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Activation of I_2-imidazoline receptors enhances supraspinal morphine analgesia in mice: a model to detect agonist and antagonist activities at these receptors

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1 This work investigates the receptor acted upon by imidazoline compounds in the modulation of morphine analgesia. The effects of highly selective imidazoline ligands on the supraspinal antinociception induced by morphine in mice were determined.

2 Intracerebroventricular (i.c.v.) or subcutaneous (s.c.) administration of ligands selective for the I_2-imidazoline receptor, 2-BFI, LSL 60101, LSL 61122 and aganodine, and the non selective ligand agmatine, increased morphine antinociception in a dose-dependent manner. Neither moxonidine, a mixed I_1-imidazoline and α_2-adrenoceptor agonist, RX821002, a potent α_2-adrenoceptor antagonist that displays low affinity at I_2-imidazoline receptors, nor the selective non-imidazoline α_2-adrenoceptor antagonist RS-15385-197, modified the analgesic responses to morphine.

3 Administration of pertussis toxin (0.25 μg per mouse, i.c.v.) 6 days before the analgesic test blocked the ability of the I_2-imidazoline ligands to potentiate morphine antinociception.

4 The increased effect of morphine induced by I_2-imidazoline ligands (agonists) was completely reversed by idazoxan and BU 224. Identical results were obtained with IBI, which alkylates I_2-imidazoline binding sites. Thus, both agonist and antagonist properties of imidazoline ligands at the I_2-imidazoline receptors were observed.

5 Pre-treatment (30 min) with deprenyl, an irreversible inhibitor of monoamine oxidase B (IMAO-B), produced an increase of morphine antinociception. Clorgyline, an irreversible IMAO-A, given 30 min before morphine did not alter the effect of the opioid. At longer intervals (24 h) a single dose of either clorgyline or deprenyl reduced the density of I_2-imidazoline receptors and prevented the I_2-mediated potentiation of morphine analgesia.

6 These results demonstrate functional interaction between I_2-imidazoline and opioid receptors. The involvement of G_i-G_o transducer proteins in this modulatory effect is also suggested.
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Keywords: Imidazoline receptors; potentiation of morphine-effects; agonists -antagonists at I_2-imidazoline receptors; supraspinal antinociception; GTP-binding proteins

Abbreviations: G proteins, GTP-binding proteins; i.c.v., intracerebroventricular; MAO, monoamine oxidase; s.c, subcutaneous

Introduction

Various imidazol(ine)-guanidine compounds, such as clonidine or idazoxan, elicit central and peripheral effects through their binding to non-adrenoceptor sites, the imidazoline receptors (Bousquet *et al*., 1984; Regunathan & Reis 1996). Decarboxylated arginine, agmatine, is proposed as an endogenous ligand for these receptors (Li *et al*., 1994; Piletz *et al*., 1995). On the bases of pharmacological profiles and tissue and subcellular distributions, the imidazoline receptors were initially divided into two main types: I_1-receptors labelled by [^{3}H]-clonidine and their derivatives (Molderings *et al*., 1993), and I_2-receptors which show high affinity for [^{3}H]-idazoxan (Michel & Insel, 1989; Ernsberger, 1992). I_1-imidazoline receptors show a discrete distribution in the brain and appear to regulate the release of prostaglandins and the influx of Ca^{2+} ions (Regunathan *et al*., 1991). I_2-imidazoline receptors, however, are widely distributed in the brain and are found in both neurones and glial cells (Regunathan *et al*., 1993; Ruggiero *et al*., 1998) although their functional role is still to be fully established. *In vitro* studies have suggested possible structural and functional relationships between I_2-imidazoline receptors and monoamine oxidase A and B (MAOs), two mitochondrial enzymes involved in the oxidate deamination of neurotransmitters (Tesson *et al*., 1995; Raddatz *et al*., 1995).

Besides the well-known influence of α_2-adrenoceptors on opioid analgesia and tolerance, imidazoline receptors have also been implicated in certain morphine effects. Administration of agmatine prevents or attenuates the development of tolerance to morphine and to other opioid agonists in the mouse (Kolesnikov *et al*., 1996). In addition, the concurrent chronic administration of idazoxan -or of more selective-potent I_2-imidazoline receptor ligands- and morphine attenuates the development of tolerance to the opioid in rats (Boronat *et al*., 1998a).

