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REVIEW ARTICLE

α_2 Adrenoceptor: A Target for Neuropathic Pain Treatment

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Abstract: Neuropathic pain is originated from different alterations of the nervous system. The difficulty of treatment strongly impairs quality of life of affected people. It is associated with severe, chronic sensory disturbances characterized by spontaneous pain, increased responsiveness to painful stimuli and pain perceived in response to normally non-noxious stimuli. The underlying mechanisms are complex and involve both peripheral and central nervous components.The noradrenergic system plays a pivotal role in the control of pain since its widespread distribution in the "pain matrix" representing a valuable therapeutic target. This review focused on the α_2 adrenoceptor subtype modulation as strategy for neuropathic pain relief. Drugs acting as direct α_2 adrenoceptor agonists (clonidine and dexmedetomidine) were analyzed as well as the indirect α_2 adrenoceptor modulators. The overview included norepinephrine reuptake inhibitors (reboxetine, maprotiline), serotonin/norepinephrine reuptake inhibitors (venlafaxine, milnacipran, amitriptyline, duloxetine, bicifadine) and the compounds characterized by a double pharmacodynamic mechanism combining the norepinephrine reuptake inhibition and the μ opioid agonist profile (tramadol and tapentadol). A summary of recent compounds was illustrated.

L. Di Cesare Mannelli

Keywords: α_2 adrenoceptor agonists, α_2 adrenoceptor, neuropathic pain, norepinephrine reuptake inhibitors, norepinephrine, serotonin/norepinephrine reuptake inhibitors, μ opioid agonist/norepinephrine reuptake inhibitors,

1. INTRODUCTION

1.1. Neuropathic Pain and Noradrenergic System

Neuropathic pain (NP) is estimated to afflict millions of people worldwide [1-3]. Many common diseases, injuries, chemotherapy drugs and interventions cause NP by producing lesions in somatosensory pathways in the peripheral or central nervous system [4]. An exaggerated response to non noxious stimuli (referred as allodynia) and an excessive response to noxious or painful stimuli (referred as hyperalgesia) are the common clinical symptoms [5]. The pathological aspects of NP involve a maladaptive response of the nervous system characterized by peripheral and central sensitization as well as by spinal disinhibition [5]. The impairment of the spinal control of pain is strictly related to the dysregulation of the descending pain modulatory system. The descending pathways originate in the midbrain (periaqueductal gray; PAG) and brainstem (rostroventromedial medulla; RVM) and project to the spinal cord dorsal horn, powerfully influencing the modulation of nociceptive information transmitted from the periphery to the brain. The prominent role of this system in reducing nociception has been recognized by Reynolds [6] and Fields and Basbaum [7].

More recently, the role of descending projections in pain facilitation and in the generation of hyperalgesic states has received increased attention. The descending modulatory system recruits norepinephrine (NE) and serotonin (5-HT) as its main transmitters. The role of NE appears to be predominantly inhibitory, while the role of 5-HT appears to be bidirectional, mediating inhibitory as well as excitatory effects [8]. The increased activity of the descending noradrenergic system and high extracellular levels of spinal NE has been linked to antinociceptive/antiallodynic/ antihyperalgesic effects in acute pain and NP. In the brain, noradrenergic cell groups are classified as A1-A7 [9]. The A1 cell group is located at the level of the area postrema, A2 is distributed throughout the dorsal vagal complex, A3 is in the medullary reticular formation, and A4 surrounds the fourth ventricle. The A5 cell group is in the ventrolateral pons, A6 (locus coeruleus) is dorsally in the pons and A7 is

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in the lateral part of the pons, close to the lateral lemniscus. The main ascending noradrenergic projection pathways in the central nervous system are the dorsal and ventral bundles and the periventricular bundle [10]. Additionally, cell groups A5-A7 have significant descending noradrenergic projections to the spinal cord [11, 12]. Moreover, the noradrenergic locus coeruleus (LC) is the main nucleus in the pons involved in pain processing, and based on classical studies of acute pain modulation, it also mediates the inhibition of pain. LC neurons are the crossroads of an elaborate network of ascending and descending projections [13]. Descending inhibitory noradrenergic fibers reach the substantia gelatinosa reducing pain at the spinal level [14, 15]. Furthermore, ascending inputs from the LC to cortical areas, such as the prefrontal cortex (PFC), modulate the cortico-thalamic loop that processes the affective component of pain [16].

NE effects are mediated by adrenergic receptors (ARs) divided into two main categories, α and β. ARs are G protein-coupled membrane receptors divided into 9 subtypes encoded by different genes: three α_1 ARs, three α_2 ARs and three β_1 ARs [17-20]. α1 subtypes are collectively characterized by their positive coupling via Gq to voltage-gated Ca^{2+} channels and phospholipase C (PLC), activation of which mobilises intracellular pools of calcium. The three classes of β ARs stimulate the activity of adenylyl cyclase by Gs leading to an increase of neuronal excitability [21]. On the contrary, the three α_2 AR subtypes (α_{2A}, α_{2B} and α_{2C}) couple with Gi/o and are related to adenylyl cyclase inhibition, K^+ current enhancement and to reduction of Ca^{2+} currents [22]. α_2 ARs are involved in different physiological functions and all the three subtypes have been identified as potential contributors to nociceptive modulation being located in key nodes of the nociceptive system [23-25]. Pharmacological or genetic manipulations demonstrated that α_2 ARs can alter nocifensive behaviours and/or neuronal activity [26-31]. α_2 AR stimulation induce analgesia by directly acting on the spinal cord [32-37] producing both post-synaptic hyperpolarization [38] and presynaptic inhibition of excitatory transmission [39]. In general, activation of postsynaptic α_2 ARs produces hyperpolarization by the activation of G protein-coupled inwardly-rectifying potassium channels via $G_{i/o}$-proteins. Presynaptic α_2 ARs reduce neurotransmitter release by inhibiting calcium influx [40]. In the noradrenergic neurons of the LC, α_2 ARs act as autoreceptors to reduce local norepinephrine release [41] and also induce postsynaptic hyperpolarization [42]. Recently, Funai *et al.* [43], suggest that systemic α_2 AR stimulation may facilitate inhibitory synaptic responses in the superficial dorsal horn to produce analgesia mediated by activation of the pontospinal noradrenergic inhibitory system.

