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Synergic stimulation of serotonin 5-HT_{1A} receptor and α_2-adrenoceptors for neuropathic pain relief: Preclinical effects of 2-substituted imidazoline derivatives☆

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ABSTRACT

Neuropathic pain affects millions of people causing disability and impairing quality of life. Commonly used analgesics are generally characterized by limited therapeutic outcomes. The serotonin 5-HT_{1A} receptor and the α_2 adrenergic receptors are involved in central nociceptive mechanisms with a pivotal role in the inhibitory descending pain pathway. Since their stimulation may modulate the nervous signaling altered by neuropathies, the purpose of the present research is the study of the combined activation of 5-HT_{1A} and α_2 receptors by rationally designed imidazoline ligands ((*S*)-(-)-**1** and **2**–**5**) in a rat model of neuropathic pain (chronic constriction injury - CCI). On day 14 after nerve damage, the acute administration *per os* (p.o.) of low doses of (*S*)-(-)-**1** (0.1–1 mg/kg) was able to significantly increase the pain threshold to mechanical noxious stimuli for more than 1 h. (*S*)-(-)-**1** efficacy was confirmed by the decrease of spontaneous pain evaluated as hind limb weight bearing alterations. The clinically-used compound gabapentin (100 mg/kg p.o.) induced a pain relieving effect similar to (*S*)-(-)-**1** administered at 100 fold lower dose. In the same model, the selected analogues, compounds **2**–**5** (1 mg/kg p.o.) were effective 30 min after administration. In particular, **5** fully reverted the CCI-induced hypersensitivity. The pain relieving activity of **5** was significantly prevented by the selective 5-HT_{1A} receptor antagonist WAY 100635 (1 mg/kg intraperitoneally, i.p.) and, at a lesser extent, by the α_2 antagonist yohimbine (3 mg/kg i.p.). A novel pharmacodynamic approach to the treatment of neuropathic pain is presented.

1. Introduction

The treatment of neuropathic pain is heavily hampered by the pathological changes of the pain pathways that characterized this condition (Garland, 2012). The use of opioids, fully active against nociceptive pain, is unsatisfactory in neuropathic patients (McNicol et al., 2013; Sun et al., 2017), in addition their long-term use is related to the development of pharmacodynamic tolerance (Ballantyne and Shin, 2008; Dumas and Pollack, 2008). Non-analgesic drugs, like anti-depressants or anti-epileptics, are currently used despite the partial efficacy and the significant side-effects (Hurley et al., 2013).

The pathological aspects of neuropathic pain involve a maladaptive response of the nervous system characterized by peripheral and central sensitization and spinal disinhibition. Disruption of the balance of descending modulatory circuits to favour facilitation may promote and maintain chronic pain (Von Hehn et al., 2012; Ossipov et al., 2010; Di Cesare Mannelli et al., 2015a). This scenario is consistent with the clinical success of drugs that enhance spinal monoaminergic activity, such as serotonin/norepinephrine reuptake inhibitors (SNRIs), in the treatment of chronic pain states. Among the serotonin receptors, the 5-HT_{1A} receptor has been one of the first to be pharmacologically characterized (Fargin et al., 1988, 1989). It is expressed in the raphe nucleus and in several postsynaptic areas involved in central nociceptive mechanisms (Kalipatnapu and Chattopadhyay, 2007). Full and partial 5-HT_{1A} receptor agonists have shown to be beneficial in pain treatments (Nadeson and Goodchild, 2002) arousing great interest as future therapeutic agents (Muthuraman et al., 2014; Panczyk et al., 2015).

☆ Dedicated to Prof. Maria Pigini, who passed away on February 8th, 2016.

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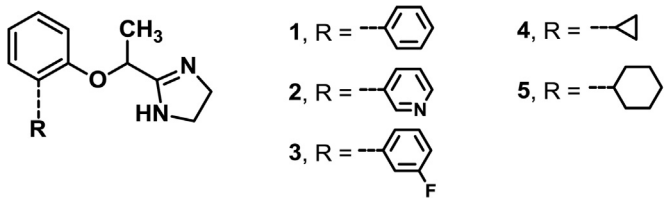

Fig. 1. Chemical structures of **1**–**5**.

