Contribution of Delta-Opioid Receptors to Pathophysiological Events Explored by Endogenous Enkephalins

Bernard P. Roques

Contents

Abstract

Very few discoveries in the neurosciences have triggered clinical speculation and experimentation regarding the etiology of psychiatric illness to the same extent as that following identification of the opiate receptor(s) and subsequent isolation of endogenous morphine-like peptides. There is overwhelming evidence in animals and in human that opioids are involved in behaviorally relevant issues such as the modulation of pain, the response to stress, motivation, addiction, sexuality, food intake, etc., but our knowledge on the possible relation between opioids and mental illness is still very limited.

These responses could be explored either by using highly selective delta agonist or by emphasizing the effects of phasically secreted endogenous opioid peptides, enkephalin. Both approaches were investigated in particular through protection of enkephalin degradation by dual enkephalinase inhibitors DENKIs such as RB101, PL37 or PL265.

The original version of this chapter was revised. A correction to this chapter is available at https://doi.org/10.1007/978-3-319-95133-1_1001.

B.P. Roques (✉)
Membre de l'Académie des Sciences (France et Europe), U1022 INSERM/UMR 8258 CNRS, Université Paris-Descartes (Paris V), CSO Pharmaleads SAS, 4 Avenue de l'Observatoire, 75006 Paris, France
e-mail: bernard.roques@parisdescartes.fr; bernard.roques@pharmaleads.com

E. M. Jutkiewicz (ed.), *Delta Opioid Receptor Pharmacology and Therapeutic Applications*, Handbook of Experimental Pharmacology 247, https://doi.org/10.1007/164_2016_17

Keywords
Synthetic opioids • Endogenous opioids peptides • Enkephalin • Enkephalinases • Aminopeptidase inhibition • DENKIs, Dual enkephalinase inhibitor • Dopamine • Depression

The apparent influence of endogenous opioids on the regulation of behavior has led to considerable efforts to associate alterations in opioidergic systems with psychiatric symptoms. The hypothesized associations have been investigated basically by three different methods. First, the effect of a selective opioid agonist or its antagonist on behavior is observed in normal volunteers as well as in psychiatric patients. The substance is usually administered intravenously or orally. The second type of study involves the determination of opioid concentrations in the CSF or serum of psychotic patients. The third method was to investigate the behavioral effects of increasing the level of endogenous opioids secreted by a stimuli in a particular brain structure though their physiological protection of degradation by specific degrading enzymes.

The biological effects of endogenous opioid peptides are mediated through three classes of naloxone-sensitive opioid receptors: mu (μ), kappa (k), and delta (∂). Each receptor is stimulated preferentially by specific endogenous peptides (Met-enkephalin, Leu-enkephalin, and β-endorphin) for the μ- and ∂-receptors. These endorphins are generated from maturation of long gene precursor called preproenkephalin for Met- and Leu-Enks, pre-proopiomelanocortin for β-endorphin, whereas dynorphin, the agonist of k-opioid receptor, is issued from preprodynorphin.

Several lines of evidence have suggested a role for opioid receptor systems in depression, including early studies investigating the potential antidepressant therapy of endogenous opioid peptides in humans. For example, it was shown that basal serum β-endorphin (an endogenous opioid peptide that binds to μ- and ∂-opioid receptors) levels were significantly elevated in patients with depression after antidepressant treatment (Darko et al. 1992; Djurovic et al. 1999). It was also reported that plasma β-endorphin levels were found to be elevated in patients after electroconvulsive shocks (ECS) for the treatment of depression, suggesting that endogenous μ- and/or ∂-opioid receptors were involved, at least in part, in the mechanisms of the ECS antidepressant activity (Emrich et al. 1979; Inturrisi et al. 1982). Consistently β-endorphin was reported to produce rapidly an antidepressant action in depressed patients (Kline et al. 1977). Some clinical reports also demonstrated the effectiveness of the μ-opioid receptor agonists oxycodone, oxymorphone, and buprenorphine in patients with refractory major depression (Bodkin et al. 1995; Stoll and Rueter 1999). Conversely, it was indicated that the nonselective opioid receptor antagonist naltrexone induced self-reported mental depression to volunteer subjects, in a placebo-controlled open study (Hollister et al. 1981). Taken together, these findings suggest that the endogenous opioid

Table 1 Affinity and selectivity for μ- and ∂-opioid binding sites of sterically constrained cyclic and linear enkephalins

	KI(∂) nM	KI(μ) nM	
Compounds	[^{3}H]DSTBULET	[^{3}H]DAGO	μ/∂
Tyr-D-Thr-Gly-Phe-Leu-Thr, DTLET	1.61	25	16
Tyr-D-Pen-Gly-Phe-D-Pen, DPDPE	8.85	993	110
Tyr-D-Ser(OtBu)-Gly-Phe-Leu-Thr, DSTBULET	2.81	374	130
Tyr-D-Ser(OtBu)-Gly-Phe-Leu-Thr(OtBu), BUBU	1.69	480	280
Tyr-D-Cys(OtBu)-Gly-Phe-Leu-Thr(OtBu), BUBUC	2.90	2,980	1,020
Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2, Deltorphin	2.40[a]	1,630[a]	679
Diallyl-Tyr-Aib-Aib-Phe-Leu, ICI 174,864	311	29,200	94
Naltrindole, NTI	1.30	74	57

[a]Using different radioligands

systems have important roles in depression, but clinical demonstration using a well-characterized exogenous opiate is still expected.

The ∂-opioid receptor was cloned in the 1990s (Evans et al. 1992; Kieffer et al. 1992). It was reported that these ∂-opioid receptors were located in the olfactory bulb, cerebral cortex, striatum, amygdala, hippocampus, brainstem nuclei, and spinal cord in rodents (Erbs et al. 2015; Pradhan et al. 2011; Le Merrer et al. 2009; Delay-Goyet et al. 1990). Madar et al. (1996) suggest that the distribution pattern of ∂-opioid receptors in the human brain using [^{11}C]-methyl-naltrindole was partially consistent with the location of the major regions involved in the modulation of mood and emotion (Madar et al. 1996). Interestingly, Filliol et al. (2000) found that ∂-opioid receptor knockout mice exhibited increases in the immobility times in the forced swimming test. This finding also suggests that the endogenous ∂-opioid receptor systems significantly contribute to the regulation of mood and emotion confirming the early results obtained in rodent using well-adapted behavioral tests and the first designed selective ∂-agonists (Table 1) (review in Roques et al. 1993).

Moreover, there are two different strategies to modulate the responses elicited by a neuropeptide. The classical approach is to use exogenous agonists that ubiquitously stimulate the peptide receptor(s), a strategy that is thought to be associated with serious drawbacks related to overstimulation of receptors (e.g., morphine). A more physiological approach is to modulate the extracellular concentrations of endogenous peptide effectors by inhibiting their metabolizing enzymes (Roques et al. 2012).

The disadvantages of peptidase inhibitors compared with exogenous agonists or antagonists could be their lower pharmacological potencies. However, this disadvantage is compensated for by their more physiological effects that correlate with the phasic release of their peptide substrates in brain structures recruited by a particular stimulus (e.g., pain, stress, or emotion) and the absence or small change in either the secretion of the peptide or expression of its targets (e.g., metabolizing enzymes and receptors).