The present review intends to be a contribution to the comprehension of the α_2 AR role in neuropathic states focusing on the pharmacological modulation.

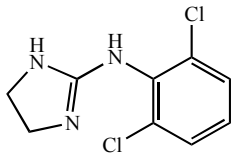
1.2. Direct α_2 AR Modulation

1.2.1. Effect of α_2 AR agonists

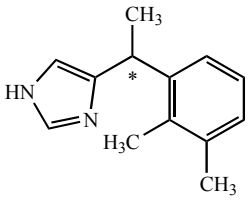
There is considerable preclinical and clinical evidence that α_2 AR agonists elicit analgesia against a diversity of painful states, including NP.

Clonidine showed anti-hyperalgesic activity after intrathecal (i.t.) administration in patients with NP [44, 45]. Clonidine inhibits nociceptive impulse by α_2 ARs in the dorsal horn of the spinal cord promoting inhibitory interactions with the pre and post synaptic primary afferent nociceptive projections [46] and the inhibition of substance P release [47]. The α_2 selective antagonist yohimbine reversed clonidine-induced analgesia [46]. Clonidine may be useful for patients tolerant, non-responsive or allergic to morphine or other opioids. Indeed, clonidine is approved by the United States Food and Drug Administration (FDA) for epidural administration in the treatment of neuropathic cancer pain and, together with drugs that enhance NE concentrations in the spinal cord (amitriptyline and duloxetine), it is considered a first-line treatment for NP [4] The efficacy of clonidine to relieve NP was tested by Feng [48] in a rat model of partial sciatic nerve ligation-induced hypersensitivity. Fourteen days after surgery 5, 10 and 20 μg clonidine, i.t. injected, was able to attenuate mechanical and thermal hyperalgesia in a dose-dependent manner. The attenuation of pain paralleled with microglia and astrocyte inhibition [48]. The inhibitory effect of clonidine on glia cells was explained by the authors as result of decreased proinflammatory cytokines (IL-1β and IL-6) [48].

Poree [49] investigated the analgesic potency and site of action of systemic (intraperitoneally; i.p.) **dexmedetomidine** in naive and neuropathic rats (L5 and L6 spinal nerve ligation model; SNL). Neuropathic animals responded to smaller doses of dexmedetomidine in comparison to naive rats. Atipamezole, a selective α_2 AR antagonist, blocked dexmedetomidine pain reliever effect both in NP and control animals. On the contrary, a peripherally restricted α_2 AR antagonist blocked the anti-hyperalgesic actions of dexmedetomidine in neuropathic rats only. A nerve injury-dependent shift of the α_2 analgesic site of action outside the blood-brain barrier was hypothesized and a peripheral restricted α_2 AR agonist was suggested for the management of NP [49]. As regards the α_2 AR subtype involved in pain modulation, Robinson *et al.* [50] showed the loss of clonidine analgesic effect in α_{2A} knockdown animals. Moreover, in mice lacking the α_{2A} AR gene the data was confirmed for both the analgesic [51] (increase of normal pain threshold) and anti-hyperalgesic [34] (increase of altered pain threshold) efficacy of dexmedetomidine i.p. or subcutaneously (s.c.) administered. This selectivity was not confirmed when α_2 AR agonists were intraplantarly injected in rat underwent SNL [52]. The anti-hypersensitivity effect of intraplantar dexmedetomidine was reversed by ipsilateral (but not contralateral) intraplantar injection of yohimbine (a non selective α_2 adrenoceptor antagonist), as well as by intraplantar injections of BRL 44408, ARC 239, and JP 1302, α_{2A}, α_{2B}, and α_{2C} AR antagonist, respectively [52].



Clonidine



Dexmedetomidine

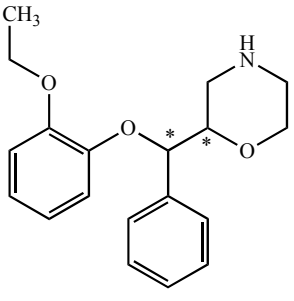
1.3. Indirect α_2 AR Modulation

1.3.1. Effect of Norepinephrine Reuptake Inhibitors

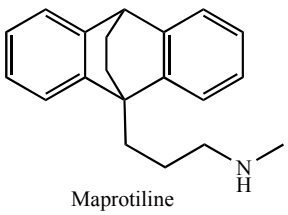
Clinical studies suggest that the efficacy of 5-HT and NE reuptake inhibitors (SNRIs) for neuropathic pain is greater than that of selective 5-HT reuptake inhibitors (SSRIs) [53]. Moreover, Nakjima *et al.* [53] demonstrated that an increase in NE in the spinal cord plays an important role in the antihyperalgesic effects of not only SNRIs but also SSRIs. Recent researches in this area have explored the use of purely noradrenergic reuptake inhibitors as analgesic treatment. Preclinical research suggests that noradrenergic antidepressants may have antinociceptive effects comparable to mixed tricyclic antidepressants [54].

The recent introduction of **reboxetine**, a selective NE reuptake inhibitor, contributed to increase the interest in the catecholamine pathway for NP treatment. Its comparatively low affinity for muscarinic acetylcholine receptors causes less dry mouth, constipation, and other side effects characteristic of tricyclic antidepressants. Moreover, nausea, diarrhea, and hypotension appear to be less common since reboxetine does not block α_1 AR or the 5-HT transporter. Reboxetine is non-sedating and widespread used in neuropathic, musculoskeletal pain and in fibromyalgia [55]. In rats underwent to tibial nerve transection, reboxetine i.t. or systemically injected reduced neuropathic pain; the antiallodynic effect was blocked by prior administration of yohimbine but not by prazosin (α_1 AR antagonist) or propranolol (β AR antagonist) [56]. As regards fibromyalgia, a disorder characterized by pain, muscle tenderness, fatigue and sleep disturbance, [57, 58] the active reboxetine enantiomer esreboxetine induced clinical benefit with the least risk of drug exposure [59].

Maprotiline was compared with amitriptyline in the treatment of postherpetic neuralgia evaluating the relief of steady pain, brief pain and pain dues to tactile stimulation. Maprotiline relieved pain but was not as effective as amitriptyline conversely it was effective when amitriptyline failed [60]. The relationship between the maprotiline pain reliever effect and α_2 AR activation was demonstrated for acute pain [61].



Reboxetine



Maprotiline

1.3.2. Effect of Serotonin/Norepinephrine Reuptake Inhibitors

The higher efficacy of SNRI in comparison to SSRI emerges from a preclinical study performed in neuropathic rat. The SNRI **Milnacipran**, produced a dose-dependent (3 – 30 mg/kg; i.p.) antihyperalgesic effect. The effect was reversed by i.t. injection of the α_2 AR antagonist idazoxan (30μg), but not by various 5-HT receptor antagonists. Paroxetine produced an antihyperalgesic effect only at the highest dose tested and it was reversed by intrathecal injection of both idazoxan and ondansetron (30μg), a 5-HT_3 receptor antagonist [53]. Milnacipran is licensed for the treatment of fibromyalgia in some countries. Efficacy seems to be similar to that induced by duloxetine and pregabalin [62] even if a recent review of literature data reports that milnacipram provides moderate pain relief in a minority of treated patients increasing adverse events and adverse withdrawals [63].

Venlafaxine is a 5-HT/NE reuptake inhibitor that in comparison with other dual reuptake inhibitors such as duloxetine has weak affinity for 5-HT and NE transporters [64] and belongs to newer antidepressant drugs [65]. Its antinociceptive effect has been demonstrated in different pain models as neurogenic pain, diabetic neuropathy and fibromyalgia [66-69]. Venlafaxine does not induce the classical side effects of tricyclic antidepressants becoming a promising pharmacological approach for NP treatment. The α_2 AR role in the pain reliever effect of venlafaxine was clarified by Hajhashemi [70] in a rat model of peripheral neuropathy (chronic constriction injury; CCI). Acute administration of venlafaxine on days 7, 14 and 21 after surgery was not able to reduce tactile and cold hypersensitivity, on the contrary repeated administrations of the drug (starting the first day after trauma) significantly prevented heat hyperalgesia and tactile hypersensitivity. Yohimbine reversed venlafaxine effect on heat hyperalgesia but not on mechanical allodynia [70]. Venlafaxine inhibits LC electrical activity through α_2 AR [71].

A study performed by Benbouzid [72] showed that the **tryciclic antidepressants** that act by blocking NE and 5-HT reuptake requires a chronic treatment to alleviate NP as observed against depression. The authors used a murine NP model induced by the unilateral insertion of a polyethylene cuff around the main branch of the sciatic nerve to produce a long lasting ipsilateral allodynia. **Amitryptiline (**5 mg/kg; lower than that used as antidepressant) was administered i.p. *bid* for 3 weeks starting 15 days after surgical procedure. Ten days after treatment, amitriptyline was able to suppress the cuff-induced allodynia measured by Von Frey test. On the contrary, the acute treatment was ineffective [72]. Zychowska [73] demonstrated that amitriptyline in CCI-exposed rats attenuated only mechanical allodynia with no significative effects on thermal hyperalgesia. These results are consistent with Berrocoso [74] that observed higher efficacy of acute amitriptyline (10 mg/kg) against mechanical allodynia in comparision to cold allodynia in a rat model of NP.

The anti hyperalgesic effect of **duloxetine** was investigated in rats which underwent to CCI with particular attention to the LC activity [75]. CCI did not modify the basal tonic activity in the LC although was able to alter the animals response to noxious stimuli. Under normal conditions, noxious stimulation evoked an early LC neuron response, dependent and comparable with gabapentin [77]. Bicifadine efficacy was also confirmed in the streptozotocine model of diabetic NP, 6 min after administration the paw withdrawal was significantly higher in comparison to vehicle-treated diabetic rats and was not different from nondiabetic rat response [77]. Corresponding to the activation of myelinated A fibers, which is followed by an inhibitory period and a subsequent late capsaicin-sensitive response, consistent with the activation of unmyelinated C fibers [76]. CCI provoked an enhanced excitatory early response in the animals and the loss of the late response. The continuous administration (by osmotic pump s.c.) over 7 days of desipramine (10 mg/kg/day) or duloxetine (5 mg/kg/day) decreased the excitatory firing rate of the early response in the CCI group [75].

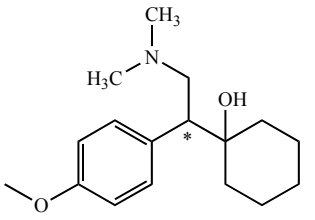
Bicifadine inhibits monoamine neurotransmitter uptake *in vitro* (recombinant human monoamine transporters) with a relative potency of NE > 5-HT > dopamine (DA) (≈1:2:17). This *in vitro* profile is supported by microdialysis studies in freely moving rats, where bicifadine (20 mg/kg i.p.) increased extrasynaptic NE and 5-HT concentrations in the prefrontal cortex, NE levels in the locus coeruleus, and DA levels in the striatum [77]. Although NE levels increased more rapidly that 5-HT, both neurotransmitters reached similar maxima 40 min after bicifadine administration [77]. However, additional aspects of the molecular pharmacology of bicifadine may contribute to the antinociceptive profile. Bicifadine is a α_2 AR agonist and a full 5-HT_{1A} receptor agonist [78, 79]. The molecule was effective in reducing mechanical and thermal hyperalgesia and mechanical allodynia in the rat NP model of SNL. The effect was dose-dependent.

