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30 Years of Dynorphins – New Insights on Their Functions in Neuropsychiatric Diseases

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Abstract

Since the first description of their opioid properties three decades ago, dynorphins have increasingly been thought to play a regulatory role in numerous functional pathways of the brain. Dynorphins are members of the opioid peptide family and preferentially bind to kappa opioid receptors. In line with their localization in the hippocampus, amygdala, hypothalamus, striatum and spinal cord, their functions are related to learning and memory, emotional control, stress response and pain. Pathophysiological mechanisms that may involve dynorphins/kappa opioid receptors include epilepsy, addiction, depression and schizophrenia. Most of these functions were proposed in the 1980s and 1990s following histochemical, pharmacological and electrophysiological experiments using kappa receptor-specific or general opioid receptor agonists and antagonists in animal models. However, at that time, we had little information on the functional relevance of endogenous dynorphins. This was mainly due to the complexity of the opioid system. Besides actions of peptides from all three classical opioid precursors (proenkephalin, prodynorphin, proopiomelanocortin) on the three classical opioid receptors (delta, mu and kappa), dynorphins were also shown to exert non-opioid effects mainly through direct effects on NMDA receptors. Moreover, discrepancies between the distribution of opioid receptor binding sites and dynorphin immunoreactivity contributed to the difficulties in interpretation. In recent years, the generation of prodynorphin- and opioid receptor-deficient mice has provided the tools to investigate open questions on network effects of endogenous dynorphins.

This article examines the physiological, pathophysiological and pharmacological implications of dynorphins in the light of new insights in part obtained from genetically modified animals.

Keywords

Opioids; Epilepsy; Anxiety; Stress; Depression; Schizophrenia

1. Introduction

In 1979, Goldstein and colleagues (1979) described the opioid properties of a tridecapeptide, which they had first isolated from porcine pituitary four years earlier (Cox et al., 1975; Teschemacher et al., 1975). The first five amino acids of this peptide (Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys) represent Leu-enkephalin. To denote its extraordinary potency, the natural peptide was named “dynorphin”. The prefix dyn- was taken from the Greek *dynamis* (power) and the ending -orphin indicates its opioid nature. Two years later, the complete sequence of 17 amino acids was identified (Goldstein et al., 1981). This peptide was renamed to dynorphin A after the isolation of the larger form

dynorphin-32 (also termed big-dynorphin), which consists of the original 17 amino acids at its amino-terminus and a novel Leu-enkephalin containing tridecapeptide now termed dynorphin B (=rimorphin) at the carboxy-terminus. The two peptides are linked by a pair of basic amino acids (Lys-Arg), which indicate a potential processing site (Fischli et al., 1982a, b). A smaller bioactive form of dynorphin A, dynorphin 1-8, was described in 1980 (Minamino et al., 1980). The first five amino acids (i.e. those representing Leu-enkephalin) were proposed as essential for binding to opioid receptors (Chavkin and Goldstein, 1981). Characterization of the precursor of dynorphins (Dyn), prodynorphin (pDyn, also termed proenkephalin B) at the mRNA (Kakidani et al., 1982) and protein level (Watson et al., 1983) also revealed the presence of α - and β -neo-endorphin (Minamino et al., 1981), leumorphin (=dynorphin B 1-29; assembled from dynorphin B and the C-terminal C-peptide) as well as a number of biologically inactive fragments, which do not contain the Leu-enkephalin motif. Their potential importance will not be discussed in this review.

Since their first description, Dyn have increasingly been thought to play a regulatory role in numerous functional pathways of the brain. In line with their localization in the hippocampus, amygdala, hypothalamus, striatum and spinal cord, these functions are related to learning and memory, emotional control, stress response and pain. Pathophysiological mechanisms that may involve Dyn/kappa opioid receptors (Dyn/KOP) include epilepsy, addiction, depression, schizophrenia, and chronic pain. Most of these functions were proposed in the 1980s and 1990s following histochemical, pharmacological and electrophysiological experiments using kappa receptor-specific or general opioid receptor agonists and antagonists in animal models. However, at that time, we had little information on the functional relevance of endogenous Dyn. This was mainly due to the complexity of the opioid system. Besides actions on all three classical opioid receptors (delta (DOP), mu (MOP) and kappa (KOP); see Box 1 for their nomenclature), Dyn were also shown to exert non-opioid effects mainly through direct effects on NMDA receptors. Moreover, discrepancies between the distribution of opioid receptor binding sites and Dyn immunoreactivity contributed to the difficulties in interpretation. Systemic or local drug applications do not really address the specific functions of endogenous pDyn. These functions strongly depend on the activation of different receptors localized on different groups of neurons. In recent years, new insights into old concepts have been provided by investigations on pDyn- and opioid receptor-deficient mice. This review focuses on the function of Dyn in neurological and psychiatric diseases. The certainly important, but also very complex role of Dyn in nociception and pain (for reviews see Lai et al., 2001; Laughlin et al., 2001) will not be discussed.

2. General aspects of dynorphins

2.1. The prodynorphin gene and transcripts

The *pDyn* gene contains four exons (1–4) and three introns (A,B,C) in humans and rodents (Horikawa et al., 1983; Douglass et al., 1989; Sharifi et al., 1999). While exons 1 and 2 encode for the majority of the 5'-untranslated region, exons 3 and 4 contain the entire coding sequence (Fig. 1). Several promoter elements have been identified within the rat pDyn promoter. An AP-1 site, representing a specific target for Jun/Fos (Naranjo et al., 1991), and a SP1-like domain, targeted by NGFI-A (McMurray et al., 1992) and a single AP-2 consensus site were proposed (Douglass et al., 1994). The influence of NF-kappa B on the expression of pDyn through specific promoter elements was also proposed (Bakalkin et al., 1994). However, the four CRE sites observed in the rat promoter were thought to be the most important, perhaps being responsible for the excitation-dependent regulation of pDyn expression (Douglass et al., 1994). In terms of suppression of pDyn expression, the downstream regulatory element (DRE) and its Ca²⁺-regulated transcriptional repressor DREAM was suggested to be important (Carrion et al., 1999; Campos et al., 2003).

DREAM appears to play a crucial role in the regulation of pDyn expression in pancreatic beta cells as a response to low glucose (Jacobson et al., 2006), but it is much broader discussed in the context of neuropathic and inflammatory pain. Low concentrations of Dyn acting on KOP located on spinal projection neurons produce analgesic effects. In contrast, a single intrathecal injection of a higher dose of Dyn produces long-lasting allodynia in mice and rats. This puzzle was solved by identification of NMDA receptors as target of high concentrations of Dyn (Vanderah et al., 1996; Laughlin et al., 1997). Noteworthy, knockout of DREAM, leading to increased expression of pDyn, markedly reduces a broad spectrum of acute and chronic pain related behaviours (Cheng et al., 2002). Although this phenotype was shown to be NMDA receptor independent, some questions such as the influence of the expression of pDyn in the ventral horn (which is not seen in wild-type mice), remain open. Meanwhile seven pDYN mRNA splice variants have been isolated from human brain (Horikawa et al., 1983; Telkov et al., 1998; Nikoshkov et al., 2005). Two of the transcripts, termed FL1 and FL2, contain the entire coding sequence of pDYN (Fig. 1). The predominant form FL1 is highly expressed in limbic structures such as the nucleus accumbens and amygdala, while the expression of FL2 is restricted to a few brain areas including the claustrum and hypothalamus (Nikoshkov et al., 2005). These two transcripts differ in their 5'-non-coding region. FL1 transcripts are initiated somewhat upstream of the proposed transcription initiation site (Douglass et al., 1994). FL2 contains a novel second exon, which extends the originally described exon 2 and is initiated within intron A close to a site previously detected in embryonic brain (Telkov et al., 1998). The exons comprising FL1 and FL2 are highly conserved in human mouse and rat genomes. In contrast, the elements detected in minor human pDYN mRNAs, which are not found in rodents, may be associated with recent evolutionary changes (Nikoshkov et al., 2005).

2.2. Processing of prodynorphin

Like all other neuropeptides, Dyn are processed from a large biologically inactive precursor protein. The first evidence for differential processing of pDyn was observed in the lobes of the pituitary. While processing to mature peptides appeared almost complete in the posterior lobe, predominantly larger precursor fragments were isolated from the anterior lobe (Seizinger et al., 1984; Day and Akil, 1989).

Processing of pDyn requires the endopeptidases, termed prohormone convertases (PC), PC1 and PC2 and carboxypeptidase E (Fig. 1). PC1 was proposed to cut at the carboxy side of three of the seven pairs of basic amino acids. The primary target is the Lys-Arg pair N-terminal to α -neoendorphin, yielding a 10 kDa C-terminal fragment containing all known pDyn-derived peptides (Fig. 1). A minor alternative pathway may be the proteolytic cleavage at the Lys-Arg pair C-terminal to α -neoendorphin. In a second step, a carboxyterminal fragment of about 2 kDa is cleaved at a single Arg, yielding an 8 kDa intermediate product (Dupuy et al., 1994). These fragments largely comprise the characteristics of those observed in the anterior lobe of the rat pituitary (Seizinger et al., 1984; Day and Akil, 1989). Further processing requires PC2, producing biologically active peptides including α -neoendorphin, big-Dyn, leumorphin, Dyn A 1-17 and 1-8 and Dyn B (Fig. 1). This processing is enhanced by the presence of carboxypeptidase E (Day et al., 1998). In line with this, mice lacking functional PC2 displayed increased amounts of the 8 kDa pDyn intermediate fragment and significant reductions in Dyn A 1-8 and Dyn B levels, but not in Dyn A 1-17 (Berman et al., 2000). This may be related to compensatory actions of PC1 as suggested by *in vitro* experiments (Seidah et al., 1998). *In vitro* studies on the ability of different pDyn-derived peptides to activate kappa opioid receptors suggested a rank order of potency with Dyn A1-17 > (10–20 times) big-Dyn = Dyn B = Dyn B 1-29 = α -neo-endorphin > (10–20 times) Dyn A 1-8 = β -neo-endorphin (James et al., 1984).

Differential processing of pDyn was also observed in the brain. Electron microscopy showed the coexistence of pDyn and Dyn within the same axon and even individual vesicles (Yakovleva et al., 2006). While the classical model suggests initiation of processing of pDyn in the trans-Golgi network, a newly hypothesized model suggests transport of pDyn to the synapse and initiation of processing in response to external stimuli. Such a regional regulation of trafficking and processing at synapses may provide local regulation of synaptic transmission (Yakovleva et al., 2006).

2.3. Distribution of pDyn mRNA and peptides

In human brain, the highest pDYN mRNA levels were measured in the amygdala, entorhinal cortex, dentate gyrus, nucleus accumbens, dorsomedial hypothalamus and premammillary nucleus. The caudate, putamen, and parahippocampal gyrus as well as the paraventricular and lateral hypothalamus display moderate to high pDYN mRNA levels. Lower levels were found in most cortical regions, the septum, bed nucleus of stria terminalis and additional hypothalamic nuclei (Hurd, 1996; Nikoshkov et al., 2005). This distribution is highly similar to that described for rat and mouse (Morris et al., 1986; Merchenthaler et al., 1997; Lin et al., 2006). However, there are some species differences, which might be important in terms of translation of results from animals to humans. The strong expression of pDYN mRNA in the human (Hurd, 1996) entorhinal cortex was not observed in the rat (Merchenthaler et al., 1997) or mouse (Lin et al., 2006). Within the amygdala, rat and mouse display the highest levels of pDyn mRNA expression in the central nucleus, while cortical subnuclei are more prominently labelled in human brain. In the rodent striatum, the differences in pDyn mRNA content between patch and matrix are less pronounced than in human tissue, but may display some lateralization effects (Capper-Loup and Kaelin-Lang, 2008).

The distribution of pDyn-derived peptides was studied by several groups in rat brain. Using an antibody recognizing Dyn 1-13 in radioimmunoassay, Höllt and colleagues (1980) reported Dyn levels of about 1200 pmol/g in the pituitary down to about 1 pmol/g in the cerebellum. The rank order of peptide levels was intermediate/posterior pituitary lobe > anterior lobe > hypothalamus > hippocampus = striatum = midbrain = thalamus = medulla/pons > cortex > cerebellum. Similar results were reported for different pDyn-derived peptides (Zamir et al., 1984a, b, c, d). These data were well reproduced in a pDyn-eGFP BAC transgenic mouse. A panel of images displaying the expression pattern of pDyn-eGFP in the brain is available from http://www.gensat.org/ShowMMRRCStock.jsp?mmrc_id=MMRRC:000240. Peptide concentrations measured in human post-mortem brain are substantially lower. Levels of about 25 pmol/g in the substantia nigra and hypothalamus represent the highest levels, followed by lower concentrations in the amygdala, hippocampus, periaqueductal grey, colliculi, pons, medulla and area postrema. Particularly low amounts or lack of DYN were detected in the posterior and anterior lobe of the pituitary, respectively (Gramsch et al., 1982). Immunohistochemical data (Khachaturian et al., 1982; Vincent et al., 1982b; Fallon and Leslie, 1986) provided more detail on the distribution of pDyn-derived peptides. The distribution of pDyn immunoreactive perikarya mostly fits in most cases with the mRNA distribution. In areas of lower pDyn expression levels, the immunohistochemical reports show several discrepancies, which may well depend on the different antisera used. However, with the exception of the pedunculopontine tegmental nucleus, all proposed pDyn-containing cells were confirmed by *in situ* hybridization.

Immunoreactive fibres that conform to output systems of the nuclei-containing pDyn immunoreactive neurons were found. These systems include major descending pathways such as striatonigral, striatopallidal, reticulospinal and hypothalamospinal projections, short projection systems such as hippocampal mossy fibres and hypothalamic–hypophyseal connections, and also local circuits in the cortex and hypothalamus (Vincent et al., 1982a; Weber and Barchas, 1983; Code and Fallon, 1986; Fallon and Leslie, 1986). Ultrastructural

evidence suggests that Dyn is also present in dendrites (Van Bockstaele et al., 1995; Hara et al., 2006).

2.4. Dual release mechanisms of dynorphins

Besides the N-type calcium channel-mediated Dyn release from axon terminals, L-type channel-dependent somatodendritic Dyn release also plays an important functional role (Simmons et al., 1995). This situation offers a variety of scenarios regarding Dyn release site and receptor interaction. Axonally released Dyn may target axonal auto- and/or heteroreceptors as well as dendritic heteroreceptors. Dendritically released Dyn, in turn, may target dendritic autoreceptors and axonal heteroreceptors. This complex situation is best demonstrated in hippocampal granule cells (for a schematic drawing see Fig. 2), but was observed also elsewhere in the brain. KOP on the dendrites of hippocampal granule cells (Mathieu-Kia et al., 2001) may bind DYN released from perforant path fibre afferents originating from the entorhinal cortex in humans (Hurd, 1996), but may also be activated through dendritically released Dyn from granule cells. Besides postsynaptic KOP on granule cell dendrites, presynaptic KOP on perforant path axons was reported for some species. In fact, high frequency stimulation of hippocampal granule cells causes inhibition of perforant path terminals on granule cell dendrites (Wagner et al., 1993; Drake et al., 1994). Inhibition of the perforant path through Dyn/KOP was originally described in the guinea pig, but also observed in mouse and hamster (Salin et al., 1995). In the rat, the presence of this effect is strain-dependent and consistent with the presence of KOP in the molecular layer (Salin et al., 1995). In the axonal compartment of the granule cells, the mossy fibres, high frequency stimulation leads to heterosynaptic inhibition of neighbouring mossy fibres (Weisskopf et al., 1993) through axon-axonal connections. Mossy fibre terminals in the stratum lucidum target dendrites of CA3 pyramidal neurons which contain postsynaptic KOP. Dyn acting on KOP described so far exerts inhibitory actions on glutamatergic neurons, thereby reducing hippocampal excitability. In fact, activation of KOP in the hippocampus was shown to be a potent modulator inhibitor of hippocampal transmission and the establishment of long term potentiation (LTP) (Wagner et al., 1993; Salin et al., 1995; Terman et al., 2000; Hugué et al., 2009). However, also a subset of GABAergic hippocampal interneurons expresses KOP (Racz and Halasy, 2002). The inhibition of these neurons would result in disinhibition of hippocampal transmission.

3. Interaction of dynorphin with opioid and non-opioid receptors

3.1. Interactions with kappa opioid receptors

pDyn-derived peptides (see Box 2 for affinities), especially Dyn A, preferentially bind to KOP (Chavkin et al., 1982). Although the existence of several KOP subtypes was proposed following early behavioural (Martin et al., 1976; Martin, 1979) and biochemical (Chang et al., 1981; Kosterlitz et al., 1981) experiments, only one KOP has been cloned so far. The pharmacological properties of this receptor mostly resemble those of kappa 1 sites (Meng et al., 1993; Yasuda et al., 1993; Mansson et al., 1994). KOP mostly couples to G_o , G_{i2} and G_{i3} and less frequently to G_z .

Therefore, KOP-mediated effects are pertussis toxin (PTX) sensitive. KOP-mediated effects include inhibition of cAMP synthesis (Sharma et al., 1977) and calcium channels (Hescheler et al., 1987; Surprenant et al., 1990; Rusin et al., 1997), activation of potassium channels (North et al., 1987) and increased intracellular Ca^{2+} levels (Jin et al., 1992). Protein kinase C and the inositol phosphatase are involved in the modulation of DNA synthesis by KOP agonists in fetal rat brain (Barg et al., 1993). Functionally significant is the activation of the MAP kinase pathways (Burt et al., 1996; Fukuda et al., 1996; Bruchas et al., 2006). The stimulation of ERK1 and ERK2 activity depends on the GTP binding protein Ras and

involves the G $\beta\gamma$ subunit (Belcheva et al., 1998). Presynaptically located KOP functioning as autoreceptors were also shown to inhibit the release of Dyn (Nikolarakis et al., 1989). However, there is a significant mismatch between Dyn A distribution and KOP-specific binding sites in the brain (Arvidsson et al., 1995). This can only partially be explained by somatodendritic release. Therefore, the original proposal of different KOP subtypes based on the different pharmacological profiles of Dyn action may be related to Dyn interactions with other opioid or non-opioid receptors.

3.2. Interactions with other opioid receptors

Binding studies also have suggested that Dyn A can interact with MOP and DOP in the brain tissue of different species (Quirion and Pert, 1981; Hewlett and Barchas, 1983; Young et al., 1983, 1986; Garzon et al., 1984). The situation is rather complex, as different pDyn-derived peptides display different affinities to the three classical opioid receptors, and the length of the peptide (Dyn A 1-17; Dyn A 1-13; Dyn A 1-8) is important. Thus, short molecules like Dyn A 1-8 display lower specificity for KOP than the long form Dyn A 1-17 (James et al., 1984). Due to the complex *in vivo* situation, the interaction of Dyn and opioid receptors was characterized *in vitro*. The receptor binding affinities of proopiomelanocortin-, proenkephalin- and pDyn-derived peptides on MOP, KOP and DOP expressed in COS-1 cells were studied by Mansour et al. (1995a). Displacement of [³H]-diprenorphine from human DOP, MOP and KOP receptors expressed in *Xenopus* oocytes by Dyn A 1-13 suggests K_i values of Dyn A in the subnanomolar range for KOP and in the low nanomolar range for DOP and MOP (Zhang et al., 1998). The affinity of Dyn A for the nociceptin receptor (NOP) was approximately 200 times lower in a displacement study (Zhang et al., 1998). In line with this, the EC₅₀ values for Dyn A activating any of the four receptors were highest for DOP [84 nM], somewhat lower for NOP and MOP [30 nM] and lowest for KOP [0.4 nM] (Zhang et al., 1998). A short list of affinities of some pDyn derived peptides, the two enkephalins and morphine for the classical opioid receptors is given in Box 2.

3.3. Interactions with non-opioid receptors

In addition to its effects mediated through opioid and opioid-like receptors, some non-opioid functions of Dyn have been proposed. An interaction with NMDA receptors in the spinal cord (Walker et al., 1982; Bakshi and Faden, 1990; Trujillo and Akil, 1991; Dubner and Ruda, 1992; Caudle and Dubner, 1998), hippocampus (Faden, 1992; Shukla and Lemaire, 1994), periaqueductal grey (Lai et al., 1998), and cochlea (Sahley et al., 2008) was proposed. Thus, Dyn A-induced neurological dysfunctions, hindlimb paralysis, and allodynia are blocked by NMDA receptor antagonists (Bakshi and Faden, 1990; Shukla and Lemaire, 1994; Vanderah et al., 1996; Tan-No et al., 2002, 2005). One potential target of Dyn on the NMDA receptor is the glycine site (Zhang et al., 1997; Voorn et al., 2007). Another study demonstrated that Dyn and Dyn 2–17 bind non-covalently to a linear conserved acidic region of the NR1 subunit via salt bridging (Woods et al., 2006). In addition, the Dyn–NMDA interaction may be pH dependent (Kanemitsu et al., 2003).

Recently other neuropeptide receptors were also suggested as potential mediators of Dyn effects. Thus, bradykinin receptors may be involved in the maintenance of neuropathic pain (Lai et al., 2006). Also translocation of Dyn across the plasma membrane targeting intracellular effectors has been suggested (Marinova et al., 2005). However, there is currently no evidence either for a functional importance of this mechanism, or for intracellular interaction partners.

4. Dynorphins in epilepsy

A considerable number of publications on the functions of Dyn in different models of epilepsy and epileptogenesis date back to the 1980s and 1990s. Due to the distribution of Dyn, it was thought most likely to act in partial complex seizures originating from the limbic system, or more precisely, the hippocampus. Since then, stimulation of KOP has been accepted as an anticonvulsant mechanism. However, the anticonvulsant properties of endogenous Dyn have only been revealed in recent years using pDyn-deficient (pDyn-KO) mice.

4.1. Potential sites of dynorphin actions

As described in the section on dual release mechanisms and illustrated in Figure 2, Dyn/KOP actions on hippocampal granule cells are complex. However, KOP density is rather low in the hippocampus, but the distribution of these receptors is strategically perfect in terms of dampening excitation in the limbic circuitry. Indeed KOP activation is capable of blocking LTP in the hippocampus (Wagner et al., 1993). Pre-synaptic KOP are located on terminals of perforant path fibres, mossy fibres and pyramidal neurons (Wagner et al., 1992; Drake et al., 1994; Terman et al., 2000). CA1 and CA3 neurons contain KOP mRNA, which might be localized pre- and/or post-synaptically (Mansour et al., 1994). In addition, somatostatin and neuropeptide Y (NPY) immuno-positive hippocampal interneurons express KOP (Halasy et al., 2000; Racz and Halasy, 2002). In guinea pigs pre-synaptic KOP were observed on terminals of supramammillary afferents innervating the inner molecular layer. Pre-synaptic KOP of perforant path fibres and mossy fibres, as well as postsynaptic KOP on CA3 pyramidal neurons and interneurons, are potential targets for Dyn released from mossy fibres during seizures. Besides this, interactions with MOP and NMDA receptors may also be involved in the mediation of Dyn effects. Pre-synaptic activation of KOP decreases N-, L- and P/Q-type Ca^{2+} currents (Rusin et al., 1997), resulting in reduction of glutamate release. Stimulation of voltage-gated K^{+} channels through post-synaptic KOP was proposed to occur in pyramidal neurons (Moore et al., 1994; Madamba et al., 1999). Dyn may inhibit the function of GABAergic interneurons through activation of MOP and KOP. The resulting inhibition of GABA release would facilitate seizures, whereas de-synchronization of interneurons might be beneficial in epilepsy (Aradi et al., 2002). Noteworthy, pretreatment with the MOP specific agonist DAMGO did not influence the seizure threshold in wild-type and pDyn KO mice (Loacker et al., 2007). Reports of Dyn actions on NMDA receptors are also controversial. While Kanemitsu et al. (2003) suggested pH-dependent inhibition of NMDA receptors others propose stimulatory effects of Dyn on NMDA responses (Caudle and Isaac, 1988; Shukla and Lemaire, 1994; Woods et al., 2006). Activation of DOP, which displays similar affinities for Dyn as MOP, may represent a potential target. Their activation is generally seen as proconvulsant (Comer et al., 1993; Broom et al., 2002a, b). Of note is the fact, that the application of SNC80, a specific DOP agonist, yielded exactly the same reduction in seizure threshold in wild-typ and pDyn KO mice (Loacker et al., 2007), which suggests that only a minor portion of DOP may be targeted by endogenous Dyn.

4.2. Alterations in dynorphinergic systems in animal models of epilepsy and epileptogenesis

Regarding the role of Dyn in epilepsy, no other structure has been described in more detail than the rat hippocampus. pDyn is expressed in granule cells of the hippocampus of rodents (McGinty et al., 1983) and human beings (Houser et al., 1990; Houser, 1992). Dyn is mostly accumulated in the mossy fibres and to a lesser extent in granule cell dendrites. At seizure onset and during initial seizures, Dyn is released in relatively large amounts, followed by a period of Dyn depletion. This effect is pronounced in the kainic acid model of temporal lobe epilepsy, in which Dyn levels recovered only after about one day when kainic acid was

injected intrastrially (Kanamatsu et al., 1986b), or were below control levels over several days when kainic acid was injected systemically in rat or mouse (Gall, 1988; Douglass et al., 1991; Lason et al., 1992b). Single electroconvulsive shocks depleted the Dyn pool for about 6 hours, while repeated shocks led to decreased Dyn peptide levels for up to 2 weeks (Kanamatsu et al., 1986a; Xie et al., 1989b). pDyn mRNA was upregulated within the first few hours after systemic injection of kainic acid and subsequently decreased gradually over a few days. Although the time course was similar, the extent of upregulation varied in different reports from 200% (Lason et al., 1992b) to 1300% (Douglass et al., 1991). This may be related to the different methods used for mRNA quantification (i.e. *in situ* hybridization and Northern blotting, respectively). In the electroconvulsive shock model, an initial decrease of pDyn mRNA within the first hour is followed by mild overexpression and subsequent repression (Xie et al., 1989b). Similar results as those obtained after intrastriatal injection of kainic acid were obtained after intrahippocampal injection of NMDA (Lason et al., 1992a; Hong et al., 1993). A transient induction of pDyn mRNA expression, followed by decreased expression levels, was also induced by local injection of subconvulsant/subneurodegenerative doses of the metabotropic glutamate receptor group I agonist (1S,3R)-ACPD (Schwarzer and Sperk, 1998). After an initial rebound of depleted Dyn pools to and above control levels, Dyn concentrations in the hippocampus appear persistently decreased (for at least 28 days) after kainic acid treatment (Rocha and Maidment, 2003).

Decreases in pDyn protein and mRNA levels were also measured 24 h after the last stimulation in several kindling models of epileptogenesis (Iadarola et al., 1986; McGinty et al., 1986; Morris et al., 1987; Lee et al., 1989; Xie et al., 1989a; Rosen et al., 1992; Harrison et al., 1995). Decreased Dyn A peptide, but not mRNA levels, was reported 7 days after kindling (Romualdi et al., 1995), but not after 14 days (McGinty et al., 1986; Lee et al., 1989; Rosen et al., 1992). Information about changes during the kindling process is rare. Lee et al. (1989) reported unchanged pDyn mRNA and Dyn A peptide levels 24 h after stage 2 seizures, while Moneta and Höllt (1990) measured decreased pDyn mRNA levels 2 h after stage 3 seizures in rats. More interesting from a functional point of view is a microdialysis study which showed that fully kindled rats displayed significantly lower extracellular opioid peptide levels during the interictal period 16 days after the last stimulation than sham-treated controls. In contrast, opioid levels reached peak levels 20 min after stimulation, which were comparable to those of partially kindled rats (Rocha et al., 1997). The complexity of changes in regard to expression levels and time course of alterations may be orchestrated by the dual influence of Ca^{2+} on the expression of pDyn. On the one hand, Ca^{2+} stimulates, through the activation of CREB which subsequently binds to CRE sites in the pDyn promoter, the expression of pDyn mRNA. On the other hand, Ca^{2+} augments the expression of DREAM (downstream regulatory element antagonizing modulator), which in turn downregulates the expression of pDyn mRNA when bound to the DRE (downstream regulatory element) sequence of the pDyn promoter. In fact, DREAM binding to DRE was shown in the mouse hippocampus (Cheng et al., 2002) and seizure-induced upregulation of DREAM is pronounced in dentate granule cells of the mouse (Matsu-ura et al., 2002).

The dynorphinergic system is also affected by pathological and morphological changes in the hippocampus under conditions of experimental epilepsy. Thus, somatostatin immunoreactive interneurons, which express KOP (Racz and Halasy, 2002), as well as CA1 and CA3 pyramidal neurons, are at least in part lost in several models of temporal lobe epilepsy. In contrast, mossy fibres, which contain Dyn, sprout to the supergranular layer (for review see Ben-Ari, 2001). Changes in the distribution of large dense core vesicles in mossy fibres, as well as in the size of the total active zone of mossy fibre terminals, were also reported after pentylenetetrazole-induced seizures (Pierce et al., 1999; Pierce and Milner, 2001). Therefore, downregulation of pDyn expression may at least in part reflect loss of the neural substrate.

