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Effects of imidazoline I_2 receptor ligands on morphine- and tramadol-induced antinociception in rats

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ABSTRACT

Currently available analgesics cannot meet the increasing clinical needs and new analgesics with better therapeutic profiles are in great demand. The imidazoline I_2 receptor is an emerging drug target for analgesics. However, few studies have examined the effects of selective I_2 receptor ligands on the antinociceptive activity of opioids. This study examined the antinociceptive effects of the opioids morphine (0.1–10 mg/kg) and tramadol (3.2–56 mg/kg), the nonselective I_2 receptor ligand agmatine (10–100 mg/kg), and the selective I_2 receptor ligands 2-(2-benzofuranyl)-2-imidazoline hydrochloride (2-BFI; 1–10 mg/kg) and 2-(4, 5-dihydroimidazol-2-yl) quinoline hydrochloride (BU224; 1–10 mg/kg), alone and in combination, in a warm water tail withdrawal procedure in rats. Morphine and tramadol but not agmatine, 2-BFI or BU224 increased tail withdrawal latency in a dose-related manner at 48 °C water. Agmatine and 2-BFI but not BU224 dose-dependently enhanced the antinociceptive effects of morphine and tramadol, shifting the dose–effect curves of morphine and tramadol leftward. The enhancement of agmatine and 2-BFI on morphine and tramadol antinociception was prevented by BU224. These results, combined with the fact that BU224 and 2-BFI share similar behavioral effects under other conditions, suggest that BU224 has lower efficacy than 2-BFI at I_2 receptors, and that the enhancement of opioid antinociception by I_2 receptor ligands depends on their efficacies.

1. Introduction

Pain, both as a symptom and as a disease, imparts high health cost and economic loss to society. Currently available analgesics are not adequate to meet the clinical needs, leaving a big population with undertreated pain. Opioids remain the most effective analgesics for many painful conditions. However, adequate dosing with opioids is limited by unwanted effects, particularly constipation, physical dependence, abuse and overdose. Although great efforts have been made to develop analgesics with novel mechanisms of action for decades, a careful analysis of the analgesics marketed in the past 50 years revealed a lack of clinically significant advances (Kissin, 2010). An alternative strategy is combination therapy, which requires combining two or more drugs for pain management. This scientifically valid strategy has been successfully practiced for treating various diseases including cancer and cardiovascular disorders, and emerging evidence suggests the validity of this strategy for treating pain (Smith, 2008). For example, the combination of a μ opioid agonist with another non-μ opioid analgesic may have increased analgesic effectiveness and/or a better safety profile (Smith, 2008). Further support comes from the finding that analgesic drugs with dual mechanisms of action (μ opioid receptor agonism and a second mechanism) tend to have improved therapeutic profiles. For instance, tramadol is a μ opioid receptor agonist that also enhances serotonin and norepinephrine transmission (Reeves and Burke, 2008). Tramadol is effective in many painful conditions and has relatively low abuse liability, presumably due to this unique pharmacological profile (Epstein et al., 2006). Tapentadol is a μ opioid receptor agonist and a norepinephrine reuptake inhibitor (Hartrick and Rozek, 2011). Clinical studies indicate that tapentadol is effective for both acute and chronic pain and has decreased unwanted effects as compared to μ opioid agonists (Etropolski et al., 2011; Hartrick and Rozek, 2011). Thus, this strategy remains promising for developing candidate analgesics.

Imidazoline receptors are a class of three novel receptors (I_1, I_2, I_3) that are widely distributed in mammalian central and peripheral nervous systems and other tissues (Regunathan and Reis, 1996). Although it is now widely recognized that the imidazoline I_1 receptor is involved in central control of blood pressure (Head and Mayorov, 2006), the physiological functions of the I_2 receptor are less well-characterized. Accumulating evidence suggests that the I_2 receptor is an emerging drug target for novel analgesics (Li and Zhang, 2011). The purported endogenous imidazoline receptor ligand, agmatine, has antinociceptive activity in several animal models of inflammatory and neuropathic pain.

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Agmatine also increases the antinociceptive effects of morphine in models of both acute and chronic pain (see Li and Zhang, 2011 for review). However, many of the early studies did not examine the receptor mechanisms underlying the antinociceptive effects of agmatine. This is problematic as, besides the I_2 receptors, agmatine also binds to several other receptors (Halaris and Plietz, 2007). Nonetheless, agmatine shows analgesic activity in patients with lumbar disc-associated radiculopathy (Keynan et al., 2010). Although selective I_2 receptor ligands are available (Dardonville and Rozas, 2004), only two studies have examined the antinociceptive effects of I_2 receptor ligands and their interactions with morphine in a mouse model of acute pain and the generality of those findings to other conditions (species, pain models, and opioids) is unknown (Gentili et al., 2006; Sanchez-Blazquez et al., 2000).