1.3.3. Effect of μ Agonist/Norepinephrine Reuptake Inhibitors

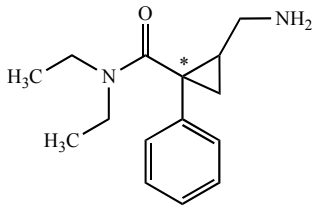
Tramadol is a synthetic opioid of the aminocyclohexanol group and is globally used for the management of moderate and severe pain, including post-operative pain, cancer pain and NP [4, 80, 81]. The pharmacodynamic characteristic of tramadol is a dual analgesic mechanism of action, it is active as a μ opioid receptor agonist and as inhibitor of 5-HT and NE reuptake [81-85]. The relevance of α_2 ARs in NP was highlighted injecting tramadol i.p. in neuropathic rats (partial spinal nerve ligation; PSNL) [86]. Tramadol acutely reduced mechanical allodynia, the effect was blocked by naloxone. Repeated administrations showed preventive and alleviative effects on the mechanical allodynia that was diminished by yohimbine, but not naloxone. Furthermore, tramadol (as well as amitriptyline) inhibited the nerve ligation-induced activation of spinal astrocytes, which was reduced by yohimbine. Tramadol and amitriptyline were ineffective against PSNL-induced microglia activation [86].

The analgesic effect of tramadol on NP is considered to be at least partly due to its interaction with presynaptic and postsynaptic α_2 ARs located on the central terminals of primary nociceptive afferents and on the spinal dorsal horn neurons, respectively [25, 87, 88]. The pain reliever potency of tramadol is higher in NP than in acute pain, [89] probably because the activity of presynaptic and postsynaptic α_2 AR is increased in neuropathic conditions [90].

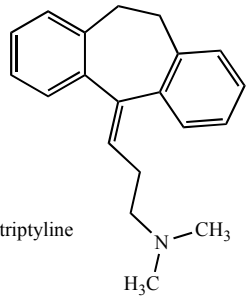
Likewise tramadol, **tapentadol** is a dual action molecule, μ opioid agonist and NE reuptake inhibitor, approved for the management of moderate and severe pain. In contrast to morphine, tapentadol elevates NE levels in the ventral rat hippocampus [91]. Moreover, the lack of significant serotonergic effects clearly differentiates tapentadol from the 'atypical opioid' tramadol, which produced significant hippocampal increases of NE as well as 5-HT [92]. Tapendadol double mechanism of action is particularly advantageous because opioids are highly efficacious against



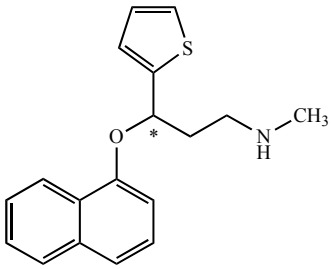
Venlafaxine



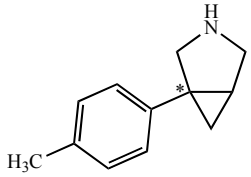
Milnacipran



Amitriptyline



Duloxetine

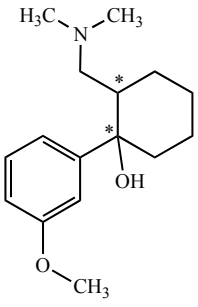


Bicifadine

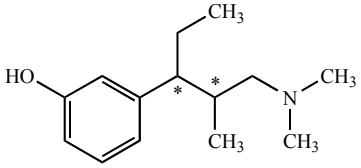
acute pain whereas noradrenergic drugs are particularly useful in chronic NP conditions [93-95]. Moreover the two mechanisms interact with each other because the opioid component activates the descending inhibitory pathway at the supraspinal level (PAG and RVM) that leads to increased NE release in the dorsal horn reducing the nociceptive transmission from the primary afferents to spinal second order neurons [96, 97]. The different contribution of tapentadol opioid and noradrenergic mechanisms was evaluated in a rat model of nociceptive and NP by Schroder [98]. The antinociceptive and antihyperalgesic effect of tapentadol was tested in naive rats, using the tail flick test, and in SNL rats, using the Von Frey test. Its effects were antagonized by naloxone, yohimbine and ritanserin (μ, α_2 and 5-HT_{2A} receptor antagonist, respectively). Intravenously administration of tapentadol induced antinociceptive and antihyperalgesic effect in naive and neuropathic rats with median effective dose (ED_{50}) values of 3.3 mg/kg and 1.9 mg/kg [98]. Naloxone produced a 6.4-fold shift to the right in the antinociceptive ED_{50} value of tapentadol while yohimbine 1.7-fold only. On the contrary, yohimbine induced a 4.7-fold shift in the antihyperalgesic ED_{50} in comparison to the 2.7-fold shift induced by naloxone; ritanserin did not induce changes neither on the antinociceptive nor on the antihyperalgeisc effect of tapentadol [98].

The predominant role of NE reuptake inhibition in NP and of μ opioid stimulation in acute pain was suggested [98]. A recent research conducted by Meske [99] studied the effects of tapentadol administration in sham and SNL rats vs morphine and duloxetine. All these drugs showed significant and time-related reversal of tactile hypersensitivity. Tapentadol (30 mg/kg) was able to significantly increase NE levels in the cerebrospinal fluid (CSF) in both sham and SNL rats even if the effect was long lasting in SNL only. Duloxetine was able to increase NE levels in both groups of rats while morphine, at the same doses able to reverse tactile hypersensitivity, reduced NE levels in spinal CSF of both groups [99] On the other hand, a broad range of preclinical and clinical studies on analgesia has demonstrated that opioid and α_2 AR mechanisms are interdependent. It is widely known that α_2 AR stimulation enhances the antinociceptive effect of μ-opioids, while α_2 AR antagonists such as yohimbine or idazoxan attenuate morphine analgesia in several pharmacological tests. Genetically-altered mice lacking NE, showed a significantly attenuation of the antinociceptive effect induced by morphine [100]. Moreover, early studies demonstrated the increase of NE release into the CSF after morphine microinjections into the PAG or RVM [101-104].

Summarizing tapentadol effects against NP are imputable to increased NE levels and to the synergy between μ and α_2 receptors.