4.3. Alterations in dynorphinergic systems in human epilepsy

Two patterns of DYN A 1-13-immunoreactivity were observed in the tissue of patients suffering from mesial temporal lobe epilepsy, which were basically due to the presence or absence of mossy fibre sprouting (Houser et al., 1990; de Lanerolle et al., 1997, 2003). Surviving hilar interneurons and CA3 pyramidal neurons displaying pDYN mRNA (de Lanerolle et al., 1992) and peptide (Gall, 1988; de Lanerolle et al., 1997) were also observed in mesial temporal lobe epilepsy, but not in mass-associated temporal lobe epilepsy or paradoxical temporal lobe epilepsy, both of which are characterized by less hippocampal sclerosis and lack of mossy fibre sprouting. No such labelled cells were described in the healthy brain (Hurd, 1996). Overall, DYN A 1-13-immunoreactivity appeared reduced in tissue obtained from patients with mesial temporal lobe epilepsy (de Lanerolle et al., 1997). However this reduction may at least in part be due to neuronal loss or DYN down-regulation. No data are available on the fate of DYN in the entorhinal cortex in which the peptide is expressed in humans, but not rodents (Hurd, 1996).

The known mismatch of DYN immunoreactivity (high in granule cells/mossy fibres) and KOP binding (high in medial CA1 and subiculum) is also evident in epilepsy patients. Patients suffering from mass-associated temporal lobe epilepsy or paradoxical temporal lobe epilepsy did not show marked differences in [³H]U69,593 binding compared to post-mortem controls. In contrast, hippocampi of mesial temporal lobe epilepsy patients displayed reduced binding in area CA1, but not subiculum, which was consistent with marked neuronal loss in CA1 but not subiculum (de Lanerolle et al., 1997). Of interest from a functional point of view may be the loss of DYN A-mediated inhibition of voltage-gated Ca²⁺ currents in hippocampal granule cells of epilepsy patients (Jeub et al., 1999). Increased Ca²⁺ currents in these cells lead to augmented glutamate release from mossy fibre terminals. The excitotoxic action of glutamate acting on NMDA receptors is seen as one of the most important inducers of neurodegeneration in epilepsy. In fact, loss of inhibition of voltage-gated Ca²⁺ currents was observed only in tissue also displaying mossy fibre sprouting and hippocampal sclerosis. The loss of DYN A effects on Ca²⁺ currents is consistent with reduced KOP in this type of tissue (de Lanerolle et al., 1997).

4.4. Dynorphins –endogenous anticonvulsant and neuroprotective agents?

There is ample evidence regarding the anticonvulsant and antiepileptic effects mediated by KOP. Different selective KOP agonists applied via different routes yielded time- and dose-dependent effects similar to those of phenytoin or phenobarbital (for a review see Simonato and Romualdi, 1996). Anticonvulsant effects of Dyn/KOP were observed in electroconvulsant models (Tortella et al., 1986, 1989, 1990; VonVoigtlander et al., 1987; Frey, 1988), chemicoconvulsant models involving injection of kainic acid or NMDA (VonVoigtlander et al., 1987; Tortella et al., 1990), herpes simplex viral seizures (Solbrig et al., 2006a, b), genetic models like audiogenic seizures (VonVoigtlander et al., 1987; De Sarro et al., 1993) and absence seizures (Przewlocka et al., 1995). In contrast, intracerebroventricular administration of Dyn caused EEG seizures in about one third of animals (Simonato and Romualdi, 1996). This effect was shown to be MOP-mediated. However, the question of which of the two effects – anticonvulsant via KOP or proconvulsant via MOP – would be elicited by endogenous pDyn-derived peptides remained unanswered for some time. Recently, Loacker et al. (2007) have shown that pDyn KO mice display a reduced seizure threshold upon pentylenetetrazole tail vein infusion, increased seizure severity and reduced delay time upon intracisternal injection of kainic acid, and increased neurodegeneration after intrahippocampal kainic acid injection. In addition, kindling progression was increased in pDyn KO mice (Loacker et al., 2007). These data need to be seen against the limitations immanent in a germ line knockout model, which might be influenced by compensatory changes during ontogeny. Although only minor

changes in MOP and no changes in DOP and KOP mRNA levels were observed in the hippocampi of these mice, significant alterations in opioid receptor binding in other brain areas relevant to epilepsy were reported from another pDyn KO mouse line (Clarke et al., 2003).

The situation in humans has been less well investigated. Increased seizure susceptibility was observed in humans with a *pDYN* gene promoter polymorphism resulting in reduced expression of pDYN (Stogmann et al., 2002). Similar results were reported by Gambardella et al. (2003). These specific associations could not be reproduced in a more recent study (Cavalleri et al., 2005), however the authors state that the mutation in the *pDYN* promoter may act as a general risk factor for epilepsy. Of note is the fact that only 50 patients out of 752 investigated in this study matched the phenotype reported by Stogmann et al. (2002), and thus the failure to reach statistical significance may be related to the small cohort.

4.5. Bottom line thoughts

The anticonvulsant and antiepileptic effects of Dyn in animal models are largely accepted. These effects appear almost exclusively mediated by KOP. Given the species dependent differences in pDyn expression and KOP distribution in the entorhinal cortex and amygdala, the preclinical data need to be confirmed in humans. In any case, seizure control may not be the only relevant feature of Dyn in epilepsy. Recent evidence led to the proposal that Dyn may also be responsible for inter- and post-ictal psychosis in epilepsy patients (Bortolato and Solbrig, 2007).

5. Dynorphins in addiction

The Dyn/KOP system plays a crucial role in reward mechanisms and addiction. Dysregulation of the Dyn/KOP system is induced by repeated drug abuse and involves the mesolimbic reward system. Thus, the dopaminergic pathway of the ventral tegmental area to the nucleus accumbens is seen as the main site of Dyn action in addiction. The importance of the Dyn/KOP systems is discussed not only with regard to habit learning and establishment, but also with regard to the reinstatement of addiction. This topic has recently been reviewed in Pharmacology and Therapeutics (Shippenberg et al., 2007).

6. Dynorphins in emotional control

While findings in epilepsy and addiction are mostly consistent and the functions of Dyn are widely accepted, the data related to emotional control mechanisms are rather inconsistent. Testing of emotions in animals is not as straightforward as EEG recordings and most of the tests were developed for rats. Interpretation of results obtained from mouse testing has to be seen in the context of an entirely different social behaviour in rats and mice. In addition, we have to deal with a large number of different mouse strains, which already vary significantly in their basal behaviour regarding anxiety, stress and activity. Therefore, inconsistent data may at least in part reflect strain-specific differences in pDyn expression (Ploj et al., 2000), housing conditions (Kudryavtseva et al., 2004) and testing setups. In addition, it is questionable to what extent rodent studies can model the complex spectrum of mood and anxiety or fear expression in human beings. Nevertheless, insight into basal mechanisms may be gained by careful interpretation of data obtained from animal models.

6.1. Potential sites of dynorphin action

Emotional control and the stress response are based on a network of brain nuclei, including the amygdala, hypothalamus, hippocampus, cortical regions and the brain stem. The expression of pDyn mRNA is especially high in key structures of these circuits (illustrated in Fig. 3). Thus, pDyn mRNA is observed at high levels in the amygdala, with the highest

concentrations in the central nucleus, in the hypothalamic paraventricular, supraoptic and medial nuclei and the olfactory tubercle, in the hippocampal granule cell layer, in the striatum and nucleus accumbens and in the nucleus of the solitary tract. Numerous pDyn-expressing cells are found scattered throughout the cortex, including the prefrontal cortex. This expression pattern is rather consistent in the human (Hurd, 1996) and rodent brain (Merchenthaler et al., 1997; Lin et al., 2006). However, within the central nucleus of the amygdala, rats and mice (Merchenthaler et al., 1997; Lin et al., 2006) display the highest levels of pDyn mRNA expression, while cortical subnuclei are more prominently labelled in the human brain (Sukhov et al., 1995; Hurd, 1996). In the rodent striatum, the differences in pDyn mRNA content between patch and matrix are less pronounced than in human tissue, but may display some lateralization effects (Capper-Loup and Kaelin-Lang, 2008). KOP binding sites reach the highest densities in the claustrum and endopiriform nucleus, but marked binding is also observed in the medial and basal amygdala, hypothalamus, nucleus accumbens, ventral pallidum, the olfactory tubercle, septum, bed nucleus of stria terminalis, central grey, substantia nigra, striatum, and throughout the cortex (Slowe et al., 1999; Clarke et al., 2003). This is consistent with the distribution of pDyn mRNA (DePaoli et al., 1994). Therefore, the prerequisites for an involvement of Dyn/KOP in major pathways involved in emotional control and stress response are present.