The presence of imidazoline receptors has recently been described in brain areas involved in perception and response to painful stimuli (Ruggiero *et al*., 1998). It might be the neural substrate acted upon by agmatine to enhance dose-dependently morphine antinociception in mice (Kolesnikov *et al*., 1996). However, agmatine binds with poor selectivity to both α_2-adrenoceptors and imidazoline (l_1 and l_2) receptors (Li *et al*., 1994). It is therefore difficult to ascribe its modulatory activity

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on morphine antinociception to its binding to one of these receptor types -more selective ligands are required. The present paper investigates the participation of I_1-I_2 imidazoline receptors in the increase of morphine antinociception produced by imidazoline compounds. Besides agmatine and idazoxan, the highly selective and potent I_2-imidazoline ligands such as [2-(2-benzofuranyl)-2-imidazoline], 2-BFI (Lione *et al*., 1996; Alemany *et al*., 1997), [2-(2-benzofuranyl) imidazole HCl], LSL 60101 (Alemany *et al*., 1995), [2-styryl-2-imidazoline HCl, valldemossine], LSL 61122 (Ozaita *et al*., 1997), [2-(4,5-dihydroimidazol-2yl)-quinoline HCl], BU 224 (Hudson *et al*., 1996), aganodine, a guanidine compound displaying nanomolar affinity for I_2-imidazoline receptors (Alemany *et al*., 1997), and the irreversible I_2-imidazoline ligand (isothiocyanatobenzyl imidazoline), IBI (Boronat *et al*., 1998b), were included in the study. The effects of moxonidine, I_1-imidazoline receptor ligand (Ernsberger *et al*., 1993; Likungu *et al*., 1996), [2-methoxy idazoxan], RX 821002, potent α_2-adrenoceptor antagonist that displays low affinity for I_2-imidazoline receptors (Galitzky *et al*., 1990; Miralles *et al*., 1993) and ((8a*R*, 12aS, 13aS)-3-methoxy-12-methane sulphonyl-5,6,8a,9,10,11,12,12a,13,13a-decahydro-8H-isoquino [2, 1-g]-naphthyridine), RS-15385-197, highly potent and selective non-imidazoline α_2-adrenoceptor antagonist (Brown *et al*., 1993), were also evaluated.

Methods

Animals and injection techniques

Male albino mice CD-1 (Charles River, Barcelona, Spain) weighing 22–25 g, were used throughout. The animals were housed in groups of 10 in a temperature- (22°C) and humidity-controlled environment for 4–5 days before experimentation, under a 12-h light-dark cycle (08.00–20.00 h). Food and water were provided *ad libitum*. Mice were housed and used strictly in accordance with the guidelines of the European Community on the Care and Use of Laboratory Animals (Council Directive 86-609-EEC). To reduce the possibility of interference from spinal events, all substances were injected i.c.v. in 4 μl volumes into the right lateral ventricle as previously described (Sánchez-Blázquez *et al*., 1995). Briefly, mice were lightly anaesthetized with ether and injections were given with a 10 μl Hamilton syringe. Animals received either the vehicle (saline) or the drug of study 30 min before the injection of morphine. When two drugs were injected, administrations were performed at 5 or 10 min intervals. All compounds were dissolved in distilled water, except for moxonidine which was dissolved in 20 mM HCl and 9.25 mM NaOH. Solutions were made up immediately before use.

Mice were i.c.v. injected with 0.25 μg pertussis toxin and the effect of I_2-ligands on morphine antinociception was evaluated 6 days later (Sánchez-Blázquez & Garzón, 1988). In another series of assays, animals were i.c.v. injected with either 40 μg of the irreversible I_2-imidazoline ligand IBI, or 10 μg clorgyline or deprenyl. At 24 h the effects of 2-BFI, LSL 61122 and aganodine were evaluated on morphine antinociception.

Thermal antinociceptive assay

The response of the animals to nociceptive stimuli was determined in the warm water tail-flick test using different temperatures. Base-line latencies ranged from 1.5–2.5 s at 52°C, and from 3.8–5.1 s at 45°C. A cut off time of 10 or 20 s, respectively, was allotted to minimize the risk of tissue damage. Antinociception was expressed as a per cent of the maximum possible effect (MPE) according to the following formula: $\%MPE = 100 \times (\text{test latency} - \text{base-line latency})\ (\text{cut off time} - \text{base-line latency})^{-1}$. A single i.c.v. dose of morphine was given and antinociception was assessed 30 min later. The results underwent analysis of variance (ANOVA) followed by the Student-Newman-Keuls test. The level of significance was set at $P<0.05$.