On the other side, among the receptors recognized by noradrenaline, α_2 receptor activation decrease the intracellular adenylyl-cyclase activity through Gi or directly modify the activity of ion channels (Summers and McMartin, 1993; Giovannoni et al., 2009). The three subtypes (α_{2A}, α_{2B} and α_{2C}) are involved in different physiological functions and their pharmacological stimulation elicits anti-hyperalgesic effects; the prototype agonist clonidine is currently in clinical use in spite of a not optimal safety profile (Szczudlik et al., 2014). Besides clonidine, other 2-substituted imidazolines are used in human or veterinary pain treatment (Giovannoni et al., 2009). Moreover, the multiplicity of the effects produced by imidazoline compounds highlights the wide bioversatility of this scaffold (Dardonville and Rozas, 2004; Krasavin, 2015).

It has been demonstrated that the (*S*)-(-) enantiomer of biphenyline (**1**, Fig. 1), described as α_2 receptor (Gentili et al., 2002) and 5-HT_{1A} receptor (Del Bello et al., 2016) agonist, behaved as a potent and long-lasting antinociceptive agent in algesiometric paradigms (Gentili et al., 2002).

Considering the important role played by both 5-HT_{1A} and α_2 receptors in nociception (Colpaert, 2006; Deseure et al., 2007; Di Cesare Mannelli et al., 2015a), in the present research we examined the pain relieving effect of (*S*)-(-)-**1** in the rat model of peripheral mononeuropathy induced by chronic constriction injury (CCI) of the sciatic nerve. Moreover, to obtain more information about the role of 5-HT_{1A} and α_2 receptors in the neuropathic pain relieving effect of (*S*)-(-)-**1**, and eventually improve its pharmacological efficacy, the structurally analogues **2**–**5** (Fig. 1) have been rationally selected to be tested in the same rat model of peripheral neuropathy.

2. Materials and methods

2.1. Drugs

Compound (*S*)-(-)-**1** ((*S*)-2-(1-([1,1′-biphenyl]-2-yloxy)ethyl)-4,5-dihydro-1*H*-imidazole) was obtained treating methyl (*S*)-2-([1,1′-biphenyl]-2-yloxy)propanoate with ethylenediamine and trimethyl aluminium at 40 °C in toluene (Gentili et al., 2002).

Compounds **2** (3-(2-(1-(4,5-dihydro-1*H*-imidazol-2-yl)ethoxy)phenyl)pyridine) and **3** (2-(1-((3′-fluoro-[1,1′-biphenyl]-2-yl)oxy)ethyl)-4,5-dihydro-1*H*-imidazole) were obtained treating 2-(2-(pyridin-3-yl) phenoxy)propanenitrile or 2-((3′-fluoro-[1,1′-biphenyl]-2-yl)oxy)propanenitrile, respectively, with ethylenediamine in the presence of sodium methoxide (Gentili et al., 2004).

Compounds **4** (2-(1-(2-cyclopropylphenoxy)ethyl)-4,5-dihydro-1H-imidazole) and **5** (2-(1-(2-cyclohexylphenoxy)ethyl)-4,5-dihydro-1*H*-imidazole) were obtained treating methyl 2-(2-cyclopropylphenoxy) propanoate or methyl 2-(2-cyclohexylphenoxy)propanoate, respectively, with ethylenediamine and trimethyl aluminium at 110 °C in toluene (Cardinaletti et al., 2009; Gentili et al., 2008).

2.2. Animals

Male Sprague-Dawley rats (Envigo, Varese, Italy) weighing approximately 200–250 g were used. Animals were housed in CeSAL (Centro Stabulazione Animali da Laboratorio, University of Florence) and used at least 1 week after their arrival. Four rats were housed per cage (size 26 × 41 cm); animals were fed a standard laboratory diet and tap water ad libitum, and kept at 23 ± 1 °C with a 12 h light/dark cycle, light at 7 a.m. All animal manipulations were carried out according to the Directive 2010/63/EU of the European parliament and of the European Union council (22 September 2010) on the protection of animals used for scientific purposes. The ethical policy of the University of Florence complies with the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health (NIH Publication No. 85-23, revised 1996; University of Florence assurance number: A5278-01). Formal approval to conduct the experiments described was obtained from the Animal Subjects Review Board of the University of Florence. Experiments involving animals have been reported according to ARRIVE guidelines (McGrath and Lilley, 2015). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.3. CCI-induced neuropathic pain model

