Table 1).

Several other effects of the melanocortins have been studied in the course of years. Many of the melanocortin fragments that were used in studies on avoidance behavior were also used in other behaviors. The effects on social behavior, motor activity and in vitro opiate effects are localized in the ACTH-(7–10) moiety. These effects are probably mediated by one of the opioid receptors, since they can be blocked by opioid receptor antagonists. Studies on the interaction of ACTH neuropeptides with [^{3}H]-dehydromorphine and [^{3}H]naltrexone binding had shown that these effects were located within the 4–10 and 7–16 position of the ACTH molecule (Terenius et al., 1975). Electrically induced contraction of the mouse vas deferens is depressed by opiates, but by ACTH neuropeptides as

Table 1

Org 2766 (Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH)		
	Potency	
Avoidance behavior	+++[a]	Greven and De Wied, 1980
Regeneration of 6-OHDA-induced nucleus accumbens lesion	+++[a]	Wolterink et al., 1990
Nerve crush regeneration	++	Van der Zee et al., 1991
Aging	+	Spruijt, 1992
Social behavior	++†	Niesink and Van Ree, 1984
Pilocarpine-induced epilepsy	+	Croiset and De Wied, 1992
Excessive grooming	0	Gispen et al., 1975
Stretching and yawning syndrome	0	Gispen et al., 1975

[a]Orally active.
+++ = Nanogram quantities s.c.
+ = Microgram quantities s.c.
0 = No effect.
† = Effect opposite to that of ACTH-(4–10).

well. The activity resides in the 7–10 position of the molecule. ACTH-(7–10) is also recognized by anti-morphine antibodies (De Wied and Wolterink, 1989). Results of structure activity studies on various other effects of the melanocortins have shown that ACTH-(1–16)NH_2 and γ_2-MSH are important for reducing after-discharge and behavioural depression following kindling of the dorsal hippocampus, ACTH-(4–10) for counteracting dorsal immobility, ACTH-(4–10) for restoring postural asymmetry following unilateral injections in the locus coeruleus, ACTH-(7–9) for heat stress induced sedation, ACTH-(4–10) for aggressive behavior, ACTH-(7–10) to enhance effects of environment on motor activity (see for review, De Wied and Wolterink, 1989). Taking these structural requirements into account, it looks as if none of these effects are mediated by a melanocortin MC receptor.

Recent studies have implicated melanocortin neuropeptides in other CNS effects, such as weight homeostasis, blood pressure, fever, inflammation and pilocarpine-induced epilepsy. Some of these effects appeared to be mediated by one of the melanocortin MC receptors. Leptin is a 167-aminoacid polypeptide secreted from adipose tissue in rodents (Cheung et al., 1997; Auwerx and Staels, 1998). It inhibits food intake through a receptor which is expressed in the hypothalamus and several sites in the hindbrain of mice and rats (Mercer et al., 1998). Several anorectic peptides have been proposed, which may mediate the inhibitory effect of leptin on food intake in particular POMC derived peptides. Ectopic overexpression of the so-called agouti peptide, an endogenous antagonist at the melanocortin MC_3/melanocortin MC_4 receptor and mutation of the melanocortin MC_4 receptor cause obesity in mice (Huszar et al., 1997). Seeley et al. (1997) and Kask et al. (1998) showed that the selective melanocortin MC_4 receptor antagonist HSO14 (Schiöth et al., 1998) completely blocked effects of leptin on food intake in rats. Fan et al. (1997) found an increase in food intake using SHU 9119. Recently, Vergoni et al. (1998) found that HSO14 increased food intake in rats while α-MSH had the opposite effect. Although these effects seem to be mediated by the melanocortin MC_4 receptor, the possibility remains that the melanocortin MC_3 receptor is also involved.