6.1.1. Mesolimbic and nigrostriatal dopamine signalling—Dopaminergic projections from the ventral tegmental area to the frontal cingulate and entorhinal cortices, central amygdala, hippocampus, hypothalamus, basal forebrain, periaqueductal grey, raphe and parabrachial nuclei, locus coeruleus and the nucleus accumbens are comprised in the term mesolimbic dopamine system (Beckstead et al., 1979; Simon et al., 1979). As a pivotal part of the limbic cortical-striatopallidal circuitry, these connections play a crucial role in mood control, motivation and habit learning (for review see Graybiel, 2005). Dysfunction of this system represents the neurochemical basis of addiction and schizophrenia. The nigrostriatal dopaminergic projections are part of the basal ganglia and important for movement control, but also involved in the regulation of mood. The influence of Dyn on forebrain dopamine release was reviewed in detail recently (Shippenberg et al., 2007). Therefore, only a brief overview of the role of Dyn in emotional control is given here. In most brain areas the correlation between KOP mRNA and binding is high, thus suggesting a somatodendritic distribution of KOP. In contrast there is a marked mismatch in the substantia nigra, pars compacta and ventral tegmental area, both of which express KOP mRNA, but display only low levels of KOP-specific binding (Mansour et al., 1994). Direct inhibition of dopaminergic neurons in the ventral tegmentum was demonstrated by electrophysiological experiments (Margolis et al., 2003), although inhibition of dopaminergic neurons in the ventral tegmental area by post-synaptic KOP appears to be restricted to cells that project to the prefrontal cortex, but not to the nucleus accumbens (Margolis et al., 2006). The inhibitory effects of Dyn acting via KOP may arise from a reduction of the duration of action potentials (Margolis et al., 2008). Alternatively it has been suggested that KOP is transported to axon terminals of dopaminergic neurons, which is consistent with the immunohistochemical labelling of KOP-positive fibres (presumably axons) but not somata in the nucleus accumbens and striatum, while fibres (presumably dendrites) plus somata are labelled in other brain areas (Mansour et al., 1995b). The immunohistochemical findings are in line with data indicating direct inhibition of dopamine release in the striatum and the nucleus accumbens through Dyn/KOP (Mulder et al., 1984; Di Chiara and Imperato, 1988; Werling et al., 1988; Spanagel et al., 1992). Dyn acting on these terminals could be released following dopamine D1 receptor stimulation (You et al., 1994) from axon collaterals of striatonigral neurons (Wilson and Groves, 1980; Kawaguchi et al., 1990) or from their dendrites (Drake et al., 1994; Simmons et al., 1995). The idea that

Dyn may also play a role in acetylcholine-regulated dopamine release (Gauchy et al., 1991) has not yet been confirmed.

6.1.2. Hypothalamic–pituitary axis—The hypothalamic-pituitary axis represents one of the most important interfaces of the central nervous system to the endocrine system. Hypothalamic neurons project either directly to the posterior pituitary (neurohypophysis) or release their transmitters into the portal blood circulation and thereby regulate the release of hormones from the anterior pituitary (adenohypophysis). Pituitary hormones themselves regulate the release of hormones from target organs and are involved in a wide range of functions from growth, metabolic state, stress, to proliferation and lactation. Neurons in the paraventricular and supraoptic nuclei, which represent two of the most important nuclei in the hypothalamic-pituitary unit, contain high amounts of pDyn (Burke et al., 2006). The distribution of KOP mRNA expression and binding suggests receptor transport from the hypothalamic paraventricular and supraoptic nuclei, which contain KOP mRNA, to the median eminence and neural lobe of the pituitary.

In terms of emotional control, the adrenal gland is probably the most important target of pituitary hormones (hypothalamic-pituitary-adrenal axis; HPA). Activation of corticotropin releasing hormone (CRH) induced ACTH release from the pituitary, triggering increased cortisol or corticosterone serum levels, is seen as one central event in the stress response. The expression of CRH and thyrotropin releasing hormone mRNAs in the paraventricular nucleus is significantly reduced in Dyn-deficient mice, pointing to a marked influence of the Dyn/KOP system on the functioning of the hypothalamic–pituitary axis. The decrease in CRH directly translates to decreased basal corticosterone serum levels (Wittmann et al., 2009). This is in line with the stimulatory effects of the selective KOP agonist MR-2034 on the rat HPA (Calogero et al., 1996) and with increased plasma ACTH and cortisol levels measured in monkeys after treatment with the KOP selective agonist U-50488H (Pascoe et al., 2008). In contrast the specific KOP antagonist norbinaltorphimine (norBNI) reduced CRH levels in the hypothalamic paraventricular nucleus and corticosterone levels in serum of mice (Wittmann et al., 2009).

6.1.3. Serotonergic signalling—Serotonin, which is released from axon terminals of dorsal raphe nucleus projections to the forebrain, is of substantial relevance to emotional control. Dysfunction of serotonergic neurotransmission leads to mood disorders such as depression (for review see Michelsen et al., 2008). Serotonin reuptake inhibitors are a major source of antidepressant drugs and alterations in serotonin transporter expression were shown to be associated with emotional regulation and social cognition, but also anxiety-related traits and susceptibility to depression (for reviews see Canli and Lesch, 2007; Lesch, 2007; Lowry et al., 2008). While antidepressant treatment usually takes weeks to become effective, pharmacological manipulation of the serotonin system causes also a number of short-term effects especially on the processing of emotional information (Merens et al., 2007). There is evidence for reduced extracellular serotonin levels caused by the KOP-specific agonist U-50488H infused along the axonal tract of serotonin fibres (i.e. in either the dorsal or median raphe or nucleus accumbens). A direct action of Dyn via KOP on serotonin release at nerve endings was suggested due to the lack of influence of KOP agonists on the electrophysiological properties of serotonergic neurons (Jolas and Aghajanian, 1997). The effect of U-50488H is reversible by norBNI, but appears delayed, starting only 1 h after infusion. Thus, increased clearance through reuptake facilitation was hypothesized to be responsible for the reduction of extracellular serotonin levels (Tao and Auerbach, 2002). No alterations in the expression of the rate-limiting enzyme of serotonin synthesis – tryptophan-hydroxylase 2 – were detected in pDyn-deficient mice (Wittmann et al., 2009), supporting the idea that the Dyn/KOP system regulates serotonin release or uptake, but not synthesis.

6.1.4. Noradrenergic signalling—Release of noradrenaline in the hypothalamus, amygdala and locus coeruleus was considered highly relevant to the provocation of anxiety and/or fear. Classical anxiolytic drugs such as benzodiazepines and MOP and DOP agonists were shown to decrease the release of noradrenaline in these brain regions (for review see Tanaka et al., 2000). KOP mRNA and protein were observed in the locus coeruleus (DePaoli et al., 1994; Mansour et al., 1994), which represents the primary source of forebrain noradrenaline (Swanson, 1976), while hypothalamic regions receive more noradrenergic input through the ventral noradrenergic bundle (Millan et al., 1984), which resembles projections from brainstem neurons. Dyn-containing axon terminals form primarily asymmetric (excitatory) synapses on noradrenergic dendrites within the locus coeruleus (Barr and Van Bockstaele, 2005; Reyes et al., 2007, 2008, 2009). These axons colocalize glutamate (Barr and Van Bockstaele, 2005) and stress-related peptides like CRH (Reyes et al., 2008) and may partially originate from the dorsal cap of the paraventricular nucleus (Valentino et al., 1992). In *in-vitro* experiments, KOP agonists produced a concentration-dependent depression of excitatory postsynaptic potential evoked by electrical stimulation of afferents to locus coeruleus neurons (McFadzean et al., 1987; Pinnock, 1992a, b). *In vivo* electrophysiological evidence for pre-synaptic inhibition of diverse afferents to the locus coeruleus through Dyn/KOP was published recently (Kreibich et al., 2008). These data suggest that the Dyn/KOP system represents a powerful means of regulating the noradrenergic locus coeruleus system, which might influence forebrain signal processing and organization of behavioural strategies in response to environmental stimuli. It is worth noting that the expression of tyrosine-hydroxylase, the rate-limiting enzyme in dopamine and noradrenaline synthesis, was not altered in the locus coeruleus of pDyn null mice (Wittmann et al., 2009).

The influence of Dyn/KOP on brainstem noradrenergic cell groups is less well understood. However, both Dyn immunoreactive fibres and perikarya were observed in many areas of the reticulate formation including monoamine-containing nuclei (Khachaturian et al., 1982). Significant amounts of KOP mRNA and binding were also reported for these areas (Mansour et al., 1994).

6.2. Dynorphins and anxiety

Anxiety is a fundamental part of the behaviour of animals and human beings. The proper response to anxiety cues prompts a state of defensive motivation. In its biological context, anxiety prepares the individual for a potential threat and leads to a faster response - either flight or fight - if danger materializes. Thus, heart rate, body temperature and corticosterone-serum levels are increased and commonly used as physiological measures of anxiety, but also stress. Disorders of anxiety and fear control, like panic disorders and phobias, show rising incidences in developed countries. In addition, anxiety disorders often are co-morbid with other mental health problems such as depression, addiction or schizophrenia. Human emotions are much more delicate in expression than those that can ever be analysed in animal experiments, but similarities in basal mechanisms exist and may help to understand the human situation. The regulation of anxiety behaviour involves several neurotransmitter systems. Beside the classical transmitters serotonin (Wise et al., 1970; Westenberg et al., 1987; Graeff, 2002) and noradrenaline (Vlachakis et al., 1974; Brunello et al., 2003), several neuropeptides have been proposed as modifiers of anxiety-related behaviour. Fear and anxiety also involve a dense network of cortical, amygdalar, hypothalamic and brainstem nuclei. However, the basolateral and central nuclei of the amygdala and the paraventricular hypothalamic nucleus appear to be most relevant (for review see Lang et al., 2000).