Chemicals

Morphine sulphate was obtained from Merck (Darmstadt, Germany), agmatine sulphate and clorgyline HCl from Sigma-Aldrich Chemicals (Madrid, Spain), BU-224, rilmenidine and AGN192403 from Tocris Cookson (Bristol, U.K.), moxonidine and aganodine from Beiersdorf (Hamburg, Germany), RS-15385-197 HCl, from Synthex (Palo Alto, CA, U.S.A.), Ro16-649 [N-(2-aminoethyl-p-chlorobenzamide] HCl from F. Hoffmann-La Roche, Ldt (Switzerland), deprenyl from R.B.I. (Natick, MA, U.S.A.) and pertussis toxin from List Biological Laboratories (Campbell, CA, U.S.A.). 2-BFI was synthesized by Dr Plá as LSL 61103 (S.A. Lasa Laboratorios, Spain), idazoxan HCl, LSL 60101, LSL 61122 and RX821002 HCl were synthesized by Dr F. Geijo (S.A. Lasa Laboratorios, Spain). IBI was generously provided by Drs D.D. Miller (University of Tennessee, Memphis, U.S.A.) and P.N. Patil (The Ohio State University, Columbus, U.S.A.).

Results

Effects of agmatine and other I_2-imidazoline ligands on morphine induced antinociception

In agreement with previous studies, agmatine (10 μg per mouse, i.c.v.) administered 30 min before morphine (1 μg per mouse, i.c.v.) increased the antinociceptive response to the opioid compared to mice receiving saline injections (Kolesnikov *et al*., 1996; Figure 1), as measured by the 52°C hot-water tail-flick test. Further, a fixed dose (10 μg per mouse) of 2-BFI, LSL 61122, LSL 60101 or aganodine also raised morphine

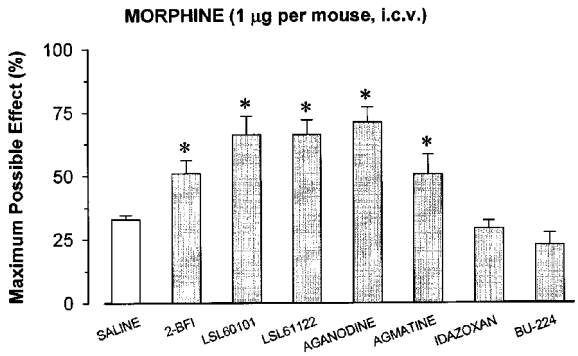

Figure 1 Effect of I_2-imidazoline ligands on the supraspinal antinociceptive effect induced by morphine in the 52°C hot water tail-flick test. Mice were injected with a fixed dose of imidazolines (10 μg per mouse) 30 min before morphine and the antinociceptive activity was evaluated by the tail-flick test. All compounds were i.c.v. injected in volumes of 4 μl. For each treatment a different group of animals were used. Latencies were measured before and 30 min after administration of morphine. Antinociception is expressed as a percentage of the maximum possible effect (MPE). Values are means ± s.e.mean from groups of 10–20 mice each. *Significantly different from the control group receiving saline. Analysis of variance, Student-Newman-Keuls test, $P<0.05$.

antinociception. In this scheme, idazoxan did not alter the analgesic effect of morphine (29.3±3.2 and 33.6±1.6% MPE in idazoxan- and saline-treated groups, respectively). Negative results were also obtained with BU-224, a highly potent and selective I_2 imidazoline compound (Figure 1). Peripheral administration of these imidazolines (10 mg kg^{-1}, s.c.) produced equivalent results on morphine antinociception (Table 1). The imidazoline compounds used alone did not affect the responses of the animals in the tail-flick test, which were identical to those of mice receiving only saline injections. The lack of effect on baseline response was confirmed using a lower temperature that gave longer latencies. Thus, when the bath temperature was set at 45°C, baseline latencies after saline, 2-BFI (10 mg kg^{-1}, i.c.v.) and LSL61122 (10 mg kg^{-1}, i.c.v.) were 4.46±0.25, 4.53±0.27 and 4.42±0.28 respectively. In this experimental protocol both imidazoline compounds were able to increase morphine-induced antinociception (40.9±3.7 to 74.7±8.7 and 68.37±7.4% MPE in saline, 2-BFI and LSL61122 treated mice). This observation discards the possibility of any additive effects between morphine and the imidazoline compounds.