Melanocortins, in particular the various γ-MSHs (γ_1, γ_2), exert cardiovascular effects (Versteeg et al., 1998). The γ-MSHs are more potent than ACTH-(4–10) to induce pressor and tachycardiac effects following intravenous administration. α-MSH and NDP-α-MSH and Org 2766 are without effect. Although γ-MSHs are selective for the melanocortin MC_3 receptor, structure activity studies showed that the pressor and tachycardiac effects reside in the γ-MSH-(6–12) moiety which appeared to have no affinity for the melanocortin MC_3 receptor. These effects are not blocked by SHU 9119 (Versteeg et al., 1998). The pressor and tachycardia effects of melanocortin are probably due to an increase of the sympathetic outflow to the cardiovascular system secondary to activation of centrally located receptors in the anteroventral part of the third ventricle, a region outside the blood–brain barrier. These receptors might be related to the MC receptor family not yet identified or related to other receptors. Versteeg et al. (1998) suggest that peptides with a C-terminal Arg-Phe sequence such as γ-MSH and FF neuropeptide, which have affinity for the FMRF amide receptor or a related receptor act through this receptor. The FF neuropeptide as well as FMRF amide have effects on the cardiovascular system similar to those of γ-MSH.

i.c.v. α-MSH has a strong antipyretic and anti-inflammatory effect (Catania and Lipton, 1993). NDP-α-MSH is more potent in this respect than α-MSH (Holdeman and Lipton, 1985). Structure activity studies indicated that the three terminal amino acid residues (α-MSH-(11–13)) are essential to counteract cytokinin-induced fever (Deeter et al., 1988). N-terminal elongation of the tripeptides to α-MSH-(9–13) reduced, but further elongation (α-MSH-(8–13)) increased the potency. A recent study showed that the antipyretic effect of α-MSH could be blocked by SHU 9119. This suggests that the antipyretic effect of α-MSH is mediated by the melanocortin MC_4 or the melanocortin MC_3 receptor. Whether α-MSH-(8–13) has affinity for this receptor is unknown. Reduction of inflammation caused by local injection of interleukin-1 is found after i.c.v. administration of α-MSH. This effect seems to be mediated by β_2-adrenoreceptors (Macaluso et al., 1994).

In a limited number of animal studies fragments of ACTH devoid of peripheral endocrine activity were found to possess anti-epileptic properties (Cottrell et al., 1983). In more recent studies it was found that the severity of pilocarpine induced epilepsy is reduced by ACTH neuropeptides. Peptides active in this respect are ACTH-(4–9), ACTH-(4–10), D-Phe[7]ACTH-(4–10), ACTH-(7–16) and Org 2766. These were able to attenuate symptoms ranging from akinesia, to motor seizures in rats following s.c. administration (Croiset and De Wied, 1992). Some of

these ACTH analogs, such as ACTH-(4–10) and D-Phe^{7}ACTH-(4–10), have been shown to possess affinity for the known melanocortin MC_3 and melanocortin MC_4 receptors. However, this was not the case for ACTH-(7–16) and Org 2766. The anti-epileptic influence of ACTH neuropeptides probably is not mediated by either of the known MC receptors.

2.2. Endorphins

POMC is also the precursor molecule of β-endorphin (βE-(1–31)) which is derived from the β-LPH region of the POMC precursor. β-endorphin is cleaved into βE-(1–17) (γ-endorphin), which can be further processed to βE-(1–16) (α-endorphin) and smaller fragments (Burbach et al., 1986; Wiegant et al., 1992). Other peptides generated from β-endorphin are βE-(1–27) and βE-(1–26) and acetylated forms of the respective endorphins. Such acetylated neuropeptides are the major forms of endorphin in the forebrain in several species. Acetylation blocks interaction with the opioid receptors and acetylated products are much less accessible to aminopeptidase attack.

β-Endorphin is one of the three major sources of endorphins, of which the enkephalins, β-endorphin and dynorphin are the prototypes. The precursors differ in characteristics and distribution in the brain. Opioid receptors are designated as μ, δ, κ. β-Endorphin has preference for the μ-, the enkephalins for δ-, and dynorphin for the κ-opioid receptor. Many subtypes of these receptors have been discovered (Dhawan et al., 1996) and the existence of an epsilon receptor specific for β-endorphin has been reported (Narita and Tseng, 1998). Novel opioid receptors have been recently detected (see for review, Van Ree et al., 1999).