The role of Dyn/KOP in anxiety control is presently not well understood. Data obtained from pDyn and KOP deficient mice are relatively rare and do not provide a uniform image

of the functions of Dyn/KOP in anxiety (see Box 3 for a summary of findings). Mostly the alterations are subtle and may be camouflaged by compensatory changes, as all models published so far are germ-line knockouts. Thus, up-regulation of both, MOP and DOP, was observed in anxiety related brain nuclei of pDyn and KOP KO mice (Slowe et al., 1999; Clarke et al., 2003). In addition, anxiety testing is strongly influenced by epigenetic and environmental conditions including the social status of mice (Kudryavtseva et al., 2004). KOP-deficient mice maintained on a mixed Sv129 $\times$ C57bl/6J background did not show marked alterations in anxiety-related behaviour (Simonin et al., 1998; Filliol et al., 2000). However, KOP deficiency does not exclude interactions of pDyn derived peptides with other opioid and non-opioid receptors. Vice versa, pDyn KO may result only in a partial loss of stimulation of KOP and other receptors, as other opioid peptides may still (or even more due to compensation) activate KOP. Thus, pDyn and KOP deficient mice have to be compared cautiously. In pDyn KO mice on a C57bl/6J background (Bilkei-Gorzo et al., 2008), zero-maze and startle response tests suggested an anxiogenic phenotype, while no effect was seen in the light-dark test. In our pDyn KO mouse line, maintained on a C57bl/6N background (Wittmann et al., 2009), a markedly anxiolytic phenotype was consistently observed in three independent tests (open field, light-dark choice and elevated plus maze), which was reproduced in wild-type mice through treatment with the KOP antagonists norBNI or GNTI. The pDyn KO phenotype was reversed by treatment with the selective KOP agonist U-50488H. While we did not find differences in stress-induced hyperthermia, Bilkei-Gorzo et al., (2008) reported a delayed subtle increase in stress-induced hyperthermia in their pDyn KO mice. Whether these minor differences depend on the different strains or different testing conditions, or simple reflect a low relevance of Dyn/KOP to anxiety control, remains debateable. In any case, the interpretation of animal behavioural data cannot be directly translated to the human situation. This holds especially true for the importance of Dyn/KOP in stress and anxiety, because marked differences in the distribution of pDyn in the amygdala of humans and rodents have been reported (Hurd, 1996; Merchenthaler et al., 1997; Lin et al., 2006). The complexity of anxiety control is also reflected in pharmacological experiments. Tsuda et al. (1996) proposed the involvement of KOP in the anxiolytic action of diazepam, and KOP agonists produced anxiolytic-like behaviour in the elevated plus-maze (Privette and Terrian, 1995). In addition, big Dyn was suggested to be an anxiolytic peptide (Kuzmin et al., 2006). Marked anxiolytic effects of KOP agonists were opposed by the finding of increased KOP-specific binding in the amygdala in chronic pain-induced anxiety in mice (Narita et al., 2006). In contrast, several other reports suggest pro-aversive effects in the elevated plus-maze mediated by KOP agonists injected into the periaqueductal grey (Motta et al., 1995; Nobre et al., 2000). Recently, Knoll et al. (2007) proposed anxiolytic effects of KOP antagonists in models of learned and unlearned fear in rats. A synopsis of these studies suggests spatial and temporal differences in the response to KOP activation. This is in line with data indicating a lack of anxiety related effects 1 h after the application of the KOP antagonist norBNI, while anxiolytic effects were observed 48 h after norBNI treatment (Wittmann et al., 2009). We therefore suggest the existence of an indirect modulation of anxiety control circuits through KOP. This is supported by specific alterations in the transmitter systems of pDyn KO mice known to be involved in emotional control. Thus, inhibition of synaptic transmission and LTP in the basolateral amygdaloid nucleus via activation of KOP stimulation has been reported (Huge et al., 2009). This nucleus plays a crucial role in anxiety control (Heilig et al., 1994). In addition, several neuropeptide systems within amygdalar and hypothalamic nuclei displayed adaptations that may be of relevance to the observed anxiolytic phenotype (Wittmann et al., 2009). The key features are an increased NPY expression in the basolateral amygdala and a concomitant reduction in CRH expression in the central amygdala and the paraventricular hypothalamic nucleus, which could be reproduced in wild-type mice by a single injection of 10 mg/kg norBNI 48 h before testing. These changes may reflect alterations in the regulatory circuit of NPY in the basolateral amygdala suppressing CRH expression in the central nucleus (Heilig

et al., 1994; Sajdyk et al., 2004). Increasing evidence supports a crucial role for NPY and Y-receptors in anxiety-related behaviour (for a review see Kask et al., 2002). Thus injection of NPY into the amygdala was shown to be anxiolytic. Y1-receptors have been proposed to mediate these anxiolytic effects (Wahlestedt et al., 1993). This was recently confirmed in Y1-receptor deficient mice (Karlsson et al., 2008). In addition, NPY is seen as the major counterpart of CRH, mediating mostly opposing effects and thereby balancing the emotional state (Heilig et al., 1994; Sajdyk et al., 2004). Furthermore, intraventricular injection of CRH or overexpression of CRH is anxiogenic in mice (Stenzel-Poore et al., 1996) and inactivation of CRH receptor 1 reduces anxiety (Smith et al., 1998; Timpl et al., 1998) whereas deletion of CRH receptor 2 is anxiogenic (Bale et al., 2000; Coste et al., 2000; Kishimoto et al., 2000).

6.2.1. Bottom line thoughts—With the presently used models and the high variability in testing conditions and paradigms the functions of Dyn/KOP in anxiety remain unclear. The application of standardized testing conditions, as suggested by the European initiative Eumorphia and available online from the EMPRESS database (<http://empress.har.mrc.ac.uk>) on mouse lines displaying different trait anxiety levels (like C57bl/6N or J; Balb/c and DBA-2) will be necessary to gain a clear picture. In addition it will be important to investigate conditional knockout models, which allow for adult onset, cell or region specific deletion of pDyn and/or KOP to dissect Dyn functions in distinct brain nuclei.

6.3. Dynorphins in the stress response

Stress is a normal element of life and humans like animals respond to stress stimuli with adaptive reactions. Inescapable, long-lasting stress however can lead to detrimental behaviours (Rhoads, 1983; Kosten et al., 1986; Najavits et al., 1998). Prolonged inescapable stress results in depression-like behaviour in rodents, which may be influenced by the Dyn/KOP system. Data in this field are somewhat contradictory, probably reflecting variations in test conditions. Thus, both slight decreases and increases in stress-induced immobility were reported for pDyn KO mice under different conditions in the forced swim and tail suspension tests (McLaughlin et al., 2003; Bruchas et al., 2007; Land et al., 2008; Wittmann et al., 2009). In addition, alterations in nociception in pDyn KO mice (Wang et al., 2001; McLaughlin et al., 2003) may influence mouse behaviour, especially in the tail suspension test (Wittmann et al., 2009).

The neurochemical and anatomical background for an involvement of the Dyn/KOP system in the acute behavioural response to stress is also rather unclear. One of the main output structures for the stress response is the HPA which regulates corticosterone release from the adrenal glands. Dyn is strongly involved in the regulation of this pathway and disruption of *pDyn* results in reduced serum ACTH and corticosterone levels under basal and stressed conditions (Bilkei-Gorzo et al., 2008; Wittmann et al., 2009), which is in line with increased corticosteroid levels after KOP activation (Vonvoigtlander et al., 1983). In pDyn KO mice, a delayed termination of the stress-induced corticosterone response was noted (Bilkei-Gorzo et al., 2008) while no difference between wild-type and pDyn-deficient mice was observed in short swim stress trials (Filliol et al., 2000). No or only a minor reduction of stress-induced hyperthermia was observed in response to pDyn knockout (Bilkei-Gorzo et al., 2008; Wittmann et al., 2009) suggesting minor differences in stress coping regardless of alterations in corticosterone levels.

In contrast to the acute stress responses, several long-term stress induced effects show a more clear-cut Dyn/KOP dependency. Stress-induced analgesia was blocked by peripheral administration of the KOP antagonist norBNI (Takahashi et al., 1990; Watkins et al., 1992; Menendez et al., 1993) and by *pDyn* disruption (McLaughlin et al., 2003). The influence of

Dyn/KOP on stress-induced reinstatement of cocaine and amphetamine seeking behaviour was proposed by several studies (for review see Koob, 2008). Apparently, this effect depends on CRH, which is significantly reduced in pDyn KO mice and after KOP antagonist treatment (Wittmann et al., 2009). A similar mechanism was described as fundamental part of stress-induced dysphoria, which is also KOP dependent (Land et al., 2008).

6.3.1. Bottom line thoughts—Given the strong interrelation of Dyn and CRH, the influence of stress on the expression of Dyn and the complexity of structures involved in the stress response, it is not surprising that no clear cut involvement of Dyn/KOP in acute stress coping was revealed by pharmacological approaches or in germ-line knockout models. However there is accumulating evidence that the Dyn/KOP system is altered under chronic stress and that Dyn influences stress related pathways, such as noradrenaline or CRH synthesis and release. Therefore, like in anxiety, Dyn may be seen as a modulator of stress related circuits, while it is not directly involved in the stress response. A detailed knowledge of adaptational processes in stress regulating systems appears to be highly relevant to the understanding of addiction and depression. However, the translation of findings from animal models to the human situation has yet to be established.

7. Dynorphins in psychotic disorders

One of the major aversive side effects of opioid treatment is dysphoria. In fact, these side effects led to the termination of clinical trials for several KOP agonists including spiradoline, enadoline and niravoline, which had been proposed as analgesics or aquaretics (Barber and Gottschlich, 1997). KOP-agonist induced dysphoria was first supposed to be mediated by the sigma-phencyclidine receptor, but finally attributed to KOP activation (Mucha and Herz, 1985; Pfeiffer et al., 1986; Shippenberg and Herz, 1986). Since then, growing evidence supports this concept, rendering dysphoria the best-accepted emotional response to KOP stimulation so far. CRH-2 receptor mediated phosphorylation of KOP in the basolateral amygdala, nucleus accumbens, dorsal raphe, and hippocampus was suggested as neurobiochemical background of stress induced dysphoria (Land et al., 2008). Brain areas affected by accelerated KOP internalization due to phosphorylation are not only involved in stress circuits, but also in psychotic disorders. Therefore, this mechanism may be essential to understand the role of Dyn in psychotic disorders.