The effects of increasing doses of 2-BFI or LSL61122 (0.01, 0.3, 10 and 30 μg per mouse, i.c.v.) were also tested upon the antinociceptive response to a fixed dose of morphine (1 μg per mouse, i.c.v.). Administration of the I_2-imidazoline selective ligands 30 min before the opioid produced a significant dose-dependent potentiation of the analgesic action of morphine (Figure 2A). Thus, injection of 0.3, 10 and 30 μg per mouse of 2-BFI significantly enhanced morphine analgesia (25.3±4.1, 52.6±5.4 and 85.3±14.0% MPE, respectively) when compared to control (saline-pretreated mice), while the lower dose of 0.01 μg had no effect on morphine antinociception. Pretreatment of mice with 10 μg of 2-BFI 30 min before morphine administration resulted in a 2.5 fold leftwards shift of the opioid dose-response curve (Figure 2B).

Modulatory effects of imidazoline ligands on morphine analgesia: lack of involvement of I_1-imidazoline receptors and α_2-adrenoceptors

The involvement of I_1 receptors and α_2-adrenoceptors in the modulation of morphine analgesia was also evaluated. Various compounds with affinity for these receptors were tested. Moxonidine, a mixed agonist at I_1-imidazoline receptors and α_2-adrenoceptors, given (1 μg per mouse, i.c.v.) 30 min before morphine did not alter the analgesic effect of the opioid (Table 2). Rilmenidine or AGN192403 (10 μg per mouse, i.c.v.) were also devoid of effect on morphine antinociception (Table 2). Negative results were obtained when RX821002, or the highly potent and selective non-imidazoline α_2-adrenoceptor antagonist RS-15385-197, were administered before the opioid (Table 2). When injected alone, these α_2-adrenoceptor and -or I_1-imidazoline receptor ligands exhibited no analgesic or hiperalgesic effects (data not shown). These results exclude the involvement of α_2-adrenoceptors and I_1-imidazoline receptors in the modulation of morphine antinociception.

Table 1 Effect of subcutaneous administration of I_2-imidazoline ligands on morphine-induced antinociception

	Morphine induced analgesia (% MPE)	
I_2-imidazoline ligands	*Saline pretreatment*	*IBI pretreatment*
None (saline)	37.2±2.7	23.7±3.4
2-BFI	59.3±2.7*	38.5±1.6
LSL61122	59.7±4.0*	22.8±2.0

Animals were pre-treated with either saline vehicle or a single dose of the I_2-imidazoline alkylating agent IBI (40 μg per mouse, i.c.v.). Twenty-four hours later the I_2-imidazoline agonists were injected at a dose of 10 mg kg^{-1}, s.c., 30 min before the administration of morphine (1 μg per mouse, i.c.v.). Values are the means±s.e.mean from groups of 10 animals. *Indicates significant differences, analysis of variance (ANOVA) followed by the Student-Newman-Keuls test, $P<0.05$.

Identification of antagonist properties of imidazoline ligands at the I_2-imidazoline receptors

The possibility of idazoxan and BU-224 acting as antagonists at I_2-imidazoline receptors was subsequently explored. Each compound was i.c.v. coadministered (10 μg per mouse) with either 2-BFI, aganodine, LSL 60101, LSL 61122 or agmatine before evaluating opioid-evoked analgesia. In these animals, idazoxan and BU-224 completely blocked the potentiation of morphine antinociception induced by the imidazoline compounds (Table 3), suggesting that these compounds behave as I_2-imidazoline receptor antagonists. Moreover, an i.c.v. dose of 40 μg per mouse IBI -an alkylating ligand at I_2-imidazoline receptors- given 24 h before studying the action of I_2 compounds on morphine-evoked antinociception, also blocked

