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Short Communication

Sub-anesthetic doses of bupivacaine or lidocaine increase peripheral ICS-205 930-induced analgesia against inflammatory pain in rats

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Abstract

Intraplantar co-administration of sub-anesthetic doses of bupivacaine (2.5 μg) or lidocaine (7.5 μg) increased the dose- and time-dependent analgesic effects of the 5-HT_3 receptor antagonist, 3-a-tropanyl-1-H-indole-3-carboxylic ester (ICS-205 930) (1–100 μg; 50 μl) against inflammatory pain induced by hindpaw inoculation with complete Freund's adjuvant. The effects of bupivacaine were greater than lidocaine at all doses of ICS-205 930 tested. These findings may reflect facilitation of ICS-205 930 effects through negative allosteric modulation by bupivacaine and lidocaine of peripheral 5-HT_3 receptors involved in nociceptive processing. © 1997 Elsevier Science B.V.

Keywords: 5-HT_3 receptors; ICS-205 930; Bupivacaine; Lidocaine; Nociception inflammation

1. Introduction

5-HT_3 receptors have been localized to peripheral C-fiber afferents (Fozard, 1984). During the inflammatory cascade, extravasated blood-borne, platelet- and mast cell-derived 5-HT can act at these receptors to evoke C-fiber depolarization and nociception (Giordano and Rogers, 1989). The 5-HT_3 site, an ionotropic receptor, exists as a component of a macromolecular complex consisting of the 5-HT binding region and a cation channel (Yakel, 1992). This receptor complex is (negatively) allosterically modulated by local anesthetics, including bupivacaine and lidocaine (Barann et al., 1993).

Previously, we have demonstrated the analgesic efficacy of peripherally administered 5-HT_3 receptor antagonists against inflammatory pain (Giordano and Rogers, 1989). In light of these findings, the combined administration of local anesthetics and 5-HT_3 receptor antagonists may interact to affect 5-HT-induced, 5-HT_3 receptor-mediated C-fiber activity contributing to inflammatory nociception. Thus, the present study examined the analgesic effects of intraplantar co-injection of sub-anesthetic doses of either bupivacaine or lidocaine and the 5-HT_3 receptor antagonist ICS-205 930 (3-a-tropanyl)1-H-indole-3-carboxylic ester) against adjuvant-induced inflammatory pain in rats.

2. Materials and methods

2.1. Subjects

Male Long-Evans Hooded rats (200–250 g) were used in all experiments. Animals were maintained in standard plastic cages, 4 per cage, were kept on a 12 h light/dark cycle, and were allowed food, water and conspecific contact ad libitum. Separate animals were used at each dose and time point in all paradigms, and each animal was used only once.

2.2. Drugs

All compounds were obtained from Research Biochemicals (Natick, MA). ICS-205 930 was dissolved in 100% dimethylsulfoxide (DMSO) and diluted to appropriate concentrations in sterile saline. Bupivacaine and lidocaine were dissolved in sterile saline; all agents were balanced to a final pH of 7.6.

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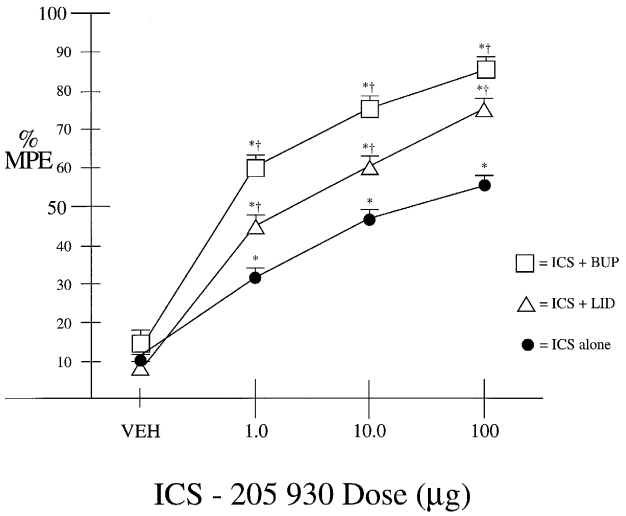

Fig. 1. Effects of intraplantar co-administration of bupivacaine (2.5 μg) or lidocaine (7.5 μg) on analgesia produced by ICS-205 930 (1–100 μg) against adjuvant-induced inflammatory pain. Total injected volumes were 50 μl in all cases. Effects of vehicle, bupivacaine and lidocaine alone are presented for comparison. Points at the vehicle represent the pH buffered vehicle alone, lidocaine (7.5 μg) in vehicle or bupivacaine (2.5 μg) in vehicle. Points represent mean scores from 4–5 determinations (±S.D.). (* = significant difference from vehicle, $P < 0.05$; + = significant difference from each dose of ICS-205 930, alone, $P < 0.05$).

