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Efficacy of Impropgan, a Non-Opioid Analgesic, in Neuropathic Pain

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Abstract

Impropgan, a non-opioid analgesic, is known to act in the rodent brain stem to produce highly effective antinociception in several acute pain tests. However, impropgan has not been studied in any models of chronic pain. To assess the efficacy of impropgan in an animal model of neuropathic pain, the effects of this drug were studied on mechanical allodynia following unilateral spinal nerve ligation (SNL) in rats. Intracerebroventricular (icv) impropgan (40 – 80 μ g) produced complete, reversible, dose-dependent attenuation of hind paw mechanical allodynia for up to one hr after administration, with no noticeable behavioral or motor side effects. Intracerebral (ic) microinjections of impropgan (5 – 30 μ g) into the rostral ventromedial medulla (RVM) also reversed the allodynia, showing this brain area to be an important site for impropgan's action. The recently-demonstrated suppression of RVM ON-cell activity by impropgan may account for the presently-observed anti-allodynic activity. The present findings suggest that brain-penetrating, impropgan-like drugs developed for human use could be effective medications for the treatment of neuropathic pain.

Keywords

neuropathic pain; allodynia; impropgan; rostral ventromedial medulla

1. Introduction

Impropgan, a compound closely related to the histamine H₂ antagonist cimetidine, attenuates acute nociceptive responses following intracerebroventricular (icv) administration in rodents (Hough, et al., 2000a; Li, et al., 1997). At doses which do not impair locomotor or rotorod behaviors (Li, et al., 1997), impropgan increases thermal (hot plate and tail flick (Hough, et al., