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Morphine-induced antinociception in the rat: Supra-additive interactions with imidazoline I_2 receptor ligands

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ABSTRACT

Pain remains a significant clinical challenge and currently available analgesics are not adequate to meet clinical needs. Emerging evidence suggests the role of imidazoline I_2 receptors in pain modulation primarily from studies of the non-selective imidazoline receptor ligand, agmatine. However, little is known of the generality of the effect to selective I_2 receptor ligands. This study examined the antinociceptive effects of two selective I_2 receptor ligands 2-BFI and BU224 (>2000-fold selectivity for I_2 receptors over α_2 adrenoceptors) in a hypertonic (5%) saline-induced writhing test and analyzed their interaction with morphine using a dose-addition analysis. Morphine, 2-BFI and BU224 but not agmatine produced a dose-dependent antinociceptive effect. Both composite additive curve analyses and isobolographical plots revealed a supra-additive interaction between morphine and 2-BFI or BU224, whereas the interaction between 2-BFI and BU224 was additive. The antinociceptive effect of 2-BFI and BU224 was attenuated by the I_2 receptor antagonist/α_2 adrenoceptor antagonist idazoxan but not by the selective α_2 adrenoceptor antagonist yohimbine, suggesting an I_2 receptor-mediated mechanism. Agmatine enhanced the antinociceptive effect of morphine, 2-BFI and BU224 and the enhancement was prevented by yohimbine, suggesting that the effect was mediated by α_2 adrenoceptors. Taken together, these data represent the first report that selective I_2 receptor ligands have substantial antinociceptive activity and produce antinociceptive synergy with opioids in a rat model of acute pain. These data suggest that drugs acting on imidazoline I_2 receptors may be useful either alone or in combination with opioids for the treatment of pain.

1. Introduction

Pain remains a major health problem that markedly reduces quality of life of a large segment of the population and imparts high health costs and economic loss to society. Opioids are the drugs of choice for many pain conditions. However, the unwanted effects related to repeated opioid use including pruritus, constipation and physical dependence limit adequate dosing in the clinic (Annemans, 2011). New analgesics that retain the therapeutic effects but circumvent some of the unwanted effects are in great clinical demand.

One strategy for improved treatment of pain is to combine one opioid with another pharmacologically unrelated drug in the hope that the drug mixture increases the analgesic efficacy while not altering or perhaps diminishing adverse effects of the opioid. However, the practice of this scientifically valid drug development strategy has achieved only modest success thus far (Smith, 2008). For example, opioids in combination with acetaminophen are widely used for pain management. However, the unwanted effects of the drug mixture are similar to those of opioids alone and non-medical use is common (Zacny et al., 2003). This underscores the need to identify new drug targets for analgesic development.

Imidazoline receptors are a group of receptors that recognize compounds with an imidazoline ring, a concept first proposed by Bousquet et al. (1984). Later studies established that the α_2 adrenoceptor agonist and imidazoline compound clonidine primarily exerts its hypotensive activity by acting on imidazoline receptors (Head and Mayorov, 2006) and the receptors that have high binding affinity with ^{3}H-para-aminoclonidine and ^{3}Hidazoxan are termed imidazoline I_1 receptors (Regunathan and Reis, 1996). Two selective I_1 receptor ligands, moxonidine and rilmenidine, are currently used for treating hypertension (Sica, 2007). Imidazoline I_2 receptors are binding sites that bind ^{3}H-idazoxan and ^{3}H-2-BFI with high affinity and ^{3}H-para-aminoclonidine and 3H-clonidine with much lower affinity (Regunathan and Reis, 1996). Imidazoline I_2 receptors might be implicated in several psychiatric disorders including depression, opioid addiction and neurodegenerative diseases as the density of I_2 receptors is significantly different in patients who suffer from those disorders as compared to control (Garcia-Sevilla et al., 1999). However, the possible functional relationship between I_2 receptors

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and these disorders remains to be elucidated (Garcia-Sevilla et al., 1999). Autoradiographical studies indicate that I_2 receptors are widely distributed in the central nervous systems, with high bindings to the area postrema, interpeduncular nucleus, arcuate nucleus, mammillary peduncle, ependyma and pineal gland (Lione et al., 1998). Emerging evidence indicates that the cationic polyamine, agmatine, possesses antinociceptive and analgesic activity both in animals and in man (Li and Zhang, 2011). Agmatine is a non-selective low-affinity imidazoline I_1 and I_2 receptor ligand but also has affinity for α_2 adrenoceptors, NMDA receptors, and nicotinic receptors, and also inhibits nitric oxide production (Berkels et al., 2004; Loring, 1990). The mechanisms of the antinociceptive effects of agmatine primarily involve I_2 receptors and α_2 adrenoceptors (Li et al., 1999; Roerig, 2003). Although the antinociceptive effects of α_2 adrenoceptor agonists are well established, there are only limited data concerning the antinociceptive effects of I_2 receptor ligands, and few studies employ selective I_2 receptor ligands (Gentili et al., 2006; Sanchez-Blazquez et al., 2000).