We have privileged this approach (review in Roques et al. 2012) for the main endogenous opioid peptides enkephalins in designing potent inhibitors of the two degrading peptidases involved in the interruption of the message conveyed by the two peptides. These two enzymes, neutral endopeptidase (NEP or neprilysin) and aminopeptidase N (APN), belong to the class of zinc metallopeptidases (Roques et al. 2012).

1 Structure, Biological, and Pharmacological Properties of Dual Inhibitors of Enkephalinase (DENKIs)

Taking into account the substantial similarities in the active sites of zinc metallopeptidases (Fournie-Zaluski et al. 2009; Oefner et al. 2004; Marie-Claire et al. 2000; Tiraboschi et al. 1999), the rational design of potent selective or dual inhibitors of NEP and APN (Roques et al. 1993; Noble and Roques 2007; Roques 2000; Fournie-Zaluski and Roques 2002) has led to the selection of molecules that contain a strong metal-coordinating group (e.g., a thiol, carboxyl, hydroxamate, or phosphinic group) and are able to satisfy all possible energetically favorable interactions with at least one of the S1–S2′ subsites surrounding the catalytic site, as evidenced by inhibitor co-crystallization (Fournie-Zaluski et al. 2009; Oefner et al. 2004) (reviewed in references Noble and Roques 2007; Thanawala et al. 2008; Roques 2000; Mina-Osorio 2008; Fournié-Zaluski and Roques 2002).

The first DENK inhibitors (Fig. 1) were designed in 1984 (Fournie-Zaluski et al. 1984) using the hydroxamate group as a zinc-chelating moiety, assuming that the strength of its coordination to the metal should counterbalance a "less-than-perfect" fit of the inhibitor side chains to the active sites of the two metallopeptidases (Roques et al. 1993) that are obviously not identical (Fournie-Zaluski et al. 2009; Oefner et al. 2004). Accordingly, kelatorphan strongly inhibits NEP ($IC_{50} = 1.8$ nM) and less efficiently inhibits APN ($IC_{50} = 380$ nM).

Kelatorphan was the first compound that completely inhibited enkephalin catabolism (Bourgoin et al. 1986). It had antinociceptive effects in numerous acute nociceptive animal models (Fournie-Zaluski et al. 1984, 1985), and after intrathecal administration, it induced longer-lasting analgesia in patients with cancer (M.C. Fournié-Zaluski and J. Meynadier, unpublished observations) than the combination of both bestatin and thiorphan (Meynadier et al. 1988). Kelatorphan was also active in complete Freund's adjuvant-induced arthritis in rats, a widely used model of chronic pain (Kayser et al. 1989; Perrot et al. 1993), and it reduced nociception by 60% in mononeuropathic rats (Kayser et al. 1989; Lee et al. 1994). The entrance of kelatorphan into the brain is very limited, and therefore, the analgesic effects observed in arthritic rats are assumed to be due to a peripheral effect at the level of injured tissues (Maldonado et al. 1994).

Due to its very weak ability to enter the brain, kelatorphan is not active in antidepressant tests. This is also the case with other class of DENKIs in which the chelating group is a phosphinic moiety (Fig. 1). Thus, these compounds such as in

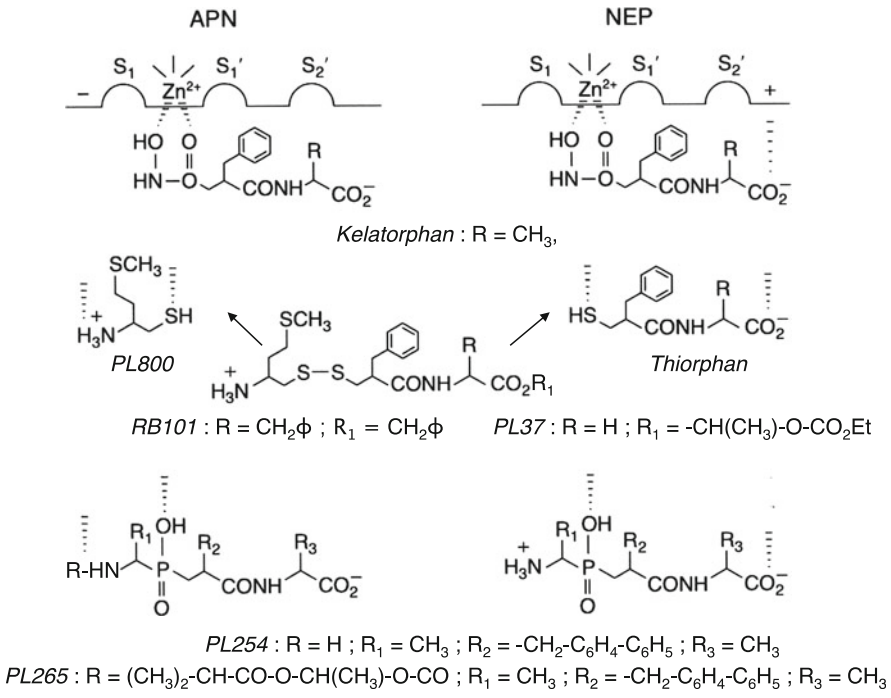

Fig. 1 Schematic inhibition of the two zinc metallopeptidases: aminopeptidase N (APN) and neprilysin (NEP) by the three different families of inhibitors

PL264 which have nanomolar NEP and APN inhibitory potency were shown to be active against neuropathic pain at the peripheral level (Poras et al. 2014).

Nevertheless, even after introduction of hydrophobic protecting group as in PL265, these phosphinic DENKIs have a very weak tendency to cross the blood-brain barrier and could not be used as putative antidepressants. In contrast, as easily shown with RB101, the DENKIs releasing in the brain (Fournie-Zaluski et al. 1992) the two highly selective APN and NEP inhibitors are able to diffuse easily in brain structures as demonstrated by autoradiographic studies (Jardinaud et al. 2004) and are the most interesting dual inhibitors for studying the role of ENKs in the control of mood. Therefore, the orally active DENKI PL37 (Fig. 1) which is in clinical trials (at this time phase II) to reduce neuropathic pain and which is about 10 times more potent by *i.v.* route than RB101 (Benoist et al. 2002) warrants an investigation in depressive disorders.

Several types of dual enkephalinase inhibitors (DENKIs) have been synthesized (Fig. 1) depending on the structure of the zinc-chelating moiety. Two families are able to recognize and inhibit directly NEP and APN with nanomolar affinity (i.e., kelatorphan or phosphinic inhibitors), while another family is able to form to two highly selective and potent inhibitors of NEP and APN issued from the cleavage of the disulfide bond (Roques et al. 2012).

In addition, potent and selective peptidic delta agonists (DSLET, Tyr-D-Ser-Gly-Phe-Leu-Thr and DTLET) derived from enkephalins and endowed with good oral bioavailability for some of them (BUBU and BUBUC) have been synthesized (Table 1).