Neuropathy was induced according to the procedure described by Bennett and Xie (1988). Briefly, rats were anaesthetized with 2% isoflurane. Under aseptic conditions, the right (ipsilateral) common sciatic nerve was exposed at the level of the middle thigh by blunt dissection. Proximal to the trifurcation, the nerve was carefully freed from the surrounding connective tissue, and four chromic cat gut ligatures (4-0, Ethicon, Norderstedt, Germany) were tied loosely around the nerve with about a 1 mm spacing between ligatures. After hemostasis was confirmed, the incision was closed in layers. The animals were allowed to recover from surgery and then housed one per cage with free access to water and standard laboratory chow. Another group of rats was subjected to sham surgery in which the sciatic nerve was only exposed but not ligated.

2.4. Pharmacological treatments

Compounds (*S*)-(-)-**1** (0.1 and 1 mg/kg), **2**–**5** (1 mg/kg) and gabapentin (100 mg/kg) were suspended in 1% carboxymethyl cellulose and administered *per os* (p.o.). WAY 100635 (1 mg/kg) and yohimbine (3 mg/kg) were dissolved in saline solution and injected intraperitoneally (i.p.) 15 min before the administration of compound **5**. The dosages of (*S*)-(-)-**1** were chosen on the basis of the already evaluated antinociceptive ED_{50} after subcutaneous administration (Gentili et al., 2002) and opportunely modified for the p.o. treatment. The doses of gabapentin, WAY 100635 and yohimbine were chosen on the basis of previously published results (Di Cesare Mannelli et al., 2015; Wang et al., 2013; Vergelli et al., 2015).

2.5. Paw-pressure test

The nociceptive threshold in the rat was determined with an analgesiometer (Ugo Basile, Varese, Italy), according to the method described by Leighton et al. (1988). Briefly, constantly increasing pressure was applied to a small area of the dorsal surface of the hind paw using a blunt conical mechanical probe. Mechanical pressure was increased until vocalization or a withdrawal reflex occurred while rats were lightly restrained. Vocalization or withdrawal reflex thresholds were expressed in grams. Rats scoring below 40 g or over 75 g during the test before drug administration were rejected (25%). The pain threshold was evaluated before and 15, 30, 45, 60 and 90 min after compound administration.

2.6. Incapacitance test

Weight-bearing changes were measured using an incapacitance apparatus (Linton Instrumentation, Norfolk, UK) to detect changes in postural equilibrium after a hind limb injury (Bove et al., 2003). Rats were trained to stand on their hind paws in a box with an inclined plane

(65° from horizontal). This box was placed above the incapacitance apparatus. This allowed us to independently measure the weight that the animal applied on each hind limb. The value reported for each animal was the mean of 5 consecutive measurements. In the absence of hind limb injury, rats applied an equal weight on both hind limbs, indicating a postural equilibrium, whereas an unequal distribution of the weight on hind limbs indicated a monolateral decreased pain threshold. Data are expressed as the difference between the weight applied to the limb contralateral to the injury and the weight applied to the ipsilateral one (Δ Weight).

2.7. Statistical analysis

Behavioral measurements were performed on 6 rats for each treatment carried out in 2 different experimental sets. Results were expressed as means ± S.E.M. and the analysis of variance was performed by Two-Way ANOVA. A Bonferroni's significant difference procedure was used as post-hoc comparison. All assessments were made by researchers blinded to cell or rat treatments. Data were analyzed using the "Origin 8.1" software (OriginLab, Northampton, USA).

3. Results

The pain relieving effects of the compounds were evaluated in rats underwent CCI of the sciatic nerve. This rat model of mononeuropathy provokes a painful syndrome beginning about 3 days after nerve ligation and plateauing between 7 and 30 days (Pacini et al., 2010; Di Cesare Mannelli et al., 2014). Fourteen days after injury, the response to a noxious mechanical stimulus was measured by the Paw pressure test. In the CCI + vehicle group the weight tolerated on the ipsilateral paw decreased to 45.3 ± 2.1 g in comparison to 67.3 ± 2.1 g of control animals (sham + vehicle) (Fig. 2). (*S*)-(-)-1 (0.1 and 1 mg/kg p.o.) was able to reduce the hypersensitivity induced by the loose ligation of the sciatic nerve. The anti-hyperalgesic activity took effect 15 min after administration. (*S*)-(-)-**1** (1 mg/kg, 30 min) showed an anti-hyperalgesic efficacy of 56%, considering as 100% the complete reversion of pain till the threshold of control animals. The effect was still statistically significant 60 min after injection and vanished at 90 min (Fig. 2). The response of the contralateral paw was unaltered

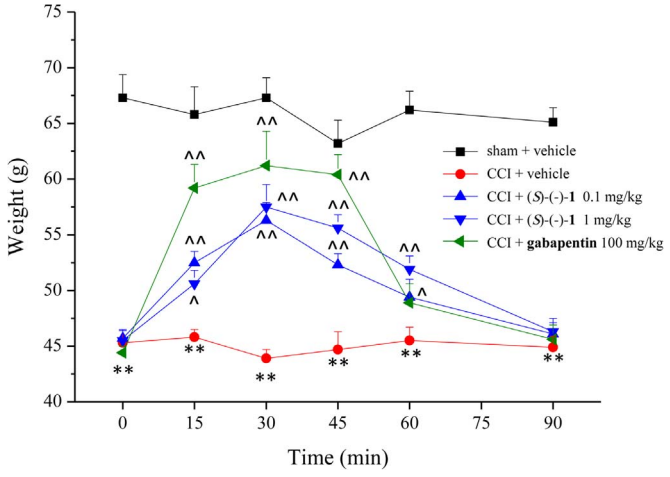

Fig. 2. Effect of acute administration of (*S*)-(-)-**1** in comparison to gabapentin on CCI-induced mechanical hypersensitivity. Peripheral neuropathy was induced by CCI of the right sciatic nerve (ipsilateral); (*S*)-(-)-**1** (0.1–1.0 mg/kg) was acutely administered p.o. on day 14 after surgery. The Paw pressure test was used to measure the sensitivity to a mechanical stimulus 0, 15, 30, 45, 60 and 90 min after compound administration. Gabapentin (100 mg/kg, p.o.) was used as reference compound; sham animals were treated with vehicle. Each value is the mean ± S.E.M. of 6 rats *per* group, performed in 2 different experimental sets. **P < 0.01 *vs* sham + vehicle; ^P < 0.05 and ^^P < 0.01 *vs* CCI + vehicle.

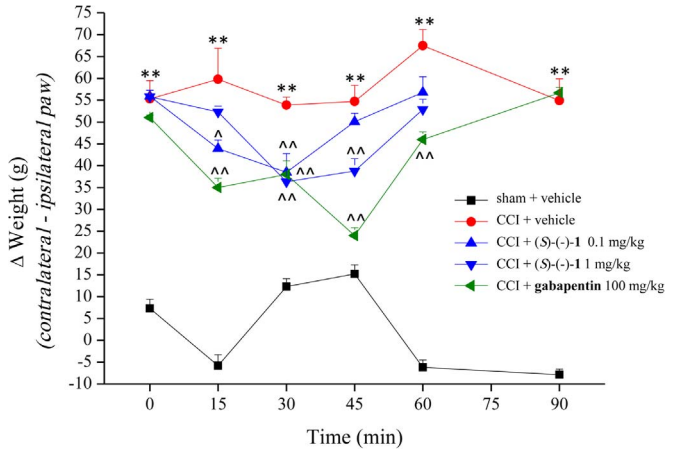

Fig. 3. Effect of acute administration of (*S*)-(-)-**1** in comparison to gabapentin on CCI-induced hyperalgesia. Peripheral neuropathy was induced by CCI of the right sciatic nerve (ipsilateral); (*S*)-(-)-**1** (0.1 – 1 mg/kg) was acutely administered p.o. on day 14 after surgery. Incapacitance test was used to measure postural unbalance related to pain 0, 15, 30, 45, 60 and 90 min after compound administration. Gabapentin (100 mg/kg, p.o.) was used as reference drug and sham animals were treated with vehicle. Data are expressed as the difference between the weight applied to the limb contralateral to the injury and the weight applied to the ipsilateral one (Δ Weight). Each value is the mean ± S.E.M. of 6 rats per group, performed in 2 different experimental sets. **P < 0.01 *vs* sham + vehicle; ^P < 0.05 and ^^P < 0.01 *vs* CCI + vehicle.

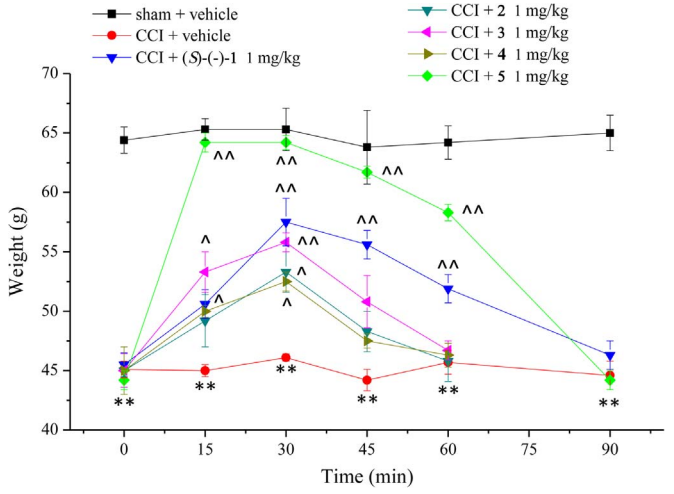

Fig. 4. Effect of acute administration of compounds **2**–**5** in comparison to (*S*)-(-)-**1** on CCI-induced mechanical hypersensitivity. Peripheral neuropathy was induced by CCI of the right sciatic nerve (ipsilateral), compounds were acutely administered p.o. (1.0 mg/kg) on day 14 after surgery. The Paw pressure test was used to measure the sensitivity to a mechanical stimulus 0, 15, 30, 45, 60 and 90 min after compound administration. Sham animals were treated with vehicle. Each value is the mean ± S.E.M. of 6 rats *per* group, performed in 2 different experimental sets. **P < 0.01 *vs* sham + vehicle; ^P < 0.05 and ^^P < 0.01 *vs* CCI + vehicle.

(data not shown) suggesting the lack of analgesic effects. Gabapentin was used as a reference compound since its relevant clinical use in neuropathic patients (Szczudlik et al., 2014). The common and accepted rat dosage of 100 mg/kg p.o. (Di Cesare Mannelli et al., 2015) was active from 15 to 45 min after treatment inducing a relief slightly higher (74% of anti-hyperalgesic efficacy) than (*S*)-(-)-**1** administered at 100 fold lower dose (1 mg/kg) (Fig. 2).

As shown in Fig. 3, monolateral pain was also able to induce hind limb weight bearing alterations (Incapacitance test). The difference between the weight burdened on the contralateral and the ipsilateral paw increased in CCI + vehicle (55.3 ± 4.2 g) in comparison to sham + vehicle (7.3 ± 2.1 g). (*S*)-(-)-**1** (1.0 mg/kg) significantly reduced the hind limb weight bearing alterations in comparison to CCI + vehicle. The effect started 15 min after administration and vanished at 60 min.

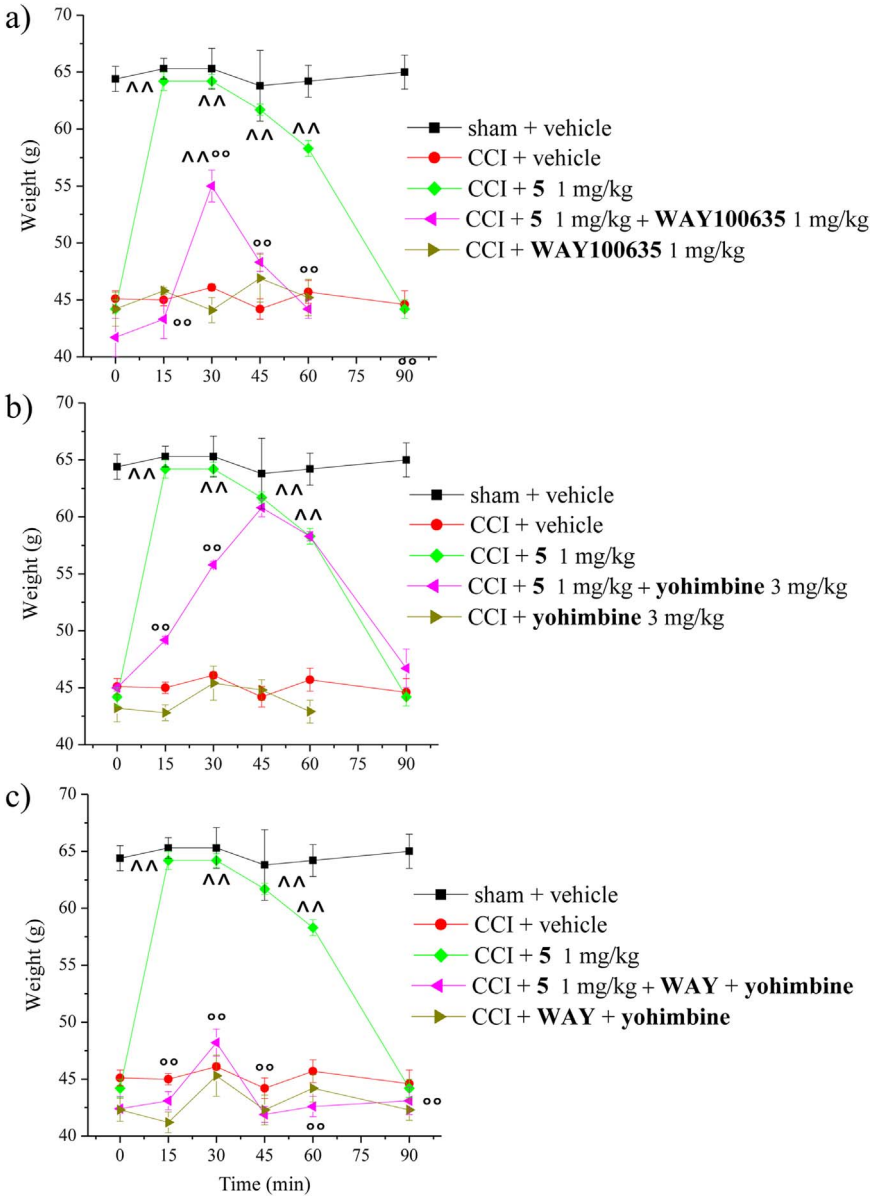

Fig. 5. Evaluation of the 5-HT_{1A} receptor and α_2-adrenoceptor involvement in the pain relieving activity of compound **5**. Peripheral neuropathy was induced by CCI of the right sciatic nerve (ipsilateral). The Paw pressure test was performed on day 14 after surgery. **5** was acutely administered p.o. (1.0 mg/kg), WAY 100635 (1 mg/kg) and yohimbine (3 mg/kg) were administered i.p. 15 min before. Sham animals were treated with vehicle. Each value is the mean ± S.E.M. of 6 rats *per* group, performed in 2 different experimental sets. Data of CCI + vehicle groups are different ($P < 0.01$) *vs* sham + vehicle; ^$P < 0.05$ and ^^$P < 0.01$ *vs* CCI + vehicle; °°$P < 0.01$ vs CCI + **5** treated animals.

A comparable effect was induced by the acute administration of 100 mg/kg gabapentin.

The imidazolines **2**–**5** were also effective in reducing the hypersensitivity induced by the loose ligation of the sciatic nerve 30 min after a single administration p.o. of 1 mg/kg. The percentages of anti-hyperalgesic efficacy (30 min) of **2**, **3**, and **4** are 37.5%, 52.5%, and 35%, respectively; whereas **5** reaches 95% (Fig. 4). The pain killer effect of **5** is long lasting in comparison to **2**, **3** and **4** and the efficacy is higher than (*S*)-(-)-**1**.

The co-administration of **5** with WAY 100635 (1 mg/kg i.p. 15 min before **5**) greatly reduced the pain relieving effect. In Fig. 5 (panel a), a full reversion of **5** activity is shown with the exception of a break-through at 30 min. The blocking effect induced by yohimbine (3 mg/kg i.p. 15 min before **5**) is significant 15 and 30 min after treatment even if lesser in efficacy as well as duration (Fig. 5, panel b). The combined administration of both the antagonists WAY 100635 (1 mg/kg i.p.) and yohimbine (3 mg/kg i.p.), 15 min before **5**, completely prevented the pain relieving effect of compound **5** (Fig. 5, panel c).

4. Discussion

The descending pain modulatory system originates in the midbrain (periaqueductal gray) and brainstem (rostroventromedial medulla) and projects to the spinal cord dorsal horn, powerfully influencing the modulation of nociceptive information transmitted from the periphery to the brain (Von Hehn et al., 2012). The role of noradrenaline in this system appears to be predominantly inhibitory, while the role of serotonin appears to be bidirectional, mediating inhibitory as well as excitatory effects (Ossipov et al., 2010). The increased activity of the descending noradrenergic system and high extracellular levels of spinal noradrenaline has been linked to antinociceptive/antiallodynic/anti-hyperalgesic effects in acute and neuropathic pain (Di Cesare Mannelli et al., 2015a). The 5-HT_{1A} receptor is expressed by all serotoninergic neurons (as autoreceptors) and by many non serotoninergic neurons (as heteroreceptors) in pain-related areas of the brain (Kalipatnapu and Chattopadhyay, 2007). As already mentioned, 5-HT_{1A} receptor agonists have shown to produce efficacious antinociceptive effect in rats

Table 1
[a]Affinity (pK_i), Antagonist Potency (pK_b), Agonist Potency (pEC_{50}), Intrinsic Activity (ia) on Human α_2 receptor Subtypes[b]; Affinity (pK_i), Agonist Potency (pD_2), Relative Efficacy (% E_{max}) on Human 5-HT_{1A} receptor.[c]

Compound	α_{2A}			α_{2B}			α_{2C}			5-HT_{1A}		
	pK_i	pEC_{50} (pK_b)	ia	pK_i	pEC_{50}	ia	pK_i	pEC_{50}	ia	pK_i	pD_2	$\%E_{max}$
1 Biphenyline	7.32[d]	6.94[d]	0.70[d]	6.30[d]	6.19[d]	0.50[d]	6.70[d]	7.24[d]	0.80[d]	7.34[e]	6.30[e]	120.2[e]
(*S*)-(-)-**1**	7.04[d]	7.13[d]	0.80[d]	6.23[d]	6.16[d]	0.65[d]	6.52[d]	7.73[d]	0.90[d]	7.60[e]	–	–
2	6.86[f]	6.50[f]	0.50[f]	6.03	6.00[f]	0.50[f]	7.19[f]	7.30[f]	1.00[f]	7.10[e]	6.58[e]	79.34[e]
3	7.83[f]	6.98[f]	0.58[f]	6.57[f]	NA[f]	–	7.77[f]	7.22[f]	1.15[f]	7.60[e]	6.85[e]	79.55[e]
4	7.64[g]	(7.00)[g]	–	6.51[g]	5.50[g]	0.70[g]	7.10[g]	7.40[g]	0.90[g]	8.03[e]	7.24[e]	92[e]
5	7.13[h]	7.10[h]	0.60[h]	6.72[h]	6.70[h]	0.70[h]	7.11[h]	7.68[h]	0.80[h]	8.25[e]	–	–

[a] The data were expressed as means of 3–6 separate experiments.
[b] According to Del Bello et al. (2010).
[c] According to Quaglia et al. (2008).
[d] See Crassous et al. (2007).
[e] See Del Bello et al. (2016).
[f] See Gentili et al. (2004).
[g] See Cardinaletti et al. (2009).
[h] See Gentili et al. (2008).

(Nadeson and Goodchild, 2002). In particular, the 5-HT_{1A} agonist F13640 provided the first evidence that the activation of this receptor produces analgesia in a model of trigeminal neuropathic pain. It is characterized by long-term analgesia in rodent models of peripheral neuropathic pain and anti-allodynic action in spinal cord injured rat (Colpaert et al., 2002; Deseure et al., 2002). As regards the α_2 adrenergic receptor, the α_{2A}, α_{2B} and α_{2C} subtypes have been identified as potential contributors to nociceptive modulation being located in key nodes of the nociceptive system. In the spinal cord, the α_2 receptor stimulation induces analgesia by post-synaptic hyperpolarization and presynaptic inhibition of the excitatory transmission (Di Cesare Mannelli et al., 2015a).

The double-face profile of the tested imidazoline compounds allows the simultaneous modulation of neurotransmitter signals of great relevance in pain mechanism. In particular, the efficacy of the 5-HT_{1A} receptor and α_2 receptor agonist (*S*)-(-)-**1** in reducing the hypersensitivity and the hind limb weight bearing alterations induced by the loose ligation of the sciatic nerve allows us to hypothesize the involvement of both the receptor systems in its anti-neuropathic activity.

The experiments performed with the selected imidazolines **2**–**5** provided further information about the role played by both 5-HT_{1A} and α_2 receptors. The compounds have been selected considering their good but different affinities for 5-HT_{1A} receptor and similar agonist activities at α_{2C} subtype. They all behave as agonists at α_{2A} subtype, except for compound **4**, which acts as an antagonist (Table 1). Based on the observation that α_{2A} subtype has been demonstrated to be critical for the pain relieving actions of α_2 receptor agonists in a mouse model of neuropathic pain (Malmberg et al., 2001), this last compound might confirm such a finding from a pharmacological point of view. Derivative **5**, showing the highest affinity for 5-HT_{1A} receptor and agonist activity at all the α_2 receptor subtypes, is the most effective compound in reducing mechanical hypersensitivity. Compared to **5**, the lower efficacy of (*S*)-(-)-**1**, **2** and **3** in in vivo studies might be attributed to their lower affinity for 5-HT_{1A} receptor (Table 1). Though derivative **4** is characterized by 5-HT_{1A} receptor affinity similar to that of **5** (Table 1), it also proved to be less effective in reducing the neuropathic pain. This behaviour might result from its α_{2A} antagonist profile, supporting the finding that α_{2A} receptor subtype is essential for the anti-neuropathic effects mediated by α_2 receptor agonists (Malmberg et al., 2001). The comparable anti-neuropathic efficacy of the α_{2A} antagonist **4** and the α_{2A} agonist **2** might be due to the significantly higher affinity and agonist activity for 5-HT_{1A} receptor of **4** with respect to **2**. The experiments performed with derivative **5** in the presence of the 5-HT_{1A} receptor antagonist WAY 100135 and the α_2 receptor antagonist yohimbine confirm the relevance of the positive modulation of both serotoninergic and adrenergic system in the pharmacodynamic mechanism of such a compound. Therefore, the double 5-HT_{1A}/α_2 agonist profile of the studied imidazoline derivatives can produce a fruitful modulation of the descending antinociceptive pathway. Moreover, the observation that yohimbine reduces the pain relieving effect at lesser extent than WAY 100635 suggests a predominance of the 5-HT_{1A} component.

5. Conclusion

In conclusion, the present study (i) highlights that the bioversatile 2-imidazoline substituted scaffold might be a valuable structure in the building of novel anti-neuropathic agents. (ii) Noteworthy, the multifunctional agent (*S*)-(-)**-1** is able to reduce neuropathic pain at the dose of 1.0 mg/kg, being 100 fold more potent than the clinically used gabapentin. (iii) Moreover, compounds **2**–**5** show different efficacies and potencies after *per os* treatment of CCI rats, providing useful information on the role played by the 5-HT_{1A} and α_2 receptors in their neuropathic pain relieving effect. In particular **5**, showing the highest affinity for the 5-HT_{1A} receptor and agonist activity at all the α_2 receptor subtypes, is the most effective compound in reducing mechanical hypersensitivity through a mechanism significantly inhibited by the pharmacological blockade of 5-HT_{1A} or α_2 receptors. Therefore, the combined 5-HT_{1A}/α_2 receptors activation might be considered a promising pharmacodynamic approach to the treatment of neuropathic pain.

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