βE-(1–31) has a wide array of effects resembling those found with morphine. Apart from having analgesic properties, it has been implicated in the central regulation of endocrine and autonomic functions, in the development of tolerance to drugs, in reward mechanisms, in learning and memory processes, sexual and social behavior and grooming (Van Ree and De Wied, 1985). The carboxyl end is of some importance for the opiate character of the endorphins' truncated peptides reduce affinity. In fact, βE-(1–27) antagonizes β-endorphin-induced analgesia and the reinforcing effect of opioids (Akil et al., 1981; Hammonds et al., 1984). The N-terminus is essential for binding to opioid receptors. Thus, acetylation or removal of the N-terminal blocks binding and also the analgesic effects and other opioid effects. There are, however, activities which do not require the intact amino terminus, as is evident for α-endorphin (βE-(1–16) and γ-endorphin (βE-(1–17). It has been found that α- and γ-endorphin possess novel effects. From a variety of neuropharmacological tests α-endorphin appeared to possess psychostimulant-like effects, whereas γ-endorphin appeared to have neuroleptic-like effects. The effects of α-endorphin and γ-endorphin are in general opposite to each other. Thus, α-endorphin delays extinction of active and facilitates passive avoidance behavior, potentiates apomorphine-induced sniffing, delays extinction of a food rewarded response and a water rewarded runway task. It attenuates problem solving, stimulates electrical self-stimulation at threshold currents in the ventral tegmental area (VTA) and heroin self-administration. Structure activity studies showed that the psychostimulant-like activity of α-endorphin resided in the 2–9 moiety (Greven and De Wied, 1980; Van Ree and Otte, 1980). In contrast to α-endorphin, γ-endorphin facilitates extinction of active and attenuates passive avoidance behavior, facilitates extinction of a water rewarded runway task, enhances problem solving, possesses positive effects on grasping responses, reduces electrical self-stimulation at threshold currents from the VTA and in the nucleus accumbens, and heroin self-administration, and antagonizes apomorphine-induced hypolocomotion. These findings suggested that γ-endorphin and related neuropeptides possess neuroleptic-like effects. The neuroleptic-like activity appeared to reside in the 6–17 moiety.

Another activity within the β-endorphin molecule appeared to be the βE-(10–16) moiety which had anti-melatonin effects. Injection of melatonin into the rat nucleus accumbens decreases locomotor activity and rearing and increases sniffing and grooming. These effects could be counteracted by both α- and γ-type endorphins. Structure activity studies showed βE-(10–16) to be the site in the molecule for this effect. Since intra-accumbal administration of serotonin also inhibited the effects of melatonin, the influence is probably mediated by the serotonergic system. In fact, 5-HT receptor antagonists administered into the nucleus accumbens induced the same effects as melatonin. Various antidepressants possess similar effects, which led to the suggestion that βE-(10–16) might have antidepressant-like effects (Gaffori and Van Ree, 1985; Van Ree and De Wied, 1985).

No receptors are known for the non-opioid endorphins. Since neuroleptic-like activity was found with γ-type endorphins, much work has been done in exploring its mode of action. Results of studies in which the displacement of neuroleptics from their binding sites was measured in the nucleus accumbens in vitro were negative. Evidence for binding sites was found, however, using in vivo binding of [^{35}S]Met-Des-enkephalin-γ-endorphin. Relatively high affinity binding sites were detected in cryosections of rat brain, in particular the orbital cortex, cingulate cortex, entorhinal cortex, amygdala and somewhat less in the pyriform cortex and nucleus accumbens, structures sensitive to γ-type endorphins (Ronken et al., 1989). The non-opioid γ-endorphins lack affinity for binding sites of other neurotransmitters and psycho-active drugs, including naloxone and fentanyl, diazepam, phenylcyclidine and *N*-methyl-D-aspartic acid (Wiegant et al., 1992). Evidence for the existence of a receptor was found when it appeared that a monoclonal anti-idiotype Des-enkephalin-γ-en-

dorphin antibody displaced the binding of [^{35}H]Met-Des-enkephalin-γ-endorphin and blocked Des-enkephalin-γ-endorphin induced apomorphine-induced hypolocomotion.