Chronic application of various mild stress has been shown to decrease the responsiveness to reward in rats. This effect, which was suggested to mimic anhedonia, one of the main symptoms observed in depressive patients, can be measured by various tests. Thus, chronic mild stress (CMS) was shown to reduce the consumption of a palatable sucrose solution and to decrease the acquisition of preferences for a distinct environment paired with a variety of reinforcing substances (Muscat and Willner 1992; Papp et al. 1993). These negative responses could be prevented by chronic treatment with tricyclic or atypical antidepressants (Smadja et al. 1995; Willner et al. 1987). The behavioral changes, induced by exposure to chronic mild stress, were shown to be associated with a number of changes in dopaminergic neurotransmission in the mesolimbic system, especially in the nucleus accumbens (Stamford et al. 1991). The nucleus accumbens contains a large number of enkephalinergic cell bodies giving rise to local collaterals and axons projecting to the globus pallidus-ventral pallidum region (for review see Groenewegen et al. 1991). Furthermore, there is evidence that this structure is instrumental in mediating the reward effects of exogenous and endogenous opioids (for reviews see Bozarth 1991; Dauge et al. 1992; Scheel-Krüger and Willner 1991). With the aim to study the possible contribution of the enkephalinergic system in the anhedonia-like state induced by chronic mild stress, microdialysis was used to measure the extracellular levels of [Met]enkephalin-like material in the rostral part of the nucleus accumbens (N-Acc) of freely moving rats exposed or not to chronically mild stress (Bertrand et al. 1997). In both groups, the basal levels of [Met]enkephalin-like material (Met-LI) were found to be similar. Exposure of the two groups to a newly introduced rat (a stressful situation) leads to increased extracellular levels of [Met]enkephalin in the controls but not in chronic mild-stressed rats. A likely explanation for the lack of effect of the fearful social confrontation on the extracellular levels of Met-LI in CMS rats could be that the stress procedure rendered the enkephalinergic system, located in the N-Acc, unable to adapt to a threatening stimulus.

It has been shown that mice with a disruption of the preproenkephalin gene displayed a reduction in locomotor activity and anxious and aggressive behavior (Konig et al. 1996). Furthermore, numerous studies, using inhibitors of enkephalins catabolism, have shown that endogenous enkephalins induce antidepressant-like effects in animal models of “depression” such as the learned helplessness, the conditioned suppression of motility test, and the Porsolt assay (Baamonde et al. 1992; Tejedor-Real et al. 1995). Thus, RB101 has also shown anxiolytic effects mainly through DOR stimulation (Nieto et al. 2005; Jardinaud et al. 2005; McNally 2005) as they remain present in MOR KO mice (Noble and Roques 2007; Nieto et al 2005). Consistent with these results, PENK KO mice exhibit anxiogenic responses, increased aggressiveness (Konig et al. 1996; Ragnauth et al. 2001), stronger anxiety, and depressive posttraumatic stress disorder (Kung et al. 2010).

A role of endogenous enkephalins in the reward process is supported by the efficiency of naloxone (injected *i.p.* or in the N-Acc.) to suppress the preferential consumption of sucrose solution by rats and to block the reinforcing effect of a sucrose solution in a place preference paradigm (Agmo et al. 1995). However, in the CMS paradigm, chronic morphine administration was reported to reverse anhedonia the first and second weeks of stress procedure, while chronic treatment with the dual inhibitor of enkephalin-degrading enzymes, RB101, failed to reproduce this effect (Smadja et al. 1995). A plausible explanation could be that the CMS procedure induces a subsensitivity of the opioid receptors rendering them unable to respond to the weak increase in enkephalin levels produced by the inhibitor. This assumption is compatible with a dysfunctioning of the dopaminergic system observed in the CMS rats. In the N-Acc, D_2 receptors are mainly located on enkephalinergic neurons, and dopamine was reported to reduce their functioning (Le Moine and Bloch 1995). Therefore, the persistent enhancement of dopamine release in the mesolimbic system and the subsensitivity of D_2 receptors (Papp et al. 1993; Stamford et al. 1991), induced by the CMS, would decrease the enkephalin-controlled reward.

However, this could also explain the lack of increased level of enkephalins in the N-Acc in animal CMS faced with a threatening situation. It is interesting to observe that the inability to respond to emotional stimuli is one of the syndromes observed in depressive states.

Most studies on the etiology of depression have been devoted to the role of catecholamines, whereas the possible involvement of peptides, especially opioids, has been poorly investigated, partly because of the lack of appropriate tools. The study with CMS of Bertrand et al. (1997) shows that the association of microdialysis techniques and pharmacological experiments should allow this new direction to be explored in particular by using the numerous recently synthesized systematically active non-peptide selective delta agonists (Saitoh and Yamada 2012).

2 Mutual Role of Dopaminergic and Endogenous Opioid Signaling in Behavioral Control

High densities of both opioid receptors and NEP are found in the nucleus accumbens and the caudate nucleus (Waksman et al. 1986; Kieffer et al. 1992), forebrain structures that are involved in emotional, cognitive, and motor functions and receive a rich innervation from dopaminergic neurons located in the VTA and substantia nigra. Various pharmacological and biochemical studies have also shown that morphine and enkephalins are involved in the control of behavior such as arousal, locomotion, self-administration, self-stimulation, learning, and memory functions through modulation of the motor (nigrostriatal) and limbic cortical (mesocorticolimbic) dopaminergic systems.

Chronic administration of antidepressant drugs increases Met-enkephalin-like immune reactivity in the striatum and N-Acc of rat brain (De Felipe et al. 1985). A

similar increase has also been observed after electroconvulsive shock or following chronic administration of lithium (Staunton et al. 1982). Moreover, chronic haloperidol treatment increases the levels of both the D2 receptor and preproenkephalin mRNA (Le Moine et al. 1991).

Several pharmacological studies have been carried out to clarify the role of opioids on dopaminergic systems. Moreover, stimulation of ∂-opioid receptors in the VTA by local injection of the selective agonists DSTBULET, DTLET, BUBU, or an inhibitor of enkephalin catabolism (kelatorphan) induced hyperactivity in familiar (home cage) or unfamiliar (open-field and four-hole box) environments. These effects were suppressed by a ∂-selective antagonist. The μ-agonist DAMGO also increased locomotion in the actimeter but decreased the activity in the open-field and four-hole box tests, possibly reflecting an increase in emotion and fear (Calenco-Choukroun et al. 1991a). The differences in the responses induced by kelatorphan or ∂-agonists with those produced by DAMGO suggest that μ- and ∂-receptors are involved in different neuronal pathways in the VTA. This is supported both by the association of only some enkephalinergic terminals with tyrosine hydroxylase-containing neurons in the rat VTA (Sesack and Pickel 1992) and by a study in which 6-hydroxydopamine-induced lesions of the rat mesoaccumbens pathway were found to abolish the effects of kelatorphan or BUBU in the VTA but not those elicited by the μ-agonist DAMGO (Calenco-Choukroun et al. 1991b). Taken together these results show that the endogenous enkephalins preferentially bind to ∂-receptors to induce hyperactivity. The conditions under which they could activate μ-receptors remain an open question.

The complexity of the interactions between dopaminergic and opioidergic systems in the N-Acc is illustrated by the results of 6-hydroxydopamine-induced lesions of the dopaminergic neurons of the VTA and chronic neuroleptic treatment, both of which potentiate the behavioral effects of exogenous opioids (Stinus et al. 1985, 1986; Kalivas and Bronson 1985) or kelatorphan (Maldonado et al. 1990b) infused into the nucleus accumbens. In agreement with the presence of D2 receptors on the forebrain enkephalin neurons, D2-dopamine receptor antagonists, such as sulpiride, or the mixed D1–D2 antagonist haloperidol, but not the D1 antagonist SCH23390, have been found to facilitate the opioid-related behavioral effects induced by kelatorphan (Maldonado et al. 1990).

Because both the enkephalinergic and dopaminergic systems in the N-Acc appear to function in parallel to increase locomotor activity (Kalivas et al. 1983), the supersensitivity of the opioid system after long-term dopaminergic blockade could be interpreted as a homeostatic mechanism to maintain normal locomotor activity. Moreover, the behavioral supersensitivity to endogenous opioids protected by kelatorphan in the N-Acc appears to be maximal after 2–3 weeks. This delay corresponds to the first appearance of the antipsychotic effects of neuroleptics, suggesting that alterations in the opioidergic system, very likely through its interrelations with the dopaminergic pathway, could be taking place in a neuronal system critically involved in the control of mood (Roques et al. 1985; MacLennan and Maier 1983; Kennedy et al. 2006). Kelatorphan also produces the same pattern

of increase in brain stimulation or locomotor activity by i.c.v. administered amphetamine and ∂-opioid agonists (Heidbreder et al. 1988).

A link between opioidergic and dopaminergic systems has also been demonstrated by the clear antidepressant-like effects observed in the forced swimming and suppression of mobility test following i.v. administration of the systemically active mixed inhibitor RB101 in mice. These effects, which were shown to be related to ∂-receptor and D1 receptor activation, produced an increase in dopamine turnover in the striatum (Baamonde et al. 1992).

Injection of DTLET or kelatorphan into the rat striatum increased locomotor activity (Roques et al. 1985; Dauge et al. 1988). This effect was reversed by the dopamine antagonist thioproperazine and could be related to a specific D2-induced increase in the spontaneous and K+ -induced release of newly synthesized striatal dopamine (Petit et al. 1986; Daugé et al. 1989).

The tonic inhibition of the striatal opioid neurons by the nigrostriatal dopaminergic input suggests that under normal conditions, dopamine release is under the control of ∂-receptors, tonically stimulated by endogenous enkephalins (Petit et al. 1986). Because haloperidol was shown to increase the expression of the striatal D2 receptors located on enkephalins neurons (Le Moine et al. 1991), the tardive dyskinesia syndrome induced by long-term treatment with neuroleptics might be, at least partially, due to excessive μ (akinesia) and ∂ (tremor) effects induced by disinhibition of the enkephalinergic neurons normally negatively controlled by the dopaminergic input. The molecular events that control the relationships between these interactions, however, are still largely unknown, and it could be interesting to study the effects of dual NEP/APN inhibitors.

The interrelationships between the opioidergic and dopaminergic systems in the mesocorticolimbic and nigrostriatal pathways provide strong support for a crucial role of endogenous opioids in the control of mood. Amphetamine enhances the release of central dopamine, and chronic use of this drug results in psychotic symptoms resembling schizophrenia. Several recent studies have shown a cross-sensitization among amphetamine, stressful stimuli, and kelatorphan. This suggests that a hypersecretion of endogenous opioid peptides in the mesocorticolimbic pathway could induce an exaggerated behavioral response to stressful environmental stimuli, whose repetition could induce psychotic symptoms (MacLennan and Maier 1983). Conversely, depression might result from a deficiency in enkephalin release, minimizing their rewarding and euphorogenic effects. Likewise, drug abuse could be caused by a deficiency in the internal opioid-controlled rewarding system.

According to their mechanism of action, the DENKIs increasing the extrasynaptic concentration of endogenous enkephalins by protecting them from degradation increase the pharmacological responses generated by the amounts of non-protected peptides release under the action of specific stimuli. This has been put to use to design DENKIs leading very efficient analgesic properties to relieve pain induced by very strong nociceptive stimuli, for example, by excess of nociception and various types of neuropathic pain (review in Roques et al. 2012).

However, another interest of this physiological manipulation of the opioidergic system is the demonstration of its very efficient association with compounds acting on the same diseases but through a different mechanism of action. These synergetic analgesic responses against cancer pain as well as various neuropathic pain were obtained by the association of various DENKIs such as PL37 and PL265 with clinically used antiepileptics such as gabapentinoids (Menendez et al. 2008; Gonzalez-Rodriguez et al. 2009; Bonnard et al. 2015) or cannabinoids agonists (Gonzalez-Rodriguez et al. 2015, in preparation).

Another interesting observation has been the very strong synergistic increase of morphine analgesia induced by its association with the DENKIs RB101 (Mas-Nieto et al. 2001) allowing to reduce by a factor 7 the efficient dose of morphine. All these results drove us to investigate the interest of associating a classical dopaminergic antidepressant such as amisulpride and the dual NEP/APN inhibitor RB101 (Cordonnier et al. 2005).

3 The Mutual Influence of Exogenous and Endogenous Opioids on the Dopaminergic Functions (Dauge et al. 1992; Di Chiara and Imperato 1988; Wood et al. 1980)

The interaction between both systems was also demonstrated by administration of more or less selective dopamine antagonists. Chronic administration of haloperidol increased both the synthesis of endogenous enkephalins in the rat striatum and, to a lesser extent, in the nucleus accumbens (Hong et al. 1978) and the preproenkephalin mRNA expression in the rat striatum and pituitary (Jaber et al. 1994; Normand et al. 1987, 1988). Whereas many studies investigated the interaction between the two systems using exogenous opioid ligands, few studies have been devoted to the regulation of the endogenous opioid system following chronic treatment with neuroleptics. As previously discussed, the best way to achieve this aim is to protect the endogenous opioids from enkephalinase-controlled inactivation. This process occurs after synaptic release and in structures only recruited by specific stimuli. Amisulpride, an antipsychotic, was selected because of its low propensity to induce extrapyramidal side effects, avoiding motor disturbances that could induce bias in behavioral tests in particular following chronic treatments.

This study has been focused on behaviors in which RB101 had already been proved to be efficient in mice, i.e., analgesia in the hot-plate test (Noble et al. 1992) and antidepressant-like effects in the forced swim test and locomotor activity (Baamonde et al 1992).

A strong potentiation of RB101-induced hyperlocomotor effects was observed after 3 weeks of treatment with amisulpride. This result reinforces previous studies showing that chronic administration of neuroleptics sensitized the enkephalinergic system, potentiating the locomotor activity induced by the dual inhibitor of enkephalin catabolism, kelatorphan (Maldonado et al. 1990). However, whereas in this previous study using sulpiride or haloperidol it was clearly demonstrated that

3 weeks of treatment was necessary to achieve these potentiating effects on opioid responses, we observed that 5 days of treatment with amisulpride were sufficient. This suggests a more rapid onset of action of this latter compound compared with other neuroleptics. This duration of treatment was retained for the remainder of the study. It is interesting to notice that the effect of a 5-day treatment could still be seen even after 3 days of withdrawal suggesting that this treatment has durable effects.

Whereas no potentiation of the analgesic effects of RB101 by the amisulpride 5-day treatment was found in the hot-plate test, its hyperlocomotor and antidepressant-like effects were potentiated as observed in locomotor activity recording and forced swim test, respectively. Altogether these results suggest that the effects of the association of amisulpride and RB101 could take place more specifically in the nigrostriatal system and the limbic system.

SNC80 (2.5 mg/kg i.p.) alone induced hyperlocomotor and antidepressant-like effects, which were potentiated by a treatment of 5 days with amisulpride. NTI (5 mg/kg s.c.) totally blocked both the effects of SNC80 and SNC80 + amisulpride, demonstrating the specific involvement of delta-opioid receptors in the observed responses.

These results indicate that a chronic treatment with amisulpride potentiates the action of RB101. This effect seems to be restricted to behavioral responses induced by opioids acting on delta-opioid receptors. This could be due to an increase in the density of the delta-opioid receptors or even a sensitization of these receptors. The presence of a high concentration of delta-opioid receptors in brain areas involved in motor and motivational control suggests the existence of selective interactions between opioids and D2 receptors in certain brain regions because of the behaviorally selective effects of the amisulpride + RB101 combination. Thus, a high density of delta-opioid receptors has been found within the limbic system which is known to control emotional responses and reward behavior (Mansour et al. 1988), and amisulpride has also a preferential action on the limbic system (Moller 2003). Another hypothesis could be an enhancement in the synthesis of preproenkephalins and an increase in the release of enkephalins, in agreement with previous studies showing an increase of preproenkephalins in mice lacking the dopaminergic D2 receptor (Baik et al. 1995; Maldonado et al. 1997), or after a chronic blockade of dopaminergic neurotransmission with antagonists.

Whatever the explanation, the results suggest an interesting new therapeutic approach in CNS disorders, for example, in the treatment of depression, owing to the implication of the delta receptor in mood regulation.

An extension of the facilitation by chronic treatment with the D2 dopamine antagonist amisulpride (Cordonnier et al. 2005) was used to investigate whether a blockade of the dopaminergic system could lead to a more physiological “opioid substitution” compared with exogenous opioid agonists such as methadone and buprenorphine which are currently used as substitutes in pharmacotherapy of opioid addiction. With these pharmacotherapies, the level of relapse unfortunately remains very high.

The goal was therefore to act on both dopaminergic and opioid systems and, particularly, to investigate whether a blockade of the dopaminergic system could potentiate the endogenous opioid system, leading to a more physiological "opiate substitution" compared with exogenous opioid agonists.

Expression of morphine-induced locomotor sensitization was abolished after combined treatment with amisulpride (20 mg kg^{-1}, i.p.) and RB101 (80 mg kg^{-1}, i.p.), whereas these drugs were not effective when used alone. These results were compared with the effects of amisulpride combined with buprenorphine (0.1 mg kg^{-1}, i.p.) or methadone (2.5 mg kg^{-1}, i.p.) upon morphine-induced behavioral sensitization. Whereas the combination of amisulpride and buprenorphine partially blocked the expression of morphine sensitization, amisulpride + methadone was not effective in this paradigm.

The combination of amisulpride + RB101 appears to be very efficient in blocking the expression of morphine-induced behavioral sensitization. This could reflect a reinstatement of a balance between the function of the dopamine and opioid systems and could represent a new approach in maintenance treatments for opiate addiction.

It is important to notice that the synergetic approach in improving the antidepressant actions of dopaminergic agents by endogenous enkephalins occurs by a concomitant increase in the levels of endogenous enkephalins protected by a DENKI and recruiting selectively delta receptors in structures where the state and syndromes of depression release the opioid peptides.

Finally, recent studies using delta-opioid receptors expressed in GABAergic forebrain neurons (DIx-DOR) (Chu Sin Chung et al. 2015) yielded curiously opposite behavioral responses since both low anxiety was found for Dlx-DOR mice which contrast with the well-known increase in anxiety produced in mice by native DOR knockout (Chu Sin Chung et al. 2015) and DOR antagonist. The dual anxiolytic and anxiogenic roles for DORs open novel perspectives in the area of DOR function and anxiety disorders and warrant investigation of their physiological function by using endogenous enkephalins protected by DENKIs.

In conclusion the development of (1) dual orally active ENK inhibitors with strong analgesic properties and immediate antidepressant effects (Noble and Roques 2007) and (2) delta agonists devoid of side effects may lead to significant improvements in the treatment of depression and mood disorders.

References

Agmo A, Galvan A, Talamantes B (1995) Reward and reinforcement produced by drinking sucrose: two processes that may depend on different neurotransmitters. Pharmacol Biochem Behav 52:403–414

Baamonde A, Dauge V, Ruiz-Gayo M, Fulga IG, Turcaud S, Fournie-Zaluski MC, Roques BP (1992) Antidepressant-type effects of endogenous enkephalins protected by systemic RB 101 are mediated by opioid delta and dopamine D1 receptor stimulation. Eur J Pharmacol 216:157–166

Baik JH, Picetti R, Saiardi A, Thiriet G, Dierich A, Depaulis A, Le Meur M, Borrelli E (1995) Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors. Nature 377:424–428

Benoist JM, Keime F, Montagne J, Noble F, Fournie-Zaluski MC, Roques BP, Willer JC, Le Bars D (2002) Depressant effect on a C-fibre reflex in the rat, of RB101, a dual inhibitor of enkephalin-degrading enzymes. Eur J Pharmacol 445:201–210

Bertrand E, Smadja C, Mauborgne A, Roques BP, Daugé V (1997) Social interaction increases the extracellular levels of [Met]enkephalin in the nucleus accumbens of control but not of chronic mild stressed rats. Neuroscience 80:17–20

Bodkin JA, Zornberg GL, Lukas SE, Cole JO (1995) Buprenorphine treatment of refractory depression. J Clin Psychopharmacol 15:49–57

Bonnard E, Poras H, Nadal X, Maldonado R, Fournie-Zaluski MC, Roques BP (2015) Long-lasting oral analgesic effects of N-protected aminophosphinic dual ENKephalinase inhibitors (DENKIs) in peripherally controlled pain. Pharmacol Res Perspect 3:e00116

Bourgoin S, Le Bars D, Artaud F, Clot AM, Bouboutou R, Fournie-Zaluski MC, Roques BP, Hamon M, Cesselin F (1986) Effects of kelatorphan and other peptidase inhibitors on the in vitro and in vivo release of methionine-enkephalin-like material from the rat spinal cord. J Pharmacol Exp Ther 238:360–366

Bozarth MA (1991) The mesolimbic dopamine system as a model reward system. In: Willner P, Scheel-Krüger J (eds) The mesolimbic dopamine system : from motivation to action. Wiley, New York, pp 301–330