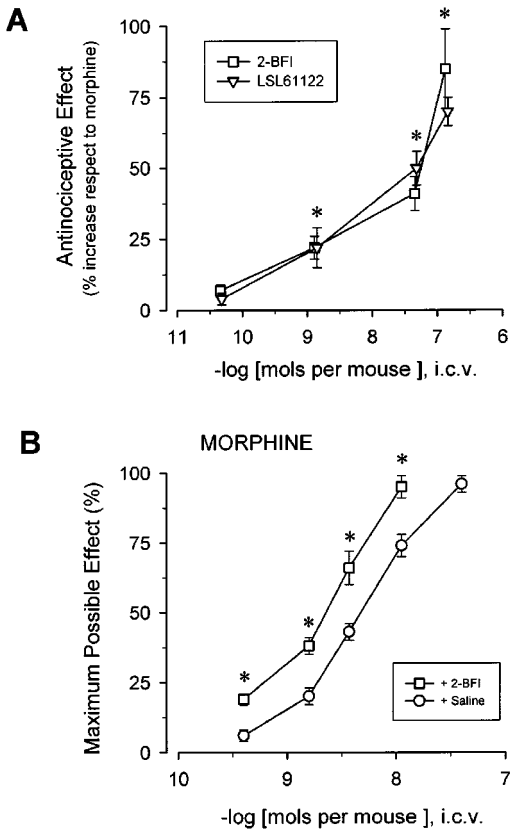

Figure 2 Effect of 2-BFI and LSL61122 on morphine antinociception (A) the effect of increased doses of 2-BFI and LSL61122 were tested upon the antinociceptive response to a fixed dose of morphine (1 μg per mouse, i.c.v.). Imidazoline compounds were given 30 min before morphine administration. The data indicate the percentage of increase respect to the antinociceptive effect induced by morphine alone (35.6±2.8% MPE). (B) Dose-response curves for morphine antinociception in mice were constructed in the absence and presence of 2-BFI (10 μg per mouse). Antinociception was evaluated in the 52°C hot-water tail-flick test and is expressed as a percentage of the maximum possible effect (MPE). All values are means±s.e.mean from groups of 10–15 mice. *Significantly different from the control group receiving saline instead of imidazoline. Analysis of variance, Student-Newman-Keuls test, $P<0.05$.

the potentiation of morphine analgesia (Figure 3). Thus, a clear modulatory role of I_2-imidazoline receptors on morphine antinociception was established.

Effect of monoamine oxidase inhibitors on morphine induced antinociception

Pre-treatment of mice with deprenyl (10 μg per mouse, i.c.v.), an irreversible inhibitor of MAO-B, 30 min before the opioid, potentiated morphine supraspinal analgesia (Table 2). The antinociceptive effect of the alkaloid significantly increased with respect to that exhibited by mice receiving only saline. Analgesic values for morphine were 35.3 ± 2.3 versus 46.5 ± 4.7% MPE in control (saline-treated) and deprenyl-treated mice. In this experimental protocol, clorgyline (an irreversible IMAO-A) and Ro 19-6327 (selective IMAO-B) did not enhance morphine antinociception (Table 2). Co-administration of deprenyl and 2-BFI produced no further increase of the analgesic potency of morphine (saline-treated: 35.3 ± 2.3% MPE, 2-BFI-, deprenyl- and 2-BFI plus deprenyl-treated groups: 52.6 ± 5.4, 46.5 ± 4.7 and 45.4 ± 4.9% MPE, respectively), suggesting that a unique binding site, presumably the I_2-imidazoline receptor, is involved in the effect of both compounds. Clorgyline or deprenyl, given 24 h before morphine prevented the potentiation of morphine analgesia induced by imidazoline compounds (not shown).

Table 2 Effect of Imidazoline compounds, α_2-adrenoceptor ligands and MAO inhibitors on morphine antinociception

Drug	n	*Percent maximum possible Effect* (% MPE)	*Affinity at I_2- imidazoline receptors* (K_i, μM)[a]
Saline	35	35.3 ± 2.3	–
Agmatine	16	51.6 ± 8.2*	294
I_2-imidazoline ligands			
2-BFI	32	52.6 ± 5.4*	0.008
LSL61122	16	65.8 ± 6.2*	0.0001
I_1-imidazoline ligands			
AGN 192403	10	33.4 ± 6.5	N.D.
Rilmenidine	10	30.5 ± 4.3	1.5
Moxonidine	10	25.7 ± 4.8	5.0
α_2-adrenoceptor ligands			
RX821002	10	27.3 ± 5.3	45
RS-15385-197	10	29.7 ± 4.3	>300
MAO inhibitors			
Clorgyline	16	17.0 ± 2.2	0.054
Deprenyl	16	46.5 ± 4.7*	1.4
Ro 19-6327	16	34.4 ± 2.6	2.1