Further studies showed that Des-enkephalin-γ-endorphin might affect dopamine neurotransmission by inhibition of the re-uptake of dopamine in slices of rat brain tissue in vitro in the nucleus accumbens (Ronken et al., 1989), but not in the striatum, where there is a low density of binding sites. Des-enkephalin-γ-endorphin increased the number of binding sites for [^{3}H]-GBR12935, a selective inhibitor of the dopamine transporter, following coincubation of nucleus accumbens membranes. This suggests a direct interaction between the occupied receptor for γ-type endorphins and the dopamine transporter. This causes enhancement of uptake inhibition, thus, a slower disappearance of dopamine from the synaptic cleft which increases the availability of neurotransmitter for neurotransmission. This is not easy to explain since neuroleptics exert their effect by reducing dopamine activity in mesolimbic dopamine neurons (see for review, Davis et al., 1991). Post mortem studies have shown high dopamine and homovanilic acid concentrations in various subcortical brain regions and greater than normal binding sites in the brain of schizophrenic patients. However, there is evidence for inhibition of prefrontal dopamine activity which according to the authors, leads to excessive dopamine activity in mesolimbic dopamine neurons. Thus, both high and low dopamine activity is found in the brain of schizophrenics. This might explain the negative symptoms as a result of prefrontal low, and the positive symptoms of mesolimbic high dopamine activity. It takes days before the antipsychotic effects occur following treatment with classical neuroleptics. Chronic treatment with Des-enkephalin-γ-endorphin does lower the release of dopamine in the nucleus accumbens (Radhakishun et al., 1988). Treatment of schizophrenics with γ-type endorphin however, was much less effective in the residual type of schizophrenia (Verhoeven et al., 1982).

3. Neurohypophyseal hormones and related neuropeptides

Arg^{8}–VP and oxytocin (OT) are synthesized as parts of larger precursor molecules. Magnocellular VP and OT containing neurons in the hypothalamic paraventricular, supraoptic and accessory nuclei, project to the posterior pituitary. Parvocellular VP- and OT-containing cells are found in the hypothalami. These project to the median eminence, the brainstem, and the spinal cord. VPergic fibers from the bed nucleus of the stria terminalis and the medial amygdala nucleus terminate in limbic-brain areas (see for review, De Wied et al., 1993). Multiple post-translational modifications of the polypeptide precursors occur during axonal transport and result in the synthesis of VP, neurophysin II and a C-terminal glycopeptide and of OT and neurophysin I. In the rat VP and OT undergo further metabolic conversion in the brain (Burbach, 1986b).

The first CNS effects of the neurohypophyseal hormones reported were on memory. VP and OT modulate memory processes. VP exerts a long term facilitating, time-dependent effect on acquisition and extinction of aversively motivated behavior (De Wied et al., 1993). VP and related peptides facilitate consolidation and retrieval processes and prevent retrograde amnesia. OT and related peptides have opposite effects and induce amnesia. These early findings were followed by results of numerous studies showing that VP neuropeptides possess a great variety of CNS effects (De Wied et al., 1993). Effects were found on attention, conditioned freezing, spatial learning, heroin self-administration and electrical self-stimulation, drug tolerance and dependence, pain, social behavior, maternal and reproductive behavior, bonding, feeding, grooming, flank marking, temperature, seizure threshold, and cardiovascular regulation.

Des-Gly^{9}[Lys^{8}]VP was isolated from hog pituitary material using acquisition of shuttlebox avoidance behavior of hypophysectomized rats as the bioassay (Lande et al., 1971). This truncated VP thus had effects which were similar to those of the parent molecule. Since Des-Gly^{9}[Lys^{8}]VP had only minor effects on blood pressure and water retention, a dissociation between the influence of VP on avoidance behavior and the classical endocrine peripheral activities was evident. This led to the notion that VP and OT, like ACTH and MSH, serve as precursor molecules for neuropeptides of the second generation (De Wied et al., 1988). Structure activity studies did not reveal the exact loci in the molecule which are responsible for the memory effect of these neurohormones. This stimulated studies on the processing of VP and OT by brain enzymes which ultimately led to the elucidation of highly selective and potent memory molecules (see for review, Burbach et al., 1998). In principle, the selective metabolites had the same effect on aversively motivated behavior as the parent molecule. The main metabolites ([pGlu^{4}-Cyt^{6}]VP-(4–9) (VP-(4–9)); VP-(4–8; VP-(5–9) and VP-(5–8) all were effective in facilitating consolidation (post-learning, administration) and retrieval (preretention administration) of passive avoidance behavior. However, there were some differences. The 4–8 and 5–8 metabolites appeared to be more effective than the 4–9, 5–9 fragments on consolidation, while 4–9, 5–9 metabolites were more effective on retrieval processes. Thus, the presence of the N-terminal pGlu^{4} is important for consolidation, that of the C-terminal Gly^{9}NH$_{2}$ for retrieval processes. VP metabolites are devoid of classical endocrine activity on blood pressure, water metabolism, uterus contraction, and the potentiating effect of corticotropin releasing hormone (CRH) on ACTH release (see for review, Reijmers et al., 1998). However, they were found to be effective on avoidance behavior, attention, spatial alternation, social recognition, conditioned freezing, ethanol tolerance and on heroin self-ad-