7.1. Dynorphins and depression

Depression is frequently seen as a disease related to maladaptation to chronic stress. Although there are many effective treatments of depression, virtually all of them target the serotonergic and/or noradrenergic systems. Development of novel antidepressants with potentially less side effects is hampered by the lack of suitable animal models. Presently stress induced immobility is interpreted as “depression like behaviour” mainly based on the fact that this behaviour can be attenuated by antidepressant drugs. However, animals display an almost immediate response to antidepressants, which does not reflect the situation in humans. Many investigations have focused on the role of hippocampal and frontal cortical regions in depression and antidepressant action. It was proposed that the nucleus accumbens and the ventral tegmental area greatly contribute to the pathophysiology and symptomatology of depression and may even be involved in its aetiology (Nestler and Carlezon, 2006). In addition, the importance of the amygdala-frontal connectivity during emotional regulation was shown by fMRI (Banks et al., 2007). However, also the dopaminergic reward pathway plays a crucial role in the aetiology of depression (Nestler and Carlezon, 2006; Martin-Soelch, 2009). Dyn/KOP was shown to influence many of the brain areas related to depression and is altered in depressive states. Thus, repeated swim stress, resulting in depression like behaviour, causes activation of KOP in the nucleus

accumbens, cortex and hippocampus of mice (Bruchas et al., 2007). In human beings with major depression, decreased pDYN mRNA levels were detected in the accessory basal amygdala and amygdalohippocampal area (Hurd, 1996). In contrast, no significant changes in pDYN or KOP expression were observed in the prefrontal cortex of highly depressed subjects (Peckys and Hurd, 2001). Hippocampal excitability is regulated by Dyn/KOP in several ways (see chapter on epilepsy), resulting in reduced excitability. In humans the effects of DYN released from perforant path fibres may be more pronounced than in rodents, due to the markedly higher pDYN mRNA levels observed in human entorhinal cortex. Hippocampal output exerts an inhibitory control of the HPA and thereby is directly involved in stress regulation. Reduced hippocampal inhibition of this axis may be the neurological background of hypercortisolaemia observed in a subset of depressed patients (Ressler and Nemeroff, 2000). The mesolimbic dopamine system is tonically inhibited by Dyn/KOP receptors, which contrasts with the effects mediated by MOP (Spanagel et al., 1992). In addition, glutamatergic innervation of medium spiny neurons in the nucleus accumbens may be regulated by presynaptic KOP receptors (Hjelmstad and Fields, 2001). Probably more important is the blockade of dopamine release in the nucleus accumbens and striatum through presynaptic KOP. In general, the actions of Dyn/KOP dampen signalling in all brain areas involved. Injection of the MOP agonist DAMGO into the ventral tegmental area produces conditioned place preference, while the KOP agonist U-50488H and the Dyn derivative E-2078 cause aversion (Bals-Kubik et al., 1993). Activation of KOP mediates phosphorylation of p38 mitogen-activated protein (MAP) kinase, which appears to be an essential step in the establishment of conditioned place aversion in response to U-50488H and stress-induced immobility (Bruchas et al., 2007) and dysphoria (Land et al., 2008). Stress produces depression-like behaviour in rodent models and worsens symptoms of depression in human beings. Furthermore, stress increased Dyn levels in limbic brain areas in animal models (Shirayama et al., 2004). This stress-induced increase was blocked by antidepressant (desipramine) treatment (Chartoff et al., 2009). While the KOP-specific agonist salvinorin A induced depressive-like behaviour in the forced swim test (Carlezon et al., 2006), KOP antagonists produced antidepressant effects (Mague et al., 2003; Reindl et al., 2008). Therefore, Dyn/KOP is seen as a mediator of dysphoria, one of the major aversive side effects of opioid treatment.

7.1.1. Bottom line thoughts—The Dyn/KOP system is involved in the regulation of virtually all circuits thought to be important in depression. Generally the activation of KOP appears pro-depressant. However, Dyn influences different pathways in distinct brain regions. By inhibiting dopaminergic neurons in the ventral tegmental area or dopamine release in the nucleus accumbens, Dyn exerts its pro-depressant activity through the mesolimbic reward system. Dyn activating KOP in the hippocampus may lead to disinhibition of the HPA. Activation of KOP in the axonal compartment of serotonergic neurons either reduces release or facilitates reuptake of serotonin, while KOP in the locus coeruleus inhibits the release of noradrenaline.

Therefore, KOP antagonists may be suitable as antidepressant drugs. However, it has to be established whether antipsychotic actions can be achieved at dosages that are low enough to avoid induction of hyperalgesia.

7.2. Dynorphins and schizophrenia

Schizophrenia is one of the most often diagnosed mental illnesses in psychiatric inpatients. Several different forms of schizophrenia can be induced by environmental factors. In addition, a strong genetic component was suggested from animal models (Desbonnet et al., 2009). Effective treatment is available through antipsychotic drugs. These are divided into typical and atypical drugs. Typical, but not atypical antipsychotics activate cells in the

dorsolateral striatum (Nguyen et al., 1992; Robertson et al., 1994; Wan et al., 1995). In contrast, atypical, but not typical antipsychotic drugs activate neurons in the prefrontal cortex (Robertson and Fibiger, 1992; Robertson et al., 1994; Wan et al., 1995). Besides these differences, both classes of drugs activate cells in the nucleus accumbens shell, central amygdaloid nucleus and thalamic centromedial nucleus (Robertson et al., 1994; Wan et al., 1995), suggesting that these nuclei might be essential for the antipsychotic actions. Of note is the fact, that representatives of both classes (clozapine and haloperidol) activated almost exclusively dynorphinergic GABA neurons in these brain nuclei (Ma et al., 2003). Despite this fact, few data are available on the possible role of Dyn in schizophrenia. Early reports of decreased DYN (1-8) levels in the CSF of schizophrenic patients (Zhang et al., 1985) are backed up by the increased levels of CSF DYN A observed in schizophrenics following administration of the typical antipsychotic drug zuclopenthixol (Heikkila et al., 1990). DYN levels were unchanged in the substantia nigra (Iadarola et al., 1991) and in the caudate, putamen and accumbens nuclei (Hurd et al., 1997) of schizophrenia patients. In addition, mRNA levels of both pDYN and KOP were unchanged in the cingulate and prefrontal cortices of schizophrenics (Peckys and Hurd, 2001). Thus, the origin of increased CSF DYN levels remains unclear. However, allelic variation in the human pDYN promoter in subjects carrying the Ser9Gly mutation in the dopamine 3 receptor may contribute to the susceptibility to this disorder (Ventriglia et al., 2002).

The potent KOP agonist Salvinorin A produces hallucinations, supporting the idea of a DYN/KOP involvement in disorders characterized by disturbed perception (Sheffler and Roth, 2003). Recently, the involvement of altered dynorphinergic transmission in epilepsy was suggested as a cause of inter- and postictal psychosis (Bortolato and Solbrig, 2007). Evidence for a role of Dyn in psychotic disorders also comes from animal experiments, where the selective KOP agonist U-50488H induced a dose-dependent reduction of pre-pulse inhibition (Bortolato et al., 2005). Pre-pulse inhibition is seen as readout of sensorimotor gating and is impaired in schizophrenics. Pre-pulse inhibition was restored by the selective KOP antagonist norBNI, as well as by the atypical antipsychotic clozapine but not by the typical antipsychotic haloperidol (Bortolato et al., 2005). Unfortunately no data addressing Dyn/KOP functions in schizophrenia are currently available from knockout animals.

7.2.1. Bottom line thoughts—There is increasing evidence for a potential involvement of Dyn/KOP in schizophrenia. Like in depression, the importance of Dyn/KOP may be seen in the modulation of emotional and stress circuits. Therefore, the underlying mechanisms may differ from those of schizophrenia risk genes like neuregulin 1, which are supposed to be involved in the proper development of neuronal circuits (Falls, 2003; Harrison and Law, 2006). However, the assessment of schizophrenia in animal models is limited to the measurement of pre-pulse inhibition, which reflects only a single aspect of the complex human pathology. Therefore, one has to raise the question, whether the emotional system of mice or rats is complex enough to model mental diseases such as depression and schizophrenia.

8. Conclusions

Valuable information about the physiological and pathophysiological implications of Dyn/KOP has been accumulated over the past 30 years. However, several questions remain open and many mechanisms require further elucidation. Given the multiplicity of functions and the drawbacks in early studies of KOP agonists in analgesia, the direct use of KOP as a drug target for pain or antiepileptic therapy may be difficult. On the other hand, KOP antagonists are more likely to turn out as antipsychotic drugs or as drugs supporting withdrawal in addiction, because their neuropsychiatric effects have been observed at lower doses than

their hyperalgesic effects. In any case, further understanding of the second messenger systems of the overlapping signalling of distinct opioid peptides through different opioid and non-opioid receptors may help to design drugs with fewer side effects. The generation of opioid peptide and receptor knockout mice was an essential tool in this respect and has only just begun to be used to revisit old concepts. However, the concept of germline knockout proved unsatisfactory not only in the field of opioid research. Compensatory changes during ontogeny together with the complex situation of whole body knockout has to be overcome by the use of other genetical approaches such as conditional knockout, or viral transfection induced over-expression of peptide precursors and receptors. With these modern techniques, which allow region or cell type specific modifications in adult animals, it will be possible to solve many open questions, which analysis of the overlapping signalling of opioid systems will remain a challenge.

Box 1

The first evidence for the existence of distinct opioid receptors was published in 1976 (Martin et al., 1976). The proposed receptor forms were named after the prototypic drugs assigned to them: μ (mu for morphine) and κ (kappa for ketocyclazocine) receptors. One year later, a third receptor termed δ (delta for deferens) was described from mouse vas deferens and guinea pig ileum (Lord et al., 1977). In 1996 (Dhawan et al., 1996), it was recommended to replace μ , δ , and κ by OP_3 , OP_1 , and OP_2 , respectively, according to NC-IUPHAR guidelines. However, this change was never accepted by the research community. Therefore, NC-IUPHAR presently recommends to keep the Greek terminology, but suggests to define it additionally as MOP, DOP, KOP and NOP when first mentioned (www.iuphar-bd.org/GPCR). Pharmacological experiments which led to the proposal of multiple KOP receptors (Horan et al., 1993; Heyliger et al., 1999) could not be verified by molecular cloning and are now interpreted as actions of dynorphin DOP and MOP.

