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Reversal of morphine tolerance by a compound with NPFF receptor subtype-selective actions

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HIGHLIGHTS

- AC-263093 previously activated type 2, but not type 1, Neuropeptide FF receptors.
- Morphine infusion induced robust tolerance to morphine analgesia (tail flick test).
- AC-263093, 10 mg/kg i.p., totally reversed this tolerance to 5 mg morphine sulfate.
- AC-263093 did not induce an analgesic effect in rats never exposed to morphine.
- AC-263093 blocked activation of type 1 receptor, further altering balance between types 1 and 2.

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ABSTRACT

Neuropeptide FF (NPFF) modulates opiate actions. It has pro-nociceptive effects, primarily through the NPFF receptor 1 subtype, and anti-nociceptive effects, primarily through the NPFFR2 subtype. AC-263093 is a small l, organic, systemically active molecule that was previously shown to functionally activate NPFFR2, but not NPFFR1. It was hypothesized that AC-263093 would attenuate morphine tolerance. Rats were tested for radiant heat tail-flick latency before and after 5 mg/kg morphine sulfate s.c. They were then rendered morphine-tolerant by continuous subcutaneous infusion of 17.52 mg/kg/day morphine sulfate. On the seventh day of infusion, they were retested for analgesia 10 and 20 min after 5 mg/kg morphine sulfate s.c. Tolerance was indicated by reduction of morphine analgesia from the pre-infusion test. Fifty minutes prior to morphine challenge, rats received either 10 mg/kg i.p. AC-263093 or injection vehicle alone. AC-2623093-treated rats had far smaller tolerance scores than control rats. This drug effect was significant, $p = 0.015$. The same dose of AC-263093 had almost no analgesic effect in non-tolerant, saline-infused rats. *In vitro* experiments revealed that AC-263093 had equal affinity for NPFFR1 and NPFFR2, and functionally inactivated NPFFR1, in addition to its previously shown ability to activate NPFFR2. Thus, altering the balance between activation of NPFF receptor subtypes may provide one approach to reversing opiate tolerance.

1. Introduction

Chronic use of opiate narcotic drugs, such as morphine, causes drug tolerance: a profound loss of potency. These drugs are commonly used to alleviate chronic pain. Opiate tolerance necessitates repeated escalation of narcotic doses, creating major problems in pain management [1–3].

Chronic morphine administration in the rat results in significantly increased levels of NPFF in cerebrospinal fluid [4]. NPFF is sometimes considered an anti-opiate peptide since it antagonizes various acute effects of opiate drugs [5–7]. Conversely, antibodies and antagonists to NPFF administered i.c.v. or s.c. have attenuated morphine tolerance in the rat [8–11]. On the other hand, spinal administration of NPFF has been shown to potently intensify morphine analgesia [12]. Moreover, spinal NPFF administration has long-acting, opiate-like analgesic effects [13–15]. These actions

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are likely mediated by the release of met-enkephalin [16] and are naloxone-reversible [17], consistent with pro-opiate actions of NPFF.

Two NPFF receptor subtypes (NPFFR1 and NPFFR2) have been discovered [18]. NPFF binds to both, while a series of related RFamide C-terminal peptides, such as NPAF and NPSF, have varying affinities for these receptor subtypes. Both subtypes are distributed in the brain, but only NPFFR2 is readily detected in the rat spinal cord [18–20]. Several reports suggest that the NPFFR2 receptor is responsible for antinociceptive, or pro-opiate, activity [12–16]. Although its receptor binding affinities were not measured, Lameh et al. [21] demonstrated that the compound AC-263093 selectively stimulates functional activation of this receptor subtype. This was indicated by receptor modulation of cAMP levels in cells transfected with NPFFR1 or NPFFR2, as well as selection amplification technology (RSAT) which quantifies cellular proliferation dependent on receptor activation. As would be expected from this receptor selectivity, it also induced antinoceptive effects in multiple *in vivo* models of rodent hyperalgesia. The present study evaluated the hypothesis that this compound would restore the analgesic effect of morphine in morphine-tolerant rats. Another experiment determined whether AC-263093 might have reduced pain sensitivity by exerting an acute analgesic effect of its own rather than actually altering morphine tolerance. The receptor binding affinities of AC-263093 and related compounds were also measured, as well as AC-263093 functional inhibition of the NPFFR1 receptor, as distinguished from binding affinity and lack of functional activation.

2. Materials and methods

2.1. Approval

These experimental procedures were approved by the UHCL Animal Care and Use Committee.