2.3. Nociceptive testing

Induction of inflammation and subsequent nociceptive testing were performed according to methods previously described (Giordano and Rogers, 1989). Briefly, rats were inoculated with 50 μl Freund's complete adjuvant (heat-killed, desiccated *Mycobacterium butyricum* in 0.85 ml paraffin oil and 0.15 mannide monooleate; Sigma Chemical, St. Louis, MO) into the hindpaw. Four days following inoculation, nociceptive sensitivity to pressure was determined by assessing withdrawal response latency to application of a blunt probe at 20 g force to the dorsal surface of the affected hindpaw. Preliminary experiments demonstrated that the average maximal response in adjuvant-inflamed animals was 2 s. Analgesia was evaluated by comparison of pre-drug withdrawal latencies to responses observed 5 min following intraplantar injection of bupivacaine alone (2.5 μg), lidocaine alone (7.5 μg), ICS-205-930 alone (1–100 μg), co-administration of bupivacaine (2.5 μg) or lidocaine (7.5 μg) and ICS-205 930 (1–100 μg), or vehicle. In time course experiments, analgesic effects were assessed from 5–240 min post-treatment. The total injected volume in all conditions was 50 μl. Drug doses were based upon preliminary experiments. In these experiments, sub-anesthetic doses of bupivacaine and lidocaine were determined as the highest tested dose at which significant anesthesia was not observed against rubbing cotton wool along the dorsal surface of a non-inflamed hindpaw. In the absence of local anesthesia, this stimulus evokes almost immediate orienting behaviors of turning toward, sniffing at and/or dorsi-flexor movement of the limb to which the stimulus is applied.

Time to peak analgesic effects was determined in pilot experiments. Drug-induced alterations in nociceptive responses were calculated as maximum possible effects, which allowed for normalization to pre-treatment baseline values for each animal. Maximum possible effect (% MPE) was defined as: (Post drug response) − (Pre-drug response)/(Maximum response) − (Pre-drug response) × 100. In this formula, maximum response values were determined as described previously in this section. Statistical analyses were performed using factorial analysis of

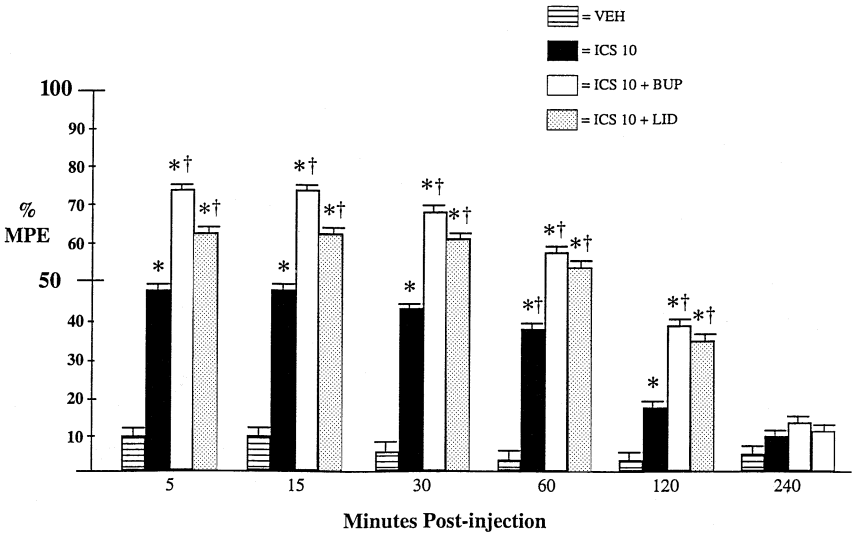

Fig. 2. Effects of intraplantar co-administration of bupivacaine (2.5 μg) or lidocaine (7.5 μg) on the time course of analgesic effects produced by ICS-205 930 (10 μg). Total injected volumes were 50 μl in all cases. Columns represent mean scores from 4–5 determinations (±S.D.). Separate animals were used to determine effects at each time point. (* = significant difference from vehicle, $P < 0.05$; + = significant difference from ICS-205 930 alone, $P < 0.05$).

variance (ANOVA), with post-hoc analysis with Dunnett's test. In all cases, significance was considered at the level of $P < 0.05$.