Calenco-Choukroun G, Dauge V, Gacel G, Feger J, Roques BP (1991a) Opioid delta agonists and endogenous enkephalins induce different emotional reactivity than mu agonists after injection in the rat ventral tegmental area. Psychopharmacology (Berl) 103:493–502

Calenco-Choukroun G, Dauge V, Gacel G, Roques BP (1991b) Lesion of dopamine mesolimbic neurons blocks behavioral effects induced by the endogenous enkephalins but not by a mu-opioid receptor agonist. Eur J Pharmacol 209:267–271

Chu Sin Chung P, Keyworth HL, Martin-Garcia E, Charbogne P, Darcq E, Bailey A, Filliol D, Matifas A, Scherrer G, Ouagazzal AM, Gaveriaux-Ruff C, Befort K, Maldonado R, Kitchen I, Kieffer BL (2015) A novel anxiogenic role for the delta opioid receptor expressed in GABAergic forebrain neurons. Biol Psychiatry 77:404–415

Cordonnier L, Sanchez M, Roques BP, Noble F (2005) Facilitation of enkephalins-induced delta-opioid behavioral responses by chronic amisulpride treatment. Neuroscience 135:1–10

Darko DF, Risch SC, Gillin JC, Golshan S (1992) Association of beta-endorphin with specific clinical symptoms of depression. Am J Psychiatry 149:1162–1167

Dauge V, Rossignol P, Roques BP (1988) Comparison of the behavioural effects induced by administration in rat nucleus accumbens or nucleus caudatus of selective mu and delta opioid peptides or kelatorphan an inhibitor of enkephalin-degrading-enzymes. Psychopharmacology (Berl) 96:343–352

Daugé V, Rossignol P, Roques BP (1989) Blockade of dopamine receptors reverses the behavioral effects of endogenous enkephalins in the nucleus caudatus but not in the nucleus accumbens : involvement of ∂ and μ opioid receptors. Psychopharmacology (Berl) 99:168–175

Dauge V, Kalivas PW, Duffy T, Roques BP (1992) Effect of inhibiting enkephalin catabolism in the VTA on motor activity and extracellular dopamine. Brain Res 599:209–214

De Felipe MC, De Ceballos ML, Gil C, Fuentes JA (1985) Chronic antidepressant treatment increases enkephalin levels in n. accumbens and striatum of the rat. Eur J Pharmacol 112:119–122

Delay-Goyet P, Zajac JM, Roques BP (1990) Improved quantitative radioautography of rat brain ∂-opioid binding sites using [(3)H]DSTBULET, a new highly potent and selective linear enkephalin analogue. Neurochem Int 16:341–368

Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 85:5274–5278

Djurovic D, Milic-Askrabic J, Majkic-Singh N (1999) Serum beta-endorphin level in patients with depression on fluvoxamine. Farmaco 54:130–133

Emrich HM, Hollt V, Kissling W, Fischler M, Laspe H, Heinemann H, von Zerssen D, Herz A (1979) beta-Endorphin-like immunoreactivity in cerebrospinal fluid and plasma of patients with schizophrenia and other neuropsychiatric disorders. Pharmakopsychiatr Neuropsychopharmakol 12:269–276

Erbs E, Paget L, Scherrer G, Matifas A, Filliol D, Vonesch JL, Koch M, Kessler P, Hentsch D, Birling MC, Koutsourakis M, Vasseur, Veinante P, Kieffeter BL, Massotte D (2015) A mu-delta opioid receptor brain atlas reveals neuronal co-occurrence in subcortical networks. Brain Struct Funct 220:677–702

Evans CJ, Keith DE Jr, Morrison H, Magendzo K, Edwards RH (1992) Cloning of a delta opioid receptor by functional expression. Science 258:1952–1955

Filliol D, Ghozland S, Chluba J, Martin M, Matthes HW, Simonin F, Befort K, Gaveriaux-Ruff C, Dierich A, LeMeur M, Valverde O, Maldonado R, Kieffer BL (2000) Mice deficient for delta- and mu-opioid receptors exhibit opposing alterations of emotional responses. Nat Genet 25:195–200

Fournie-Zaluski MC, Roques BP (2002) New selective aminopeptidase N inhibitors and potential therapeutic. In: Langner J, Snyder A (eds) Ectopeptidases : CD13/Aminopeptidase N and CD26/Dipeptidyl peptidase IV in medicine and biology. Kluwer Academic/Plenum Publishers, New York, pp 51–94

Fournie-Zaluski MC, Chaillet P, Bouboutou R, Coulaud A, Cherot P, Waksman G, Costentin J, Roques BP (1984) Analgesic effects of kelatorphan, a new highly potent inhibitor of multiple enkephalin degrading enzymes. Eur J Pharmacol 102:525–528

Fournie-Zaluski MC, Coulaud A, Bouboutou R, Chaillet P, Devin J, Waksman G, Costentin J, Roques BP (1985) New bidentates as full inhibitors of enkephalin-degrading enzymes: synthesis and analgesic properties. J Med Chem 28:1158–1169

Fournie-Zaluski MC, Coric P, Turcaud S, Lucas E, Noble F, Maldonado R, Roques BP (1992) "Mixed inhibitor-prodrug" as a new approach toward systemically active inhibitors of enkephalin-degrading enzymes. J Med Chem 35:2473–2481

Fournie-Zaluski MC, Poras H, Roques BP, Nakajima Y, Ito K, Yoshimoto T (2009) Structure of aminopeptidase N from Escherichia coli complexed with the transition-state analogue aminophosphinic inhibitor PL250. Acta Crystallogr D Biol Crystallogr 65:814–822

Gonzalez-Rodriguez S, Pevida M, Roques BP, Fournie-Zaluski MC, Hidalgo A, Menendez L, Baamonde A (2009) Involvement of enkephalins in the inhibition of osteosarcoma-induced thermal hyperalgesia evoked by the blockade of peripheral P2X3 receptors. Neurosci Lett 465:285–289

Gonzalez-Rodriguez S, Menendez L, Lastra A, Baamonde A, Roques BP, Fournié-Zaluski MC, Poras H (2015) Antihyperalgesic effects of PL265, an orally active dual enkephalinase inhibitor (DENKI) in a mice model of tibial bone cancer and synergistic responses when combined with gabapentin, the purinergic antagonist □-317491 or a cannabinoid CB2 agonist. In preparation

Groenewegen HJ, Berendse HW, Meredith GE, Haber SN, Voorn P, Woters JG, Lohman AHM (1991) Functional anatomy of the ventral, limbic system-innervated striatum. In: Willner P, Scheel-Krüger J (eds) The mesolimbic dopamine system: from motivation to action. Wiley, New York, pp 19–59

Heidbreder C, Roques B, Vanderhaeghen JJ, De Witte P (1988) Kelatorphan, a potent enkephalinases inhibitor, presents opposite properties when injected intracerebroventricularly or into the nucleus accumbens on intracranial self-stimulation. Neurochem Int 12:347–350