ministration in the same way as VP (see for review, Reijmers et al., 1998). In contrast to VP, metabolites were ineffective on scratching and grooming, antinociception, flank marking, on which VP had a positive effect and on locomotor activity and rearing which are attenuated by VP.

Social recognition is also used as a test for memory. The assessment of social recognition is based on the tendency of rodents to investigate unfamiliar conspecifics more intensively than familiar ones. In the laboratory mammalian conspecifics are used as the social stimulus. When a juvenile is presented for the first time, it is intensively investigated. A second presentation shortly after the first one elicits less attention. It can be stimulated by VP and VP metabolites and attenuated by OT and OT metabolites. In this test, both desglycinamide–arginine^{8}–VP and VP-(4–8) are active, the latter in about the same dose as AVP. Also VP-(4–9) and VP-(5–8) and VP-(5–9) were active in this respect (see for review, Popik and Van Ree, 1998). These effects are detectable 2 h after administration, but not after 24 h. Other metabolites, desglycinamide–arginine^{8}–VP, VP-(1–7) or VP-(1–6) facilitated social recognition for at least 24 h after the first encounter of the adult rat with the conspecific juvenile. Thus, this long term effect depends on the covalent ring-structure of VP. OT in equimolar doses as VP had the opposite effect, but very low doses of OT also facilitate social recognition. The high dose attenuating effect of OT could be brought about by OT, OT-(1–8), OT-(7–9) and OT-(4–9). The low dose facilitating effect which is located in the pre-optic area, could be found only with OT, OT-(7–9) and OT-(8–9). Popik and Van Ree (1998) suggested that the facilitating effect of OT on social memory resides in the C-terminal part while the attenuating effect may reside in the 5–7 region of OT.

Desglycinamide–arginine^{8}–VP reduces heroin self-administration in rats. This was also found with VP-(4–8). The profile for this effect resembles that for inhibition of extinction of pole-jumping avoidance behaviour. Surprisingly, VP-(5–9) had an effect opposite to that of VP-(4–8). OT and in particular OT-(7–9) exhibited opposite effects and facilitated heroin self-administration. The same was found for electrical self-stimulation from the VTA, which is reduced by VP neuropeptides and stimulated by OT neuropeptides. Here again VP-(5–8) and VP-(5–9) were more potent than VP. The effects are found when the threshold for electrical self-stimulation is low. These peptides may therefore have a modulatory influence on brain reward. In general, one can say that these metabolites mimic the effects of VP and OT on CNS functions which involve learning and memory processes.