3. Results

None of the pharmacologic treatments produced observed changes in respiration, grooming, or overt motor behavior. When administered singularly, neither intraplantar bupivacaine (2.5 μg) or lidocaine (7.5 μg) produced significant analgesia when compared to vehicle (Fig. 1). Administered alone, ICS-205 930 produced significant dose-dependent analgesia at all doses tested ($P < 0.05$). As shown in Fig. 1, both bupivacaine and lidocaine produced statistically significant increases in the analgesic effects of ICS-205 930, at all doses of the 5-HT_3 receptor antagonist ($P < 0.05$ as compared to effects of ICS-205 930 alone, at each dose tested). The effects produced by bupivacaine were significantly greater than those produced by lidocaine at all doses of ICS-205 930 ($P < 0.05$).

Fig. 2 illustrates the effect of co-administration of either bupivacaine (2.5 μg) or lidocaine (7.5 μg) on the time course of analgesia produced by intraplantar injection of ICS-205 930 (10 μg; 50 μl). Both bupivacaine and lidocaine extended the analgesic effect of ICS-205 930 ($P < 0.05$ vs. ICS-205-930 alone). Although the effects of bupivacaine were significantly greater than lidocaine at all time points where significant ICS-205 930-induced analgesia was observed ($P < 0.05$, in all cases), the time courses of bupivacaine- and lidocaine-increased ICS-205 930-induced effects did not significantly differ.

4. Discussion

Co-administration of a sub-anesthetic concentration of either bupivacaine or lidocaine increased the dose- and time-dependent analgesic effects of intraplantarly injected ICS-205 930 against inflammatory pain. Bupivacaine was more effective than lidocaine in increasing ICS-205 930-induced analgesia. Given that local anesthetics allosterically modulate the 5-HT_3 site (Barann et al., 1993), several possibilities exist to explain these findings. First, bupivacaine and lidocaine may synergize ICS-205 930-induced 5-HT_3 receptor antagonism by direct or indirect inhibition of ions, released during inflammatory tissue disruption, that exert pro-nociceptive activity (Williams, 1996). However, the analgesic inefficacy of bupivacaine or lidocaine administered alone argues against such a general effect. Second, bupivacaine and lidocaine may inhibit the flux of cations specific to the 5-HT_3 receptor-linked channel. The local anesthetics could potentiate the antagonist effects of ICS-205 930 at the 5-HT_3 site by interfering with receptor-mediated ionic conductance. In this scenario, bupivacaine and lidocaine may de-sensitize domains of the 5-HT_3 receptor structurally affiliated with the ion channel. Although local anesthetics have been shown to affect the structure and function of membrane proteins (Seeman, 1975; Hille, 1977) it is unlikely that these local anesthetics completely alter 5-HT_3 receptor total structural configuration or membrane orientation; distortions of the binding region would negatively affect the affinity of ICS-205-930 at the 5-HT_3 receptor, and thereby decrease its pharmacologic effect. Lastly, the observed increase in ICS-205 930-induced analgesic effects by bupivacaine or lidocaine may reflect non-competitive antagonist interactions of the local anesthetics and ICS-205 930 at the 5-HT_3 receptor (Barann et al., 1993). Such action might facilitate more complete antagonism of 5-HT_3 receptors, inhibit a greater number of 5-HT_3 receptors, or both. That bupivacaine was more effective than lidocaine in increasing ICS-205-930-induced analgesia may be due to its higher lipophilicity and/or greater protein binding of bupivacaine (Williams, 1996); both properties could possibly affect any of the aforementioned processes. Clearly, these putative mechanisms warrant further study, and are currently being investigated by our group.

In sum, these findings are promising in that combined delivery of low doses of local anesthetics and 5-HT_3 receptor antagonists may have some utility as a site-specific, peripheral analgesic preparation; providing inhibition of pain while avoiding area anesthesia, thus sparing tactile sensitivity.

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