Compounds (10 μg per mouse) were injected 30 min before morphine (1 μg per mouse). Results in per cent MPE are expressed as means$\pm$s.e.mean, from *n* mice per group. *Indicates significant differences, analysis of variance (ANOVA) followed by the Student-Newman-Keuls test, $P<0.05$. [a]Binding parameters (K_i values) for I_2-imidazoline receptors were taken from Alemany *et al.* (1995; 1997); Lione *et al.* (1996); Miralles *et al.* (1993); Olmos *et al.* (1993) and Ozaita *et al* (1997).

Effect of pretreatment with pertussis toxin on the modulation of morphine antinociception by I_2-imidazoline ligands

Administration of pertussis toxin (0.25 μg per mouse, i.c.v.) 6 days before the analgesic test does not modify basal latencies but leads to a loss of morphine antinociceptive potency (Sánchez-Blázquez & Garzón, 1988; present work). The present work shows that in mice with pertussis toxin-impaired GTP-binding G_i-G_o proteins, the ability of the I_2-imidazoline agonists to potentiate morphine antinociception is completely blocked (Figure 4). Thus, the involvement of G_i-G_o transducer proteins in the modulation of morphine supraspinal antinociception exerted by I_2-imidazoline receptors cannot be disregarded.

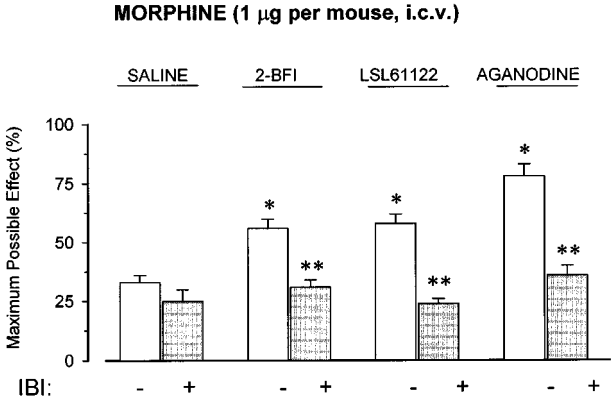

Figure 3 Blockade by IBI of the potentiation of morphine analgesia induced by I_2-imidazoline agonists. The alkylating ligand for the I_2-imidazoline receptors, IBI (40 μg per mouse, i.c.v.), was injected 24 h before the antinociceptive test. Imidazoline compounds (10 μg per mouse) were given 30 min before morphine administration. Latencies were measured 30 min after morphine. For each treatment a different group of animals was used. Antinociception was evaluated in the 52°C hot-water tail-flick test and is expressed as a percentage of the maximum possible effect (MPE). Values are means$\pm$s.e.mean from groups of 10–15 mice each. *Significantly different from the control group receiving saline; **from the corresponding group not receiving IBI. Analysis of variance, Student-Newman-Keuls test, $P<0.05$.

Table 3 Antagonist effect of idazoxan and BU-224 on the potentiation of morphine antinociception induced by I_2-imidazoline compounds

Drug	n	*+ Saline*	n	*+ Idaxozan*	n	*+ BU-224*
Saline	35	35.3 ± 2.3	10	29.8 ± 3.2	10	23.8 ± 4.8
2-BFI	26	51.4 ± 5.1*	12	26.7 ± 4.4	12	25.1 ± 2.4
LSL 60101	17	58.5 ± 7.3*	10	24.4 ± 3.8	10	24.6 ± 2.2
LSL 61122	20	62.7 ± 4.8*	8	37.2 ± 8.9	8	35.5 ± 6.8
Aganodine	21	75.3 ± 5.3*	8	36.4 ± 4.1	8	53.8 ± 10.5
Agmatine	10	51.7 ± 7.3*	8	29.4 ± 2.6	8	32.2 ± 4.1

Imidazoline ligands (10 μg per mouse, i.c.v.) were injected before a fixed dose of morphine (1 μg per mouse), following the protocol described in Methods. Results in per cent MPE are expressed as means$\pm$s.e.mean, from *n* mice per group. *Indicates significant differences versus saline-treated animals, analysis of variance (ANOVA) followed by the student-Newman-Keuls test, $P<0.05$.