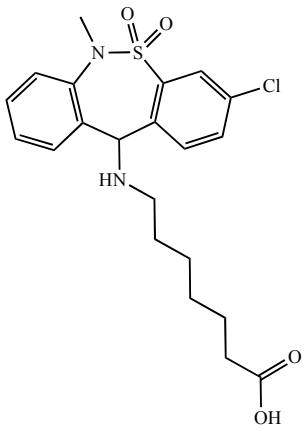
Tramadol



Tapentadol

1.4. An Overview of Compounds Present in the Recent Literature

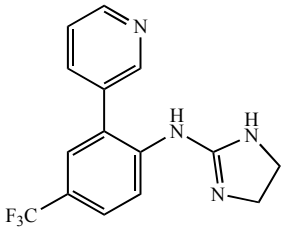
1.4.1. Tianeptine



((RS)-7-(3-chloro-6-methyl-6,11-dihydrodibenzo[c,f][1,2]thiazepin-11-ylamino) heptanoic acid S,S-dioxide)

Tianeptine is a 3-chlorodibenzothiazepine nucleus with an aminoheptanoic side chain. Tianeptine is considered an atypical antidepressant with some structural similarities to TCAs but with distinct neurobiological properties [105]. In a recent study, intrathecal administration of tianeptine was performed to assess the effect of tianeptine on mechanical allodynia in a neuropathic model induced by ligation of L5 and L6 spinal nerves [106]. Tianepine significantly increased the paw withdrawal threshold in a dose-dependent manner with a ED_{50}= 102.1 μg and a maximum effect 15-30 min after administration; its antinociceptive effect was reversed by yohimbine and dihydroergocristine. In the same study an extracellular increase of 5-HT and NE concentration in the spinal dorsal horn was observed within 15 min after tianepine administration (about 15-fold the baseline value).

1.4.2. A-1262543

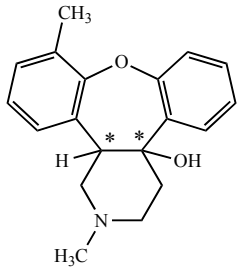


(N-(2-(pyridin-3-yl)-4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-imidazol-2-amine)

A-1262543, an imidazoline derivative, is a potent and selective agonist for rat α_{2B} adrenergic receptor with an EC_{50} 4.3 nM (no agonistic activity was recorded at rat α_{2A} and α_{2C}

at concentration up to 10 μM). This compound, tested in the SNL model of NP, is able to produce a dose-dependent anti-allodynic effect, decreasing the hypersensitivity of 42%, 66% and 84% at the doses of 1, 3 and 10 mg/kg ip respectively. In the same study it was demonstrated that the anti-allodynic effect is mediated via the ascending nociceptive system [107].

1.4.3. Beloxepin



[(±) cis-1,2,3,4,4a,13b-hexahydro-2,10-dimethyldibenz[2,3:6,7]oxepino[4,5-c]pyridine-4a(2H)-ol

Beloxepin (Org-4428) belongs to the family of diphenylossepine [108] and it was studied as antidepressant agent at the end of 1990 [109]. It is a selective inhibitor of synaptosomal noradrenalin reuptake being about 100-fold less affine for other monoamine carriers [110]. The values of NE transporter affinity of the raceme (±) and of the pure enantiomers, clearly indicate that the (-)-enantiomer binds with about 8-fold higher affinity at the transporter with respect to (+)-enantiomer (Ki(±) 700 nM, Ki(-) 390 nM and Ki(+) 2960 nM) [111]. Although (±) beloxepin is a weak inhibitor of NE reuptake, it is active in the treatment of the neuropathic pain. It produces a dose-dependent antiallodynic effect in L5 SNL rats 16 days post surgery (3, 10 and 30 mg/kg i.p.). A potent antiallodynic affect was recorded after 30 mg/kg injection reaching threshold values very similar to that of sham-operated rats. Moreover, (±) beloxepin and (-) beloxepin are effective in the same model of neuropathic pain when administered per os. They produce significant antiallodynic effect tested at 60 mg/kg, 60 and 120 minutes post drug. Finally, in the same conditions, the (+) enantiomer doesn't show significant antiallodynic effect [110, 111].

1.4.4. Conopeptides

It is well known the potency and the chemical stability of the venom peptides, properties which make these natural products a very attractive source for the discovery and the development of new drugs [112-114]. At the beginning of 2000, studies performed on conotoxin, **Mr1A**, belonging to χ-conopeptide family [115, 116], showed its ability to produce antinociception after intrathecal administration in mice [117] and was found to be a selective and reversible inhibitor of neuronal NE transporter [115, 116, 118]. **Xen2174** is another χ-conopeptide with chemical stability, efficacy and safety profile more favorable with respect to Mr1A, and it was extensively studied [113]. The intrathecal administration of this compound produced dose-dependent relief of mechanical allodynia induced in rats by two different models of NP, CCI and L5/L6 spinal-nerve injury. At the doses able to reverse allodynia, side-effects were mild with absence of sedation. The anti-allodinic effect was reverted by intrathecal co-administration of yohimbine (100 nmol), this data confirming the noradrenergic system involvement [119]. Brust and co-workers [120] proposed a pharmacophoric model for the allosteric binding of Xen2174 to NE transporter. In this model the interaction between Xen2174 and NE transporter is predominantly with aminoacids in the first loop, while the second loop interacts with a big hydrophobic pocket within the transporter (Fig. **1**).

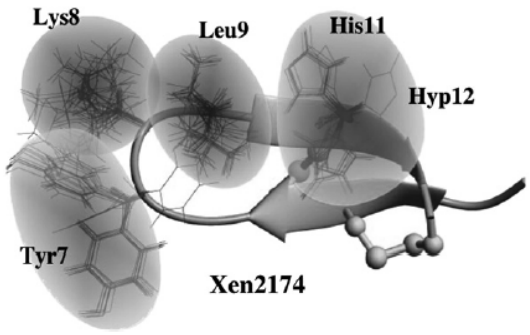
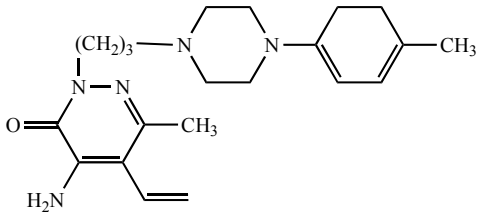


Fig. (1). Proposed pharmacophore model for the χ-conopeptides (from Brust *et al.*) [120].

1.4.5. Miscellaneous

In the past years the Authors of the present review published some articles on antinociceptive agents whose effect was demonstrated to be mediated by noradrenergic system [121-124]. They report here new data about the most interesting compound, 6a [124] (now renamed ET-1), a potent and orally active antinociceptive agent acting as α_2 AR agonist [124].



ET-1

4-amino-6-methyl-2-(3-(4-(4-methylcyclohexa-1,3-dien-1-yl)piperazin-1-yl)propyl)-5-vinylpyridazin-3(2*H*)-one

As shown in Fig. (**2**), repeated treatment with oxaliplatin, an anticancer agent able to induce a dramatic peripheral neuropathy [125-127], was able to reduce the licking latency of mice placed on a cold surface (Cold plate test) from 21.5± 1.4 s (vehicle + vehicle) to 10.8±1.7 s (oxaliplatin + vehicle). Acute administration of 10 mg kg^{-1} ET-1 significantly increased the pain threshold reaching a peak between 15 and 30 min after administration and vanishing 45 min later. ET-1 showed a dose dependent effect, completely reverting

oxaliplatin-induced pain at 40 mg kg^{-1}. The pain reliever properties of the compound were completely reverted by the pretreatment with the α_2 AR antagonist yohimbine (3 mg kg^{-1}), confirming the α_2 AR-mediated pharmacodynamic also in NP (Fig. **2**).

Moreover, the pain reliever ET-1 dosages were able to significantly increased NE extracellular levels in rat cerebral cortex (Fig. **3**).

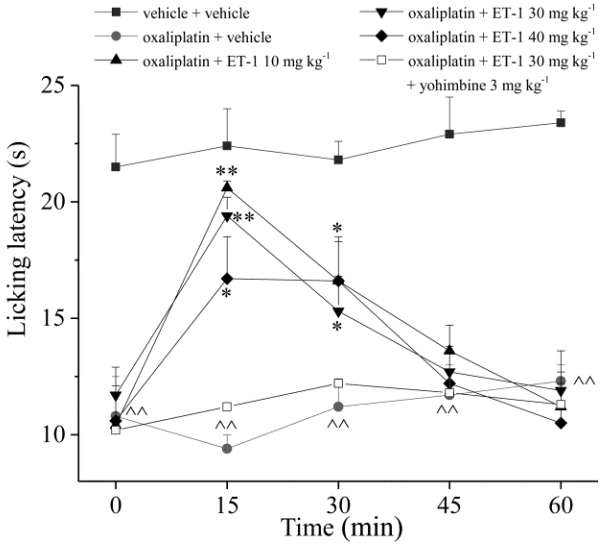


Fig. (2). Effect of ET-1 on oxaliplatin-induced neuropathic pain, Cold plate test. Mice were daily intraperitoneally treated with oxaliplatin 2.4 mg kg^{-1} for 14 days. On day 14, ET-1 (10, 30 and 40 mg kg^{-1}) was per os acutely administered. The response to a thermal stimulus was evaluated by the Cold plate test measuring the latency to pain-related behaviour (lifting or licking of the paw). Control animals were treated with vehicles. ^^P<0.01 vs vehicle + vehicle treated mice; *P<0.05 and **P<0.01 vs oxaliplatin + vehicle treated mice. Each value represents the mean of 10 mice.

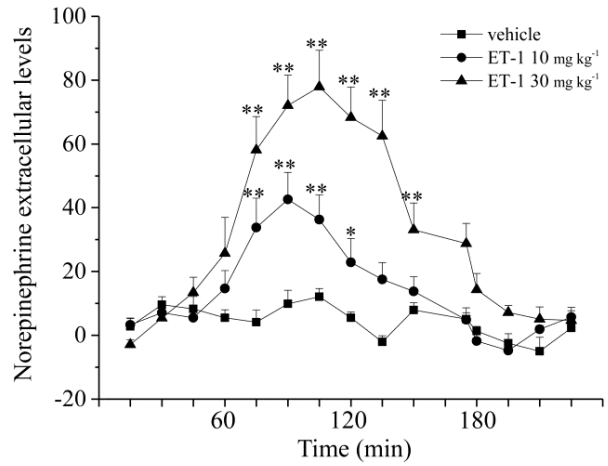
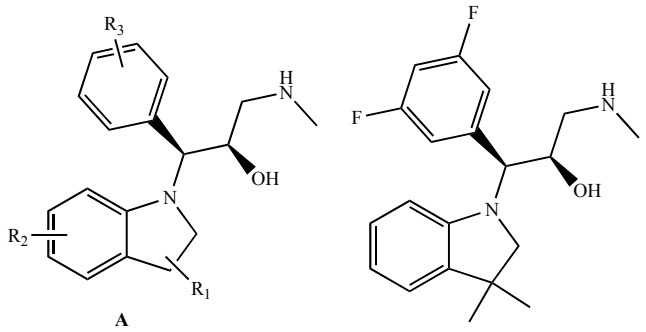


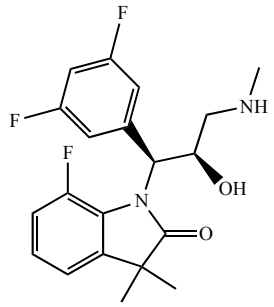
Fig. (3). Effect of ET-1 (10 – 30 mg kg^{-1} i.p.) on noradrenaline extracellular levels from rat cerebral cortex. **P<0.01 vs vehicle.. Each value represents the mean of 6 rats.

The Pfizer (Wyeth) Group has extensively investigated the field of norepinephrine reuptake inhibitors, producing a large number of studies [128-134]. Maintaining the 3-amino-2-hydroxy-1-phenylpropyl fragment as suitable template for CNS drug, they selected a series of scaffolds such as aniline [128], indole [129] and benzimidazole [130]. Many compounds belonging to the series of 1-(indolin-1-yl)-1-phenyl-3-propan-2-olamines (**A**) exhibited potent inhibition of NE transporter with an IC_{50} in the nanomolar range and a good selectivity toward DA and 5-HT transporter. The most interesting compounds also showed favorable drug like properties, such as lipophilicity value and molecular weight. Compound **9p**, a potent NE transporter inhibitor (NE uptake inhibition IC_{50} 6.5 nM; NE transporter binding IC_{50} 4.1 nM) and very selective (814-fold) over the 5-HT transporter, was selected for evaluation in a rat SNL model of NP. Results showed that it is able to reverse the mechanical hyperalgesia in dose-dependent manner at doses of 10 and 30 mg/kg per os [129].



[(1S,2R)-1-(3,5-difluorophenyl)-1-(3,3-dimethylindolin-1-yl)-3-(methylamino)propan-2-ol]

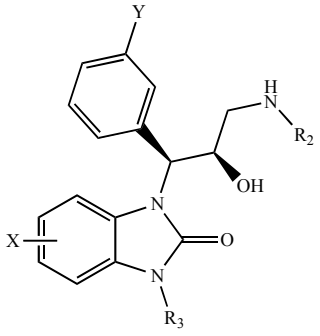
The 7-fluoroanalogue of 9p, **WAY-318068** [131, 132] was studied for its antidepressant properties and as antihyperalgesic agent in pre-clinical model of neuropathies. It is a potent and selective inhibitor of NET showing a Ki = 8.7 nM in a radioligand binding assay and an IC_{50} = 6.8 nM in a functional NE uptake assay. Moreover, microdialysis conducted after WAY-318068 administration in rats showed a significantly increase of NE but not 5-HT levels in 90 to 240 min post-dosing range. It is orally bioavailable and a significant amount of drug was found in the CNS after oral administration. In the NP model induced by SNL, oral administration of this compound 3 weeks after surgery produced a dose-dependent reduction in mechanical hyperalgesia with the maximum efficacy achieved after 5 h following the 100 mg/kg dose. In the STZ model of diabetic NP, oral administration of WAY-318068 led to an increased latency to nociceptive response to thermal stimulation 3 h after administration at the doses of 3–30 mg/kg. The good pharmacological and pharmacokinetic properties of WAY-318068, together with its potency and selectivity make this compound a good candidate for further development [132].



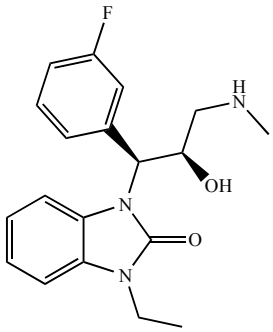
WAY-318068

(1-[(1S,2R)-1-(3,5-difluorophenyl)-2-hydroxy-3-(methylamino)propyl]-7-fluoro-3,3-dimethyl-1,3-dihydro-2H-indol-2-one)

The replacement of indole with benzimidazolone let to obtain a series of 3-(3-amino-hydroxy-1-phenylpropyl)-1,3-dihydro-2H-benzimidazol-2-ones derivatives (**B**) [130]. SARs analysis showed the importance of the size of the substituent at N-1 on the NE transporter potency: the activity is maximum for the terms bearing an ethyl or a propyl group at position 1 (IC_{50} = 13.4-186 nM), whereas it decreases when at the same position a smaller (methyl) or a bigger group (t-butyl, c-butyl, or c-pentyl) was inserted. Compound **20** is one of the most interesting terms, (NE transporter inhibition IC_{50} = 7.4 nM, NE transporter binding IC_{50} = 6.4 nM, 5-HT transporter 15% inhibition at 1 μM, DA transporter 2% inhibition at 10 μM) was selected for further investigation in both acute and neuropathic pain. In particular, in the model of rat SNL, it significantly and dose-dependent reversed the mechanical hyperalgesia at 3 and 10 mg/kg p.o., suggesting its potential use for the treatment of NP.



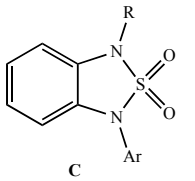
B



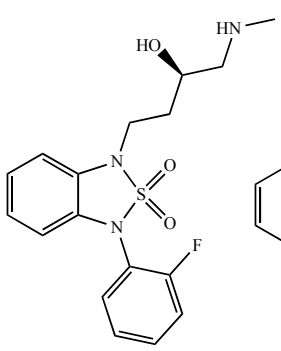
20

(1-ethyl-3-((1S,2R)-1-(3-fluorophenyl)-2-hydroxy-3-(methylamino)propyl)-1H-benzo[d]imidazol-2(3H)-one)

The same research group successively focused their attention on the benzothiadiazole scaffold [133, 134], synthesizing a series of derivatives **C**, evaluating structure-activity relationships and finally identifying two lead compounds, **S-17b** (**WYE-103231**, 4-[3-(2-fluorophenyl)-2,2-dioxo-2,3-dihydro-2λ^6-benzo[1,2,5]thiadiazol-1-yl]-1-methylamino-butan-2-ol) [133] and **10b** (**WYE-114152**, 1-(2-fluorophenyl)-3-(2-morpholin-2-yl-ethyl)-1,3-dihydro-benzo[1,2,5]thiadiazole 2,2-dioxide) [134] with the best balance among potency as inhibitor of the NE transporter, selectivity over both 5-HT and DA transporter and pharmacokinetic profile. Compound S-17b (NE transporter inhibition IC_{50} = 1 nM, NE transporter binding IC_{50} = 6 nM) showed high to moderate clearance, high volume of distribution, a long half-life (3.2-6.3 h, depending on the species), a good brain penetration and finally a bioavailability from 41% to 100% [133]. S-17b, tested on SNL model of NP, was found able to significantly reduce in a dose dependent manner, the mechanical hyperalgesia at 5, 7, 10 and 30 mg/kg. The second one lead, compound 10b (NE transporter inhibition IC_{50}=15 nM, NE transporter binding IC_{50}=5 nM) [134] is an analogue of S-17b in which the NH and OH groups of the lateral chain have been cyclized into the morpholine ring and it was selected for its pharmacokinetic parameters, such as clearance, good brain penetration and moderate half-life. Moreover, microdialysis studies in rats demonstrated that administration of 10b at 30 mg/kg po increased till 390% the levels of NE in the medial prefrontal cortex without effect on 5-HT levels. Finally, 10b reduced hyperalgesia in SNL model of NP at the doses of 3, 10 and 30 mg/kg po [134]. This compound is a good candidate for further development.