Consistent with the effects of agmatine, selective I_2 receptor ligands enhance the antinociceptive effects of morphine and attenuate the development of tolerance to morphine antinociception for pain following thermal stimulation (Boronat et al., 1998; Sanchez-Blazquez et al., 2000). However, previous studies only employed one procedure (radiant tail flick) and a single dose is typically used. Thus, it is unclear of the extent to which these findings relate to other models of pain and the nature of the interaction between I_2 receptors and opioid receptors. This study investigated the antinociceptive effects of agmatine, morphine and two selective I_2 receptor ligands 2-BFI and BU224 (Fig. 1) using a hypertonic saline-induced writhing test in the rat. Furthermore, potential receptor mechanisms were explored using pharmacological antagonists and the application of quantitative pharmacological analysis.

2. Materials and methods

2.1. Subjects

Two groups of adult male Sprague–Dawley rats (Harlan, Indianapolis, IN) were housed individually under a 12/12-h light/dark cycle beginning at 6:00 a.m. (experiments were conducted during the light period) with free access to standard rodent chow and water in the home cage. One group of 9 rats contributed to the data shown in Figs. 2–4 and a second group of 8 rats contributed to the data of Figs. 5–6. Animals were maintained and experiments were conducted in accordance with the Institutional Animal Care and Use Committee, University at Buffalo, and with the 1996 Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, National Academy of Sciences).

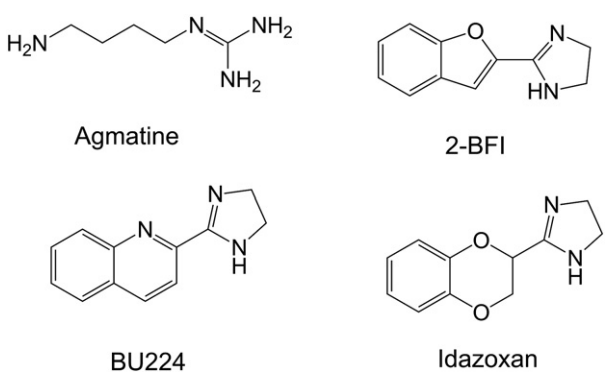

Fig. 1. Chemical structures of agmatine and three imidazoline compounds (2-BFI, BU224 and idazoxan).

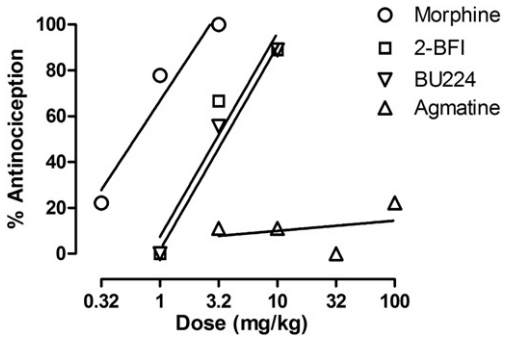

Fig. 2. Antinociceptive effects of morphine, 2-BFI, BU224 and agmatine in a hypertonic saline induced writhing test in rats. Ordinate: percentage of antinociception, expressed as the percentage of rats that did not demonstrate writhing response (n = 9). Abscissa: dose of drugs in milligram per kilogram.

2.2. Behavioral test

A hypertonic saline solution-induced writhing test was used in the current study as described previously with minor modification (Fukawa et al., 1980). Preliminary study showed that an injection of 5% saline (intraperitoneally, i.p.) at a volume of 2 ml/kg elicited a reliable writhing response in more than 80% of the rats and the writhing response was stable during repeated injections with an inter-injection period of 20 min. This dosing paradigm was used throughout the study and no more than 4 saline injections (4 cycles) were administered in a given test session. Tests were separated by at least 3 days to minimize the potential drug interactions between test sessions and to decrease the stress due to repeated testing.

All the studies were conducted in a quiet behavioral test room next to the animal colony room, with similar lighting, environmental temperature and humidity. Each rat was weighed and put into a clear cage, which served as an observation arena, for a 30 min habituation period. The observation arena was identical to the home cage except that corn cob bedding was used to facilitate observation, whereas wood chip bedding was used for home cages. For each cycle, rats were injected with 5% saline and then immediately put into the observation cage and the number of writhing response was recorded for up to 5 min. All drugs were administered immediately prior to the injection of 5% saline solution. Dose–effect relationships were determined using a cumulative dosing procedure with 0.5 log unit increments. For antagonism studies, an antagonist was administered 10 min before the start of the session.

2.3. Drugs

Morphine sulfate, agmatine sulfate, idazoxan hydrochloride ((±)-2-[1,4-benzodioxan-2-yl]-2-imidazoline hydrochloride) and yohimbine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA).

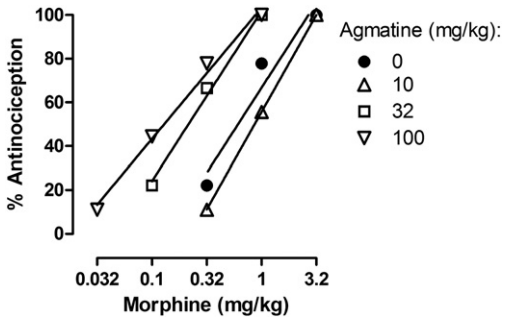

Fig. 3. Antinociceptive effects of morphine in the presence and absence of different doses of agmatine treatment (n = 9). See Fig. 2 for other details.