The purpose of the current study was to extend previous observations in two important dimensions. First, a single dose is typically used in previous studies and it is unclear the magnitude of the drug interactions. This study exploited full dose–effect functions of both I_2 receptor ligands and opioids in an acute pain procedure in a different species. This is important because the potential clinical utility of drug combinations for pain management is based on the robustness of the effect, and a full understanding of the drug interactions can only be achieved by examining the complete dose–effect functions. Second, previous reports exclusively used morphine for the I_2 ligand–opioid interaction studies. Because opioids with dual mechanisms (e.g. tramadol and tapentadol) may have favorable therapeutic profiles (Etropolski et al., 2011), and I_2 receptor ligands such as 2-BFI and BU224 can modulate brain monoamine transmission (Hudson et al., 1999), it seems warranted to examine the I_2 ligand–opioid interactions by using opioids with different mechanisms of action. Thus, the current study examined the interactions between three I_2 receptor ligands (2-BFI, BU224, and a non-selective I_2 receptor ligand, agmatine) and two opioids (morphine and tramadol) in a warm water tail withdrawal procedure in rats. It was hypothesized that I_2 receptor ligands enhanced the antinociceptive effects of the dual mechanism opioid tramadol, and that the magnitude of enhancement was greater for tramadol than for morphine.

2. Materials and methods

2.1. Subjects

Adult Sprague–Dawley rats (Harlan, Indianapolis, IN) were housed individually on a 12/12-h light/dark cycle (behavioral experiments were conducted during the light period) with free access to water and food except during experimental sessions. 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, Washington DC).

2.2. Apparatus

Prior to initiation of the studies, rats were habituated to the procedure room, the experimenter handling and the experimental procedure. Two Dual Poly Pro water baths were used (model RS-PB-200; Revolutionary Science, Lindstrom, Minnesota, USA). Each water bath has two chambers with the inside dimensions of 32 cm × 17 cm × 13.3 cm (W × D × T). Tap water was heated to the pre-set temperature (44°, 48°, or 52 °C) and remained stable throughout the experimental session with a range of no more than 0.4 °C. The readings of the digital display on the water bath were regularly compared with an Oakton® water-resistant digital thermometer (Oakton Instruments, Vernon Hills, Illinois, USA) to ensure temperature accuracy. Tail-withdrawal latencies were recorded with a hand-operated digital stopwatch (resolution = 1/100 s).

2.3. Procedure

The warm water tail withdrawal procedure was conducted as described in detail previously (Li et al., 2007). A multiple-cycle procedure was used to determine the dose–effect curves of the study drugs with an inter-cycle time of 15 min. Briefly, rats were slightly restrained and the distal 5 to 10 cm of the tail was immersed in the water baths with different temperatures (44°, 48°, and 52 °C). Testing with different temperatures varied nonsystematically among rats and across cycles. When a subject failed to remove its tail within 20 s, the experimenter removed the tail from the water and a latency of 20 s was recorded. Test sessions began with control (no drug) determinations for each temperature. For each cycle (e.g., 15 min), tail withdrawal latencies were measured for each of the three temperatures with ~1 min between determinations. Test was conducted no more than once per week to minimize the possibility of inter-test interactions. Dose–effect relationships were determined using a cumulative dosing procedure with the first cycle administered with vehicle followed by cumulative dose increasing by 0.25 (tramadol) or 0.5 log unit in the following cycles. For drug combination studies, the pretreatment drug was administered with the first dose of the opioids during the first min of the cycle and increasing doses of the opioids were administered during the following cycles. For antagonism studies, the drug was administered 10 min before the first opioid dose.

2.4. Data analyses

Tail withdrawal latency was expressed as a percentage of the maximal possible effect (MPE) using the following formula: % MPE = [(test latency − control latency)/(20 s − control latency)] × 100, where the control latency was defined as the latency determined in the absence of drug. The MPE was calculated for each individual and then averaged to obtain a group mean. Within the dose range studied, neither morphine nor tramadol produced an effect of >50% MPE in 52 °C water, thus only data from 48 °C water was used for data analysis. Dose–effect relationships were analyzed by log-linear regression of individual values by using Prism (GraphPad Software, Inc., San Diego, CA), with the following equation: effect = slope × log (dose) + intercept. Deviations from linearity were examined by the replicates test. F ratio tests in Prism were used to compare dose–effect curves with respect to their slopes and intercepts. For example, a nonsignificant F ratio for slopes and a significant F ratio for intercept show that dose–effect curves are parallel but occupy different positions on the dose axis (Koek et al., 2009). Potencies were obtained by estimating the dose required to produce 50% of the MPE (ED_{50}) using linear regression, along with 95% confidence limits (95% CL). Potency ratios of morphine and tramadol in the absence and presence of imidazoline I_2 receptor ligands were calculated for each individual and then averaged for group mean (±95% CL) to estimate potency differences. Significant changes in potencies were detected when the 95% CLs of the potency ratios averaged across rats did not include 1.

2.5. Drugs

Morphine sulfate, agmatine sulfate, and tramadol hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2-BFI hydrochloride (2-(2-benzofuranyl)-2-imidazoline hydrochloride) and BU224 hydrochloride (2-(4, 5-dihydroimidazol-2-yl) quinoline hydrochloride) were synthesized according to the published procedures (Ishihara and Togo, 2007). All drugs were dissolved in 0.9% physiological saline and administered i.p. Doses are expressed as milligrams of the form indicated above per kilogram of body weight. Injection volumes were 1 ml/kg. Drugs were studied up to doses that produced marked antinociceptive effects (morphine and tramadol), up to doses that significantly potentiated the antinociceptive effects of