2.2. Experiment 1: The ability of AC-263093 to reverse morphine tolerance

2.2.1. Materials

AC-263093 was synthesized as described previously as "compound 2" [22]. The chemical formula for AC-263093 is 2-(3,4-dibromobenzylidene) hydrazine carboximidamide hydrochloride. The structure is shown in Fig. 3.

2.2.2. Subjects

Eight male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 405 ± 27 g ($M \pm$ SD) were maintained on a 12-h light/dark cycle with food and water *ad lib*.

2.2.3. Apparatus

A radiant heat tail flick apparatus (Stoelting, Wood Dale, IL) was employed. The device was calibrated such that a normal, drug-free rat would remove its tail after approximately 3–4 s of heat exposure. The trial was terminated after a maximum of 12 s in order to prevent tissue damage.

2.2.4. Inducing morphine tolerance

Under isoflurane anesthesia, each rat was implanted s.c. with an Alzet 2ML1 osmotic pump filled with morphine sulfate in saline. Each rat was infused over seven days with 17.52 mg/kg/day of morphine sulfate. This treatment previously resulted in robust morphine tolerance [11,12].

2.2.5. Pre-infusion testing (before morphine sulfate infusion)

Rats were injected s.c. with 1.0 ml/kg saline as a control for the subsequent morphine sulfate injection. The average of three tail flick latencies was recorded at 10 and 20 min after saline injection and the average latency was computed for each test. Rats were then injected i.p. with 1.0 ml/kg of 20% dimethyl sulfoxide (DMSO)/80% saline. (This was a control for the injection vehicle subsequently used for AC-263093.) Fifty minutes after i.p. injections, rats received 5 mg/kg morphine in saline s.c. This dose was chosen as the smallest dose that induced near maximal analgesia (80–90% of the maximum possible effect), thereby providing optimal sensitivity to detecting tolerance. At 10 and 20 min after these injections, the rats were retested for tail flick latencies using the same protocol. Ten and twenty minutes morphine analgesia scores were calculated as the increase in average latency from pre- to post-morphine injection at each post-injection interval expressed as a percentage of the maximum possible increase, given the 12 s cutoff. Analgesia score as a percentage = 100 × (post-morphine latency − pre-morphine latency)/(12 s − pre-morphine latency).

2.2.6. Post-infusion testing (after morphine sulfate infusion)

On the seventh day of infusion, rats were pretested using the same method as the pre-infusion pretest. AC-263093 was dissolved in 0.2 ml DMSO and brought up to 1.0 ml with saline. An experimental group of four morphine-tolerant rats were given i.p. injections of 10 mg/kg AC-263093 while a control group of four morphine-tolerant rats were administered the injection vehicle (DMSO/saline) alone. This 10 mg/kg dose of AC-263093 was previously shown to produce analgesia in hyperalgesic carrageenan-treated rats and in hyperalgesic rats following spinal nerve ligation, but not in untreated rats [21]. Fifty minutes after i.p. injection, rats received morphine injections (5 mg/kg s.c.) and were tested for tail flick latencies 10 and 20 min later. The morphine analgesia scores in response to injection of 5 mg/kg morphine sulfate were computed as before. Each morphine tolerance score was calculated as the change in morphine analgesia score from pre-infusion testing to post-infusion testing. A negative tolerance score (decrease in morphine analgesia score) indicated morphine tolerance.

2.3. Experiment 2: Effect of 10 mg/kg AC-263093 on tail flick response in morphine-naïve rats

2.3.1. Subjects

Subjects included eleven male Sprague-Dawley rats with an average weight of 293 ± 29 g ($M \pm$ SD), maintained as in Experiment 1.

2.3.2. Procedure

The goal of this procedure was to evaluate any acute analgesic effect of AC-263093 by itself (without morphine infusion or injection) in opiate-naïve rats exposed to the same non-drug conditions as in Experiment 1. Therefore, the same procedures as in Experiment 1 were repeated with the following exceptions: all rats were infused s.c. for seven days with saline alone, to control for any effects of osmotic minipump implantation in Experiment 1; only saline injections were administered before each pre-infusion and post-infusion set of tail flick tests, so that the subjects were never exposed to morphine; although each rat experienced pre-infusion testing as before, the effect of AC-263093 was evaluated only by post-infusion testing since that compound had been administered post-infusion in Experiment 1; six rats were assessed for analgesia before and 60 and 70 min after i.p. injection of 10 mg/kg AC-263093 in DMSO/saline (10 and 20 min after s.c. saline injection); five control rats were assessed for analgesia at the same intervals after injection of DMSO/saline vehicle alone, followed 50 min later by injection by saline s.c.; each rat's AC-263093 analgesia scores were the change in tail flick latency from pre- to 60 and 70 min post-i.p injection as a percentage of the maximum possible change, given