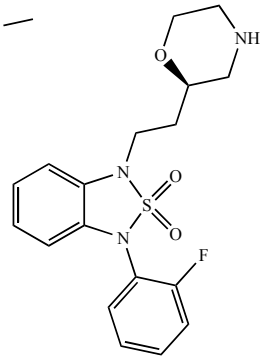


C



(WYE-103231)

4-[3-(2-fluorophenyl)-2,2-dioxo-2,3-dihydro-2λ^6-benzo[1,2,5]thiadiazol-1-yl]-1-methylamino-butan-2-ol)

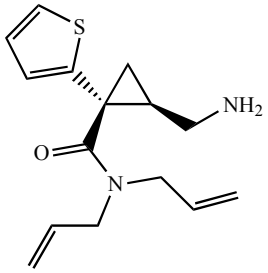


(WYE-114152)

1-(2-fluorophenyl)-3-(2-morpholin-2-yl-ethyl)-1,3-dihydro-benzo[1,2,5]thiadiazole 2,2-dioxide)

A research performed by Chen and coworkers [135] on analogues of Milnacipran afforded potent NE/5-HT transporter inhibitors, whose pharmacological and pharmacokinetic profile has been widely investigated. The most interesting compound of the series, **5c**, exhibited IC_{50} = 2.3 and 32 nM at NE and 5-HT transporters respectively, more than 10-fold better than Milnacipran. Moreover, it was tested in a panel of binding assay of about 70 different receptors, enzyme, transporter and ion channels at 10 mM, but no significant activity was recorded. Studies of

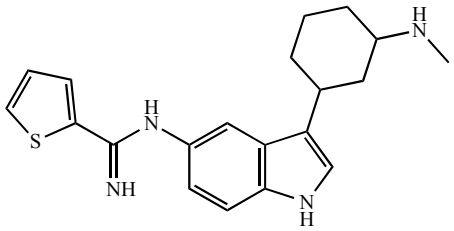
metabolism indicated that 5c is less stable than Milnacipran. The pharmacokinetic parameters were determined in different species and it was seen that 5c had the highest oral bioavailability in mice (92%), followed by dog (78%) and monkey (34%). Also for 5c was demonstrated the correlation between NE and 5-HT transporter inhibition and its ability to reverse mechanical hyperalgesia induced by SNL. Tested at the dose of 30 mg/kg it produced an excellent antihyperalgesic effect (about 60% reversal) comparable with that of Milnacipran tested at higher doses (60 and 100 mg/kg).



5c

((1R,2R)-N,N-diallyl-2-(aminomethyl)-1-(thiophene-2-yl)cyclopropanecarboxamide)

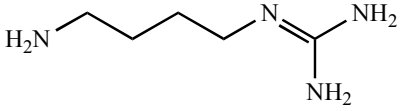
Among the drugs that act specifically on multiple targets, (classified as Designed Multiple Ligands, DMLs) other than the above reported, a new class has been discovered by Mladenova and coworkers [136]. Taking into account that both NE and nitric oxide are involved in pain, and starting from the similarities between the NE transporter and the nitric oxide synthase (NOS) pharmacophore models, they designed and synthesized a series of 3,5-disubstituted indole derivatives with dual action, as human neuronal NOS and NE reuptake inhibitors. They found that the cis analogues showed better selectivity for neuronal NOS (nNOS) over the endothelial one (eNOS), while the differences of conformation were not important for the NE transporter activity. **Cis-(+)-37** was selected as lead compound, exhibiting excellent potency for nNOS (IC_{50} = 0.56 μM) and NE transporter (IC_{50} = 1.0 μM) with a good selectivity over eNOS and inducible NOS (iNOS). Compound cis-(+)-37 was evaluated for the contractile response on human resistance arteries (inhibition of acetylcholine-mediated relaxation), in order to find cardiovascular effects associated with eNOS inhibition, but no effect was recorded and this data is in agreement with the low inhibitory potency in human eNOS (IC_{50} = 49.3 μM). In the SNL model of NP, the i.p. administration of cis-(+)-37 (30 mg/kg) led to a reversion of allodynia and thermal hyperalgesia and the maximum effect was observed at 30 min. (90% allodynia and 96% thermal hyperalgesia). These results allowed the Authors to assert that cis-(+)-37 shows a dual action activity, since their previously reported pure nNOS compounds reverted only the thermal hyperalgesia [137]. This compound, further investigated in a panel of different receptors, ion channels and transporter at 10 μM, showed a good profile and at the present is under evaluation in NP.



cis-(+)-37

(N-(3-(3-(methylamino)cyclohexyl)-1H-indol-5-yl)thiophene-2-carboxamide)

Finally, it is interesting to mention **Agmatine**, an endogenous substance synthesized from L-arginine by the enzyme arginine decarboxilase [138]. It was identified in mammalian and it is widely distributed in a variety of tissues including the brain [139, 140] and it acts as antagonist of glutamate receptors and as inhibitor of NOS but also binds with high affinity the α_2 AR [141, 142]. Fairbanks *et al.* [143] first demonstrated that i.t. administration of agmatine relieved carrageenan-induced mechanical and muscle inflammatory hyperalgesia in mice; afterwards it was demonstrated that agmatine is active (10-400 mg/kg) on NP in a rat model [144] and that this effect involves the reduction of NO levels as well as noradrenergic activity in the brain. These results were also confirmed by Paszcuk *et al.* [145] which observed that agmatine (30 mg/kg i.p.) significantly reduced the mechanical hypernociception caused by partial sciatic nerve ligation in mice during 6 h, with inhibition of 81%.



2-(4-aminobutyl)guanidine

CONCLUSIONS

Chronic pain management is a challenge entrusted to a limited number of effective therapeutic options [146] and novel pharmacological targets [114, 147, 148]. α_2 AR emerges as a key player in the pathological nervous network underlying neuropathic pain. Direct and indirect stimulation offers an interesting approach to relieve pain and favor homeostasis. New potent α_2 AR modulators are currently being evaluated.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

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