Hollister LE, Johnson K, Boukhabza D, Gillespie HK (1981) Aversive effects of naltrexone in subjects not dependent on opiates. Drug Alcohol Depend 8:37–41

Hong JS, Yang HY, Fratta W, Costa E (1978) Rat striatal methionine-enkephalin content after chronic treatment with cataleptogenic and noncataleptogenic antischizophrenic drugs. J Pharmacol Exp Ther 205:141–147

Inturrisi CE, Alexopoulos G, Lipman R, Foley K, Rossier J (1982) beta-Endorphin immunoreactivity in the plasma of psychiatric patients receiving electroconvulsive treatment. Ann N Y Acad Sci 398:413–423

Jaber M, Tison F, Fournier MC, Bloch B (1994) Differential influence of haloperidol and sulpiride on dopamine receptors and peptide mRNA levels in the rat striatum and pituitary. Brain Res Mol Brain Res 23:14–20

Jardinaud F, Banisadr G, Noble F, Melik-Parsadaniantz S, Chen H, Dugave C, Laplace H, Rostene W, Fournie-Zaluski MC, Roques BP, Popovici T (2004) Ontogenic and adult whole body distribution of aminopeptidase N in rat investigated by in vitro autoradiography. Biochimie 86:105–113

Jardinaud F, Crete D, Canestrelli C, Ledent C, Roques BP, Noble F (2005) CB1 receptor knockout mice show similar behavioral modifications to wild-type mice when enkephalin catabolism is inhibited. Brain Res 1063:77–83

Kalivas PW, Bronson M (1985) Mesolimbic dopamine lesions produce an augmented behavioral response to enkephalin. Neuropharmacology 24:931–936

Kalivas PW, Widerlov E, Stanley D, Breese G, Prange AJ Jr (1983) Enkephalin action on the mesolimbic system: a dopamine-dependent and a dopamine-independent increase in locomotor activity. J Pharmacol Exp Ther 227:229–237

Kayser V, Fournie-Zaluski MC, Guilbaud G, Roques BP (1989) Potent antinociceptive effects of kelatorphan (a highly efficient inhibitor of multiple enkephalin-degrading enzymes) systemically administered in normal and arthritic rats. Brain Res 497:94–101

Kennedy SE, Koeppe RA, Young EA, Zubieta JK (2006) Dysregulation of endogenous opioid emotion regulation circuitry in major depression in women. Arch Gen Psychiatry 63:1199–1208

Kieffer BL, Befort K, Gaveriaux-Ruff C, Hirth CG (1992) The delta-opioid receptor: isolation of a cDNA by expression cloning and pharmacological characterization. Proc Natl Acad Sci U S A 89:12048–12052

Kline NS, Li CH, Lehmann HE, Lajtha A, Laski E, Cooper T (1977) Beta-endorphin--induced changes in schizophrenic and depressed patients. Arch Gen Psychiatry 34:1111–1113

Konig M, Zimmer AM, Steiner H, Holmes PV, Crawley JN, Brownstein MJ, Zimmer A (1996) Pain responses, anxiety and aggression in mice deficient in pre-proenkephalin. Nature 383:535–538

Kung JC, Chen TC, Shyu BC, Hsiao S, Huang AC (2010) Anxiety- and depressive-like responses and c-fos activity in preproenkephalin knockout mice: oversensitivity hypothesis of enkephalin deficit-induced posttraumatic stress disorder. J Biomed Sci 17:29

Le Merrer J, Becker JA, Befort K, Kieffer BL (2009) Reward processing by the opioid system in the brain. Physiol Rev 89:1379–1412

Le Moine C, Bloch B (1995) D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive mRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. J Comp Neurol 355:426–481

Le Moine C, Normand E, Bloch B (1991) Phenotypical characterization of the rat striatal neurons expressing the D1 dopamine receptor gene. Proc Natl Acad Sci U S A 88:4206–4209

Lee SH, Kayser V, Guilbaud G (1994) Antinociceptive effect of systemic kelatorphan, in mononeuropathic rats, involves different opioid receptor types. Eur J Pharmacol 264:61–67

MacLennan AJ, Maier SF (1983) Coping and the stress-induced potentiation of stimulant stereotypy in the rat. Science 219:1091–1093

Madar I, Lever JR, Kinter CM, Scheffel U, Ravert HT, Musachio JL, Mathews WB, Dannals RF, Frost JJ (1996) Imaging of delta opioid receptors in human brain by N1'-([11C]methyl) naltrindole and PET. Synapse 24:19–28

Maldonado R, Dauge V, Feger J, Roques BP (1990) Chronic blockade of D2 but not D1 dopamine receptors facilitates behavioural responses to endogenous enkephalins, protected by kelatorphan, administered in the accumbens in rats. Neuropharmacology 29:215–223

Maldonado R, Valverde O, Turcaud S, Fournie-Zaluski MC, Roques BP (1994) Antinociceptive response induced by mixed inhibitors of enkephalin catabolism in peripheral inflammation. Pain 58:77–83

Maldonado R, Saiardi A, Valverde O, Samad TA, Roques BP, Borrelli E (1997) Absence of opiate rewarding effects in mice lacking dopamine D2 receptors. Nature 388:586–589

Mansour A, Khachaturian H, Lewis ME, Akil H, Watson SJ (1988) Anatomy of CNS opioid receptors. Trends Neurosci 11:308–314

Marie-Claire C, Tiraboschi G, Ruffet E, Inguimbert N, Fournie-Zaluski MC, Roques BP (2000) Exploration of the S(')(1) subsite of neprilysin: a joined molecular modeling and site-directed mutagenesis study. Proteins 39:365–371

Mas-Nieto M, Pommier B, Tzavara ET, Caneparo A, Da Nascimento S, Le Fur G, Roques BP, Noble F (2001) Reduction of opioid dependence by the CB(1) antagonist SR141716A in mice: evaluation of the interest in pharmacotherapy of opioid addiction. Br J Pharmacol 132:1809–1816

McNally GP (2005) Facilitation of fear extinction by midbrain periaqueductal gray infusions of RB101(S), an inhibitor of enkephalin-degrading enzymes. Behav Neurosci 119:1672–1677

Menendez L, Hidalgo A, Meana A, Poras H, Fournie-Zaluski MC, Roques BP, Baamonde A (2008) Inhibition of osteosarcoma-induced thermal hyperalgesia in mice by the orally active dual enkephalinase inhibitor PL37. Potentiation by gabapentin. Eur J Pharmacol 596:50–55

Meynadier J, Dalmas S, Lecomte JM, Gros C, Schwartz JC (1988) Potent analgesic effects of inhibitors of enkephalin metabolism administered intrathecally to cancer patients. Pain Clin 2:201–206

Mina-Osorio P (2008) The moonlighting enzyme CD13: old and new functions to target. Trends Mol Med 14:361–371

Moller HJ (2003) Amisulpride: limbic specificity and the mechanism of antipsychotic atypicality. Prog Neuropsychopharmacol Biol Psychiatry 27:1101–1111

Muscat R, Willner P (1992) Suppression of sucrose drinking by chronic mild unpredictable stress: a methodological analysis. Neurosci Biobehav Rev 16:507–517