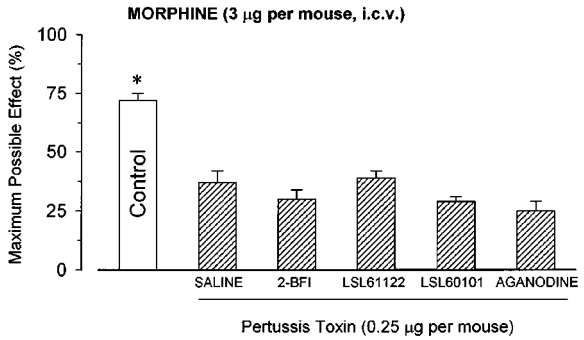

Figure 4 Effect of pertussis toxin on the potentiation of morphine analgesia induced by the putative I_2-imidazoline agonists. The toxin (0.25 μg per mouse, i.c.v.) was injected 6 days before the analgesic test (see Methods). Imidazoline compounds (10 μg per mouse) were given 30 min before morphine administration and latencies were measured 30 min after administration of morphine. For each treatment a different group of animals was used. Antinociception was evaluated in the 52°C hot-water tail-flick test and is expressed as a percentage of the maximum possible effect (MPE). Values are the means ± s.e.mean from groups of 10–12 mice each. *Significantly different from the group treated with pertussis toxin and receiving saline. Analysis of variance, Student-Newman-Keuls test, $P<0.05$.

Discussion

The present investigation suggests the existence of functional interactions between imidazoline and opioid receptors. The most relevant findings of the work are that, (i) central (i.c.v.) or peripheral (s.c.) administration of various I_2-imidazoline ligands, but not I_1-imidazoline or α_2-adrenoceptor ligands, potentiated morphine-induced supraspinal antinociception in mice; (ii) the potentiation of morphine antinociception induced by I_2-imidazoline ligands (agonists) was completely reversed by co-treatment with idazoxan or BU-224, which would act as putative I_2-imidazoline antagonists; and (iii) the impairment of the functional state of G_i-G_o proteins by *in vivo* administration of pertussis toxin hindered the effect of I_2-imidazoline ligands on morphine analgesia.

The literature describing the potential biological effects mediated by I_2-imidazoline receptors is incomplete since no intracellular signal transduction pathway has yet been identified. Ligand binding studies suggest a linkage between some types of K^+ channels and I_2-imidazoline receptors (Sakuta & Okamoto, 1994). There are also reports describing connections with insulin secretion, modulation of noradrenaline release and the modulation of ion fluxes (Regunathan & Reis, 1996). Recent studies have investigated the effects of the putative endogenous imidazoline receptor ligand agmatine in spinal nociception. This endogenous substance produces, *via* non-adrenergic receptors, inhibition of the reflex responses to noxious stimuli in spinal rats (Bradley & Headley, 1997). Kolesnikov and co-workers (1996) have also demonstrated that imidazoline receptors are responsible for the potentiation of intrathecal opioid analgesia. Moreover, BU-224 reduces the responsiveness of dorsal horn neurons to noxious stimuli, presumably by acting at I_2-imidazoline receptors (Diaz *et al*., 1997). However, in an acute arthritis model, intrathecal RS-45041-190 was shown to be hyperalgesic. These observations suggest that spinal I_2-imidazoline receptors control hyperexcitability in inflammation (Houghton & Westlund, 1996).

The putative I_2-imidazoline agonists used in the study exhibited no antinociceptive or hyperalgesic effects by themselves, but were able to potentiate in a dose-dependent manner the supraspinal antinociception induced by morphine. This regulatory effect agrees with a previous study showing that a single dose of agmatine (10 mg kg^{-1}, s.c.) enhances morphine antinociception in naive mice (Kolesnikov *et al*., 1996). However, in naive rats, agmatine and other I_2-imidazoline ligands lack this effect (Boronat *et al*., 1998a). This discrepancy might be a consequence of species-related variations or may be due to the differences in experimental protocols used.

Despite the effort devoted to the study of imidazoline compounds and their receptors, it has remained uncertain whether ligands binding to this type of receptor display agonist or antagonist properties. However, the results of present work discriminate agonist and antagonist activities at the I_2-receptors in the modulation of supraspinal opioid antinociception. The potentiation of morphine effects induced by I_2-imidazoline agonists was completely reversed by the I_2-imidazoline ligands idazoxan and BU-224. The possibility that idazoxan binds to I_2-imidazoline receptors as an antagonist is of interest since it would account for the inefficacy of this compound to inhibit the MAO (Carpéné *et al*., 1995), and the inefficacy of GTP and its analogues to reduce [^{3}H]-idazoxan binding at these I_2-receptors (Langin *et al*., 1990; Zonnenschein *et al*., 1990).