As yet, the receptors involved in the memory effects of the neurohypophyseal hormones and related neuropeptides are not exactly known. Except for the VP V_2 receptor the respective peripheral VP and OT receptors have also been detected in the brain. In the hippocampus, which is the most sensitive site for the memory effects of VP and metabolites (Kovács and De Wied, 1994), both OT and VP V_{1A} receptors have been found. The VP V_{1B} receptor has been detected in multiple brain regions (Lolait et al., 1995). The influence of VP and OT, but also that of the metabolites, can be blocked by rather selective VP V_1, VP V_2 as well as OT receptor antagonists (Elands et al., 1992). In particular, the OT receptor antagonist appeared to be potent in blocking the influence of VP, OT and VP-(4–8) on passive avoidance behavior. This was confirmed by Alescio-Lautier and Soumireu-Mourat (1998) who showed that the memory effect of VP, administered into the ventral hippocampus, on a discriminative learning task in mice could be blocked by a VP V_1 and OT receptor antagonist, but not by a VP V_2 receptor antagonist. We postulated that the memory effects may be mediated by an OT receptor in the ventral hippocampus, for which VP acts as an agonist and OT as an ''inverse'' agonist. The receptor may also have the characteristics of a receptor for the ancestral hybrid peptide vasotocin. Vasotocin in "low" doses possesses an OT-like effect (amnesic), but in "high" doses enhances passive avoidance behavior (VP-like effect) (De Wied et al., 1991). Vasotocin indeed can be converted into VP-(4–9) and related peptides (Wang et al., 1983). Interestingly, Ingram et al. (1998) reported that a few neurons in the suprachiasmatic nucleus show highly preferential responses to vasotocin as found by Mihai et al. (1994). They suggest that a vasotocin receptor may be expressed in several parts of the mammalian brain including the suprachiasmatic nucleus.

As mentioned above, the VP metabolites with effects on passive avoidance behavior are also blocked by VP V_1, VP V_2 and OT receptor antagonists (De Wied et al., 1991). They do not seem to act through the known VP receptors (Burbach et al., 1998). The identity of the metabolite receptor(s) has remained obscure. Ligand binding studies have demonstrated that VP-(4–9) and VP-(4–8) are not able to displace labelled VP from VP V_{1A} and VP V_2 receptors and also fail to display intrinsic activity on any of the known VP receptors. In addition, autoradiographic binding studies on brain slices revealed binding sites for ^{35}S-labelled VP-(4–9) anatomically different from classical VP/OT receptors (De Kloet et al., 1985). Metabolite binding sites were mainly associated with circumventricular organs in the brain lacking the blood–brain barrier. The binding of VP-(4–9) in the brain could not be competed for with VP or Arg^{8}-vasotocin (Jurzak et al., 1995). Du et al. (1998) detected VP-(4–8) binding sites in hippocampal pyramidal cells and dentate granule cells using ^{35}S labelled peptide in an autoradiographic study. Radiological studies with the same peptide on brain synaptosomal membranes found binding prominent in the amygdala, hypothalamus/pituitary and the anterior cortex. No detectable competition with VP was found. More evidence for the existence of separate receptors came from studies which showed that VP-(4–8) and VP-(4–9) increase extracellular Ca^{2+} in 8% of neurons and astrocytes cultured from the

subfornical organ or organum vasculosum of the lamina terminalis (Jurzak et al., 1995). Only a subpopulation of these cells responded to VP. The metabolite receptors may also belong to the G-protein coupled receptor family because both VP-(4–9) and VP-(4–8) stimulated inositol phosphate (IP) turnover in hippocampal slices and primary cultures (Gu and Du, 1991; Brinton et al., 1994) and stimulated accumulation of cAMP in hippocampal cultures (Brinton and Brownson, 1993). Using ^{35}S labelled GTPγS it was found that VP-(4–8) stimulated ^{35}S GTPγS binding in hippocampal synaptic membranes (Du et al., 1998). This effect was blocked by pertussis toxin, a selective inhibitor of G protein coupled receptors. This also suggests that the metabolite receptor belongs to the G protein coupled receptor family. Du et al. (1998) further found that VP-(4–8) stimulated inositol triphosphate (IP_3) metabolism in hippocampal tissue slices as compared to VP. VP-(4–8) was much more potent in this respect. Also phosphorylation of GAP-43/B-50 was enhanced. In addition they found that Ca^{2+}/CaM-dependent protein kinase II in adult brain is activated by VP-(4–8). Also Mitogen-Activated Protein Kinase (MAPK) increases in the hippocampus after VP-(4–8). This activity could not be blocked by pertussis toxin. Du et al. believe therefore that the receptor for VP-(4–8) may be a G protein coupled receptor and that the IP_3-associated signal transduction is a branching pathway one to CaM, CaMKII needed for long term potentiation (LTP), which is readily induced by VP-(4–8) and the other to Protein Kinase C via MAPK to gene expression. Whether or not the memory effects of VP are mediated by the metabolites as we have postulated is not known. Evidence for this assumption has not been obtained (Stark et al., 1989). Down-regulation of the VP V_{1a} receptor with an antisense oligodeoxy nucleotide, inhibits the effect of VP on social recognition (Landgraf et al., 1995). This suggests that VP can affect a memory-related behavior independent of a VP metabolite. It is possible that VP and the metabolites act on memory processes in a similar way as has been found for neuropeptide Y (NPY) and its metabolites as suggested by Reijmers et al. (1998). This pancreatic 36-amino acid polypeptide possesses memory effects. It improves retention of a T-maze active avoidance test and it reverses amnesia induced by scopolamine or protein synthesis inhibition. Its main influence, however, is on food intake, which is stimulated by a postsynaptic NPY_1 receptor. The influence on T-maze performance is localized in the fragment NPY-(20–36) which effect is mediated by a presynaptic Y_2 receptor which also recognizes the whole molecule (Flood and Morley, 1989).