Current NC-IUPHAR-recommended Nomenclature	Previous Nomenclature
μ ; mu; MOP	MOR; MOR-1; OP_3
δ ; delta; DOP	DOR; DOR-1; OP_1
κ ; kappa; KOP	KOR; KOR-1; OP_2
NOP	ORL1; LY322; N/OFQ receptor; OP_4

Box 2

Affinities of some prodynorphin derived peptides for the three classical opioid receptors as compared to enkephalins and morphine. Data represent the range of pK_i published.

Ligand/receptor	MOP	KOP	DOP
Dyn 1-17	8.1 ¹	10.8-8.3 ^{1,6}	7.4 ¹
Dyn 1-13	8.3 ¹	10.7-9.3 ^{1,3}	7.8 ¹
Dyn 1-8	8.4 ¹	9.9-8.0 ^{1,6,7}	8.4 ¹
Dyn B	8.5 ¹	9.9-8.1 ^{1,3,4}	7.8 ¹
Leu-Enk	8.1 ¹	6.0 ³	8.7-8.4 ^{1,8}

Ligand/receptor	MOP	KOP	DOP
Met-Enk	9.2 ⁸	6.0 ³	7.4 ⁹
Morphine	9.0-7.9 ^{1,8}	7.3-6.7 ^{1,3,10}	6.9 ¹

¹ Toll et al., 1998² Li et al., 1993³ Meng et al., 1993⁴ Simonin et al., 1995⁵ Zhu et al., 1995⁶ Zhu et al., 1997⁷ Yoshino et al., 1990⁸ Raynor et al., 1994⁹ Yasuda et al., 1993¹⁰ Chen et al., 1993

Box 3

Behavioural effects observed in pDyn and KOP knockout mice or after KOP antagonist treatment. The data have to be interpreted in the light of different testing conditions and genetic backgrounds of animals tested. In addition, all experiments were carried out on germ-line KOs, resulting in ontogenetic compensation and overlapping effects due to whole-body knockout.

Test paradigm	pDyn KO mice	KOP KO mice	KOP antagonists (mice and rats)
Locomotion	No change (Wittmann et al., 2009; Sharifi et al., 2001; Bilkei-Gorzo et al., 2008)	No change (Simonin et al., 1998)	No change (Wittmann et al., 2009)
Nociception	Increase of thermal and inflammatory pain (Wang et al., 2001); lack of stress induced analgesia (McLaughlin et al., 2003)	No change except hyperalgesia in writhing test (Simonin et al., 1998; Larsson et al., 2008); increase in pain response in neurogenic inflammation model (Schepers et al., 2008)	No change (Gardell et al., 2002; McLaughlin et al., 2003); hypoalgesia in flinch test (Wittmann et al., 2009)
Open field	Reduction of anxiety like behaviour in all test parameters (Wittmann et al., 2009)		Reduction of anxiety like behaviour in all test parameters (Wittmann et al., 2009); no effect (Knoll et al., 2007)
Elevated plus maze	Reduction of anxiety like behaviour in all test parameters (Wittmann et al., 2009)	No change (Simonin et al., 1998; Filliol et al., 2000)	Reduction of anxiety like behaviour in all test parameters (Wittmann et al., 2009; Knoll et al., 2007); tendency

Test paradigm	pDyn KO mice	KOP KO mice	KOP antagonists (mice and rats)
			towards decrease of anxiety related test parameters (Marin et al., 2003; Marco et al., 2005)
Elevated O-maze	Increase of anxiety like behaviour in one test parameter (Bilkei-Gorzo et al., 2008)	No change (Simonin et al., 1998)	
Light-dark	Reduction of anxiety like behaviour at 150 + 400 lux (Wittmann et al., 2009); no change at 1000 lux (Bilkei-Gorzo et al., 2008)	No change (Filliol et al., 2000)	
Tail suspension	Prolongation of immobility (depression like behaviour) in naive animals, no change in pre-stressed mice (Wittmann et al., 2009)		
Forced swim test	Reduction of immobility (depression like behaviour) at 30°C (McLaughlin et al., 2003); minor increase of immobility at 23°C (Wittmann et al., 2009)	No change (Filliol et al., 2000)	Reduction of immobility (depression like behaviour) (Mague et al., 2003; McLaughlin et al., 2003; Zhang et al., 2007; Reindl et al., 2008); no change (Fichna et al., 2007)
Stress induced hyperthermia	Minor increase after 20 min (Bilkei-Gorzo et al., 2008); no change after 10 min (Wittmann et al., 2009; Bilkei-Gorzo et al., 2008)		
Corticosterone serum levels	No change at baseline, but altered time course after stress (Bilkei-Gorzo et al., 2008); reduction of baseline and stress induced levels (Wittmann et al., 2009)		No change at baseline (Marco et al., 2005)
Alcohol consumption	Reduction (Blednov et al., 2006)	Reduction (Kovacs et al., 2005)	Reduction (for review see Shippenberg et al., 2007)

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Abbreviations

ACTH adrenocorticotrophic hormone

CRE	cAMP responsive element
CREB	CRE binding protein
CRH	corticotropin-releasing hormone
CSF	cerebrospinal fluid
DOP	delta opioid receptor
DRE	downstream regulatory element
DREAM	DRE antagonist modulator
Dyn	dynorphins
DYN	human dynorphins
Dyn/KOP	dynorphin - kappa opioid receptor system
HPA	hypothalamic-pituitary-adrenal axis
KO	knockout
KOP	kappa opioid receptor
LTP	long term potentiation
MAP	p38 mitogen-activated protein
MOP	mu opioid receptor
NMDA	N-Methyl-D-Aspartat
NOP	nociceptin receptor
norBNI	norbinaltorphimine
NPY	neuropeptide Y
PC	prohormone convertase
pDyn	prodynorphin
pDyn	prodynorphin gene
pDYN	human prodynorphin
PTX	pertussis toxin
SNP	single nucleotide polymorphism

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Figure 1. Biosynthesis of pDYN derived peptides

The entire coding sequence of pDYN is contained in exons 3 and 4 (dark grey shading) of the pDYN gene. Several differentially spliced transcripts are derived from this single gene, however only the two full-length mRNAs FL1 and FL2 are found in humans and rodents. These two splice variants differ only in the 5'-non-coding region with FL2 being transcribed from an extended exon 2 (light grey). An identical 254-amino acid preprodynorphin is translated from both mRNAs. The first 20 amino acids represent the signal peptide, responsible for targeting the protein towards the endoplasmic reticulum. This peptide is immediately cleaved by the signal peptidase, resulting in pDYN. Further processing is differentially regulated in distinct brain regions, resulting in pDYN as well as mature peptides in axon terminals. Maturation is dependent on prohormone convertases PC1 and PC2. Processing of the mature peptides at the paired arginine residues yields Leu-enkephalin from β-neoendorphin, DYN A and DYN B.

Figure 2. Potential sites of Dyn/KOP interactions in the hippocampus

Dyn immunoreactivity is observed mainly in axons of granule cells (gc) termed mossy fibers (mf). Dyn released from these axons may target presynaptic KOP on mossy fibers, either as autoreceptor or on axon collaterals. Postsynaptic KOP on hilar somatostatin - neuropeptide Y - GABA interneurons, or on dendrites of CA3 pyramidal neurons represent another pool of targets. Also the dendrites of granule cells contain large dense core vesicles loaded with Dyn. Dendritic release in the molecular layer (ml) may target presynaptic KOP on perforant path (pp) terminals, or on postsynaptic KOP located on granule cell dendrites in the inner and outer molecular layer. In humans, also perforant path fibers were shown to contain Dyn. These fibers innervate the molecular layer of the dentate gyrus (DG), but also dendrites of CA1 pyramidal neurons. In the inner molecular layer of the dentate gyrus presynaptic KOP on terminals of supramammillary projections were shown in guinea pigs. a...alveus; so...stratum oriens; sr...stratum radiatum; sml...stratum lacunosum moleculare.

Figure 3. Potential sites of Dyn/KOP interactions in emotional control

pDyn mRNA and Dyn peptides are widely distributed in brain nuclei involved in emotional control. For KOP, there is a certain mismatch of mRNA and binding sites, suggesting axonal transport of the receptor mainly in serotonergic and mesolimbic dopaminergic projections. Presynaptic KOP on these axons was also suggested from pharmacologic studies. Most other forebrain nuclei involved in emotional control display both, Dyn and KOP labeling, supporting a strong influence of Dyn on these circuits. Not all projections and tracts are clearly identified, but presently the suppression of dopamine release by KOP activation from VTA - NAc projections appears highly important in depression and addiction. Dyn controls dopamine release from these fibers potentially at least in part through presynaptic KOP autoreceptors. Serotonin release from fibers originating in the dorsal raphe is stimulated by MOP and decreased by KOP, which was made responsible for effects on anxiety like behavior. The noradrenergic innervation of the forebrain is also controlled through Dyn, mainly directly in the locus ceruleus. This includes postsynaptic KOP on noradrenergic neurons, but also presynaptic KOP on excitatory fiber terminals in the locus ceruleus. KOP acting as autoreceptors were described on granule cell dendrites in the molecular layer of the hippocampus. This position allows them to regulating the excitability of the main hippocampal input structure, the granule cell layer. Also on dendrites of arginine-vasopressin expressing neurons in the supraoptic nucleus autoreceptors were observed, most probably being responsible for the opioid induced reduction of arginine-vasopressin and oxytocin release. Somatodendritic autoreceptors are targeted by somatodendritically released Dyn. Acb...nucleus accumbens; Amy...amygdaloid complex; Arc...arcuate hypothalamic nucleus; Ce...central amygdaloid nucleus; CPu...caudate putamen (striatum); DR...dorsal raphe nucleus; Hipp...hippocampus; LRt...lateral reticular nucleus; PFC...prefrontal cortex; Pit...pituitary gland; PVN...paraventricular hypothalamic nucleus; Sept...septum; SO...supraoptic nucleus; Sol...nucleus of the solitary tract; VTA...ventral tegmental area;