Nieto MM, Guen SL, Kieffer BL, Roques BP, Noble F (2005) Physiological control of emotion-related behaviors by endogenous enkephalins involves essentially the delta opioid receptors. Neuroscience 135:305–313

Noble F, Roques BP (2007) Protection of endogenous enkephalin catabolism as natural approach to novel analgesic and antidepressant drugs. Expert Opin Ther Targets 11:145–159

Noble F, Soleilhac JM, Soroca-Lucas E, Turcaud S, Fournie-Zaluski MC, Roques BP (1992) Inhibition of the enkephalin-metabolizing enzymes by the first systemically active mixed inhibitor prodrug RB 101 induces potent analgesic responses in mice and rats. J Pharmacol Exp Ther 261:181–190

Normand E, Popovici T, Fellmann D, Bloch B (1987) Anatomical study of enkephalin gene expression in the rat forebrain following haloperidol treatment. Neurosci Lett 83:232–236

Normand E, Popovici T, Onteniente B, Fellmann D, Piatier-Tonneau D, Auffray C, Bloch B (1988) Dopaminergic neurons of the substantia nigra modulate preproenkephalin A gene expression in rat striatal neurons. Brain Res 439:39–46

Oefner C, Roques BP, Fournie-Zaluski MC, Dale GE (2004) Structural analysis of neprilysin with various specific and potent inhibitors. Acta Crystallogr D Biol Crystallogr 60:392–396

Papp M, Muscat R, Willner P (1993) Subsensitivity to rewarding and locomotor stimulant effects of a dopamine agonist following chronic mild stress. Psychopharmacology (Berl) 110:152–158

Perrot S, Kayser V, Fournie-Zaluski MC, Roques BP, Guilbaud G (1993) Antinociceptive effect of systemic PC 12, a prodrug mixed inhibitor of enkephalin-degrading enzymes, in normal and arthritic rats. Eur J Pharmacol 241:129–133

Petit F, Hamon M, Fournie-Zaluski MC, Roques BP, Glowinski J (1986) Further evidence for a role of delta-opiate receptors in the presynaptic regulation of newly synthesized dopamine release. Eur J Pharmacol 126:1–9

Poras H, Bonnard E, Dange E, Fournié-Zaluski MC, Roques BP (2014) New orally active dual ENKephalinase inhibitors (DENKIs) for central and peripheral pain treatment. J Med Chem 57:5748–5763

Pradhan AA, Befort K, Nozaki C, Gaveriaux-Ruff C, Kieffer BL (2011) The delta opioid receptor: an evolving target for the treatment of brain disorders. Trends Pharmacol Sci 32:581–590

Ragnauth A, Schuller A, Morgan M, Chan J, Ogawa S, Pintar J, Bodnar RJ, Pfaff DW (2001) Female preproenkephalin-knockout mice display altered emotional responses. Proc Natl Acad Sci U S A 98:1958–1963

Roques BP (2000) Novel approaches to targeting neuropeptide systems. Trends Pharmacol Sci 21:475–483

Roques BP, Dauge V, Gacel G, Fournie-Zaluski MC (1985) Selective agonists and antagonists of delta-opioid receptors and inhibitors of enkephalins metabolism. Potential use in mental illness. In: Shagass C, Josiassen RC, Bridger WH, Weiss KJ, Stoff D, Simpon GM (eds) Biological psychiatry, developments in psychiatry, vol. 7. Elsevier, New York

Roques BP, Noble F, Dauge V, Fournie-Zaluski MC, Beaumont A (1993) Neutral endopeptidase 24.11: structure, inhibition, and experimental and clinical pharmacology. Pharmacol Rev 45:87–146

Roques BP, Fournie-Zaluski MC, Wurm M (2012) Inhibiting the breakdown of endogenous opioids and cannabinoids to alleviate pain. Nat Rev Drug Discov 11(4):292–310

Saitoh A, Yamada M (2012) Antidepressant-like effects of ∂ opioid receptor agonists in animal models. Curr Neuropharmacol 10:231–238

Scheel-Krüger J, Willner P (1991) The mesolimbic system: principles of operation. In: Willner P, Scheel-Krüger J (eds) The mesolimbic dopamine system: from motivation to action. Wiley, New York, pp 559–597

Sesack SR, Pickel VM (1992) Dual ultrastructural localization of enkephalin and tyrosine hydroxylase immunoreactivity in the rat ventral tegmental area: multiple substrates for opiate-dopamine interactions. J Neurosci 12:1335–1350

Smadja C, Ruiz-Taron F, Turcaud S, Roques BP, Maldonado R (1995) Effects induced by endogenous and exogenous opioids in different animal models of depression: modulation by the CCK system. In: Traynor J (ed) Analgesia, vol 1(4–6). INRC Proceedings 1995, pp 742–745

Stamford JA, Muscat R, O'Connor JJ, Patel J, Trout SJ, Wieczorek WJ, Kruk ZL, Willner P (1991) Voltammetric evidence that subsensitivity to reward following chronic mild stress is associated with increased release of mesolimbic dopamine. Psychopharmacology (Berl) 105:275–282

Staunton DA, Deyo SN, Shoemaker WJ, Ettenberg A, Bloom FE (1982) Effects of chronic lithium on enkephalin systems and pain responsiveness. Life Sci 31:1837–1840

Stinus L, Winnock M, Kelley AE (1985) Chronic neuroleptic treatment and mesolimbic dopamine denervation induce behavioural supersensitivity to opiates. Psychopharmacology (Berl) 85:323–328

Stinus L, Nadaud D, Jauregui J, Kelley AE (1986) Chronic treatment with five different neuroleptics elicits behavioral supersensitivity to opiate infusion into the nucleus accumbens. Biol Psychiatry 21:34–48

Stoll AL, Rueter S (1999) Treatment augmentation with opiates in severe and refractory major depression. Am J Psychiatry 156:2017

Tejedor-Real P, Mico JA, Maldonado R, Roques BP, Gibert-Rahola J (1995) Implication of endogenous opioid system in the learned helplessness model of depression. Pharmacol Biochem Behav 52:145–152

Thanawala V, Kadam VJ, Ghosh R (2008) Enkephalinase inhibitors: potential agents for the management of pain. Curr Drug Targets 9:887–894

Tiraboschi G, Jullian N, Thery V, Antonczak S, Fournie-Zaluski MC, Roques BP (1999) A three-dimensional construction of the active site (region 507-749) of human neutral endopeptidase (EC.3.4.24.11). Protein Eng 12:141–149

Waksman G, Hamel E, Fournie-Zaluski MC, Roques BP (1986) Autoradiographic comparison of the distribution of the neutral endopeptidase "enkephalinase" and of mu and delta opioid receptors in rat brain. Proc Natl Acad Sci U S A 83:1523–1527

Willner P, Towell A, Sampson D, Sophokleous S, Muscat R (1987) Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology (Berl) 93:358–364

Wood PL, Stotland M, Richard JW, Rackham A (1980) Actions of mu, kappa, sigma, delta and agonist/antagonist opiates on striatal dopaminergic function. J Pharmacol Exp Ther 215:697–703