4. Concluding remarks

The studies reviewed here have revealed a large number of neuropeptides derived from two important neuropeptide generating systems, POMC and the precursor proteins of the neurohypophyseal hormones, which affect a great variety of different behaviors. These neuropeptides play a role in brain development, in normal brain functions and in brain pathology. The marked effects of the melanocortins on aging and nerve regeneration as found in animal experiments, the effects of VP and related peptides on learning and memory and the influences of the non-opiate neuropeptides α- and γ-type endorphins with, respectively, psychostimulant-like and neuroleptic-like effects, suggest that these molecules might in some way be involved in brain disorders. Several prototypes of these neuropeptides have been used in men in the treatment of dementia, aging, schizophrenia, memory disturbances and neuropathy. Nearly no side effects have been found following neuropeptide treatment in men. However, the results in general were modest and clinically relevant in approximately 25% of the patients only. However, the effect of these endogenously formed neuropeptides, suggest that they may be natural ligands for orphan receptors, that have been cloned and others that will be identified in the further sequencing of the genome. Poor penetration into the brain, and the susceptibility of these peptides to enzymatic degradation in the blood and the gastrointestinal tract, may be serious drawbacks. These may be overcome, however. The ACTH-(4–9) analog, Org 2766, synthesized more than 20 years ago, is an example of a peptide protected against enzymatic degradation which was found orally active, much more potent in some nervous system effects and highly selective. Several non-peptidergic compounds for the neurohypophyseal hormones (Pettibone and Friedinger, 1997), the tachykinins (Saria, 1999), e.o. have already been synthesized.

Our studies have shown that the influence of neurohormones (neuropeptides of the ''first generation'') can be dissociated from their classical peripheral endocrine activities by their conversion into neuropeptides of the ''second generation''. Short sequences of only a few amino acids are required for a particular effect on the nervous system. It took more than 20 years before receptors for the neuropeptides of the first generation, the melanocortins, β-endorphin and the neurohypophyseal hormones were found and subsequently cloned. Structure activity studies on many different behaviors showed distinct differences in the active locus within ACTH/MSH molecules. Few of these have affinity for the MC receptor family. This suggests the existence of related or other receptors in the nervous system. The metabolites of the neurohypophyseal hormones are even more selective, since they are active in the brain only in memory-related behaviors. However, the metabolites have no affinity for the known neurohormone receptors present in the brain, while the receptors which mediate the influence of metabolites have remained obscure.

In the beginning of the next century, the whole human genome will be known and reveal the structure of all the

approximately 100,000 genes. Among these, receptors may be found for these so-called neuropeptides of the second generation. Nearly all neuropeptide receptors so far identified are G-protein coupled receptors. There are between 600 and 1000 of these in the human genome. Approximately 300 have been cloned and ligands are known (R.A.H. Adan, personal communication). Then it will be possible with the use of combinatorial chemistry, to synthesize highly selective agonists and antagonists and to modify neuropeptides on a large scale, and to make more stable products which will be orally active and be better able to penetrate the blood–brain barrier. In addition it may then be possible to determine from the genetic profile of individual patients who will be susceptible to treatment with a particular neuropeptide. The neuropeptides reviewed in this chapter, as well as many others that have been studied during the last three decades, may be future leads in the development of potential therapeutics for the treatment of nervous system disorders.

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