The manner in which I_2-imidazoline agonists influence opioid-induced antinociception is unclear. Certainly ATP-sensitive potassium channels seem to be implicated in the production of morphine antinociception (Ocaña *et al*., 1990), and several imidazolines are described as being able to block K_{ATP} currents, though by a mechanism not well understood (Sakuta & Okamoto, 1994). However, while the antinociceptive effect of morphine was antagonized by gliblenclamide, a compound which blocks ATP-sensitive potassium channels, I_2-imidazoline compounds increased morphine analgesia. Thus, it is unlikely that the blockage of ATP-sensitive potassium channels by imidazolines can be directly related to the modulation of opioid antinociception. Still, the inhibition of MAO activity by imidazoline compounds could explain some biological effects of these substances. In fact, imidazoline ligands are reported to regulate certain processes in CNS that involve MAO activities (Tesson & Parini, 1991; Sastre & García-Sevilla, 1993). The present work reveals that the profile of imidazoline agonists in the modulation of morphine antinociception is much like that of the MAO-B inhibitor and the I_2-imidazoline ligand, deprenyl. Administration to mice of this IMAO 30 to 60 min before the opioid results in MAO-B inhibition and an increase in morphine supraspinal analgesia (Fuentes *et al*., 1977; present work). However, another selective MAO-B inhibitor -but not an I_2-imidazoline ligand- Ro 19-6327, lacked this effect on morphine antinociception. Also, clorgyline, an inhibitor of the MAO-A given 30 min before morphine, displayed no effect on morphine antinociception (Fuentes *et al*., 1977; present work). In addition, the present data show the quality of the effect of deprenyl on morphine analgesia to depend upon the dose employed and the interval allowed before the opioid is given. Twenty four hours after a single dose of deprenyl or clorgyline, which reduced the number of I_2-imidazoline binding sites (Olmos *et al*., 1993), these MAO inhibitors did not alter morphine analgesia but blocked the potentiation induced by I_2-imidazoline agonists on this opioid activity. Similar effects were obtained alkylating the I_2-imidazoline receptors with IBI. These findings argue against a direct role for the MAO enzyme in the modulation of morphine antinociceptive effects.

An additional issue of interest is the participation of G proteins in the regulatory effect exerted by imidazoline compounds on morphine antinociception. That the binding

of ligands to I_2-imidazoline receptors is not affected by GTP apparently excludes coupling to G proteins. However, the blocking effect of pertussis toxin on the effects originated at I_2 receptors suggests the involvement of G-proteins coupled receptors. Thus, the possibility exists that I_2-imidazoline agonists release an endogenous substance that acts upon certain receptors of the G family. In this respect, 2-BFI causes transient increases of noradrenaline levels in some brain regions (Lalies & Nutt, 1995). Nevertheless, under the present experimental conditions, antagonists at the α_2-adrenergic receptor failed to block the increase of morphine antinociception induced by I_2-imidazoline agonists (not shown). It is well known that morphine supraspinal antinociception involves the activation of pertussis toxin-sensitive and -insensitive G protein classes (Sánchez-Blázquez *et al*., 1995; 1999; Garzón *et al*., 1997; 1998). Whether I_2-imidazoline receptors couple to G-proteins or regulate the magnitude of certain G_i-G_o protein signalling pathways activated by morphine remains to be seen.

In summary, the present study provides evidence of the involvement of I_2-imidazoline receptors in the modulation of opioid analgesia. In addition, the data reveal that the potentiation of morphine antinociception induced by I_2-imidazoline ligands (agonists) is completely reversed by co-treatment with idazoxan or BU-224. Therefore, this experimental approach allows both agonist and antagonist properties to be ascribed to these compounds at I_2-imidazoline receptors. This indicates the involvement of a receptor-mediated mechanism for the action of I_2-imidazoline compounds. These findings, together with those of previous studies, suggest that I_2-imidazoline ligands (agonists) could be promising therapeutic co-adjuvants in the management of chronic pain with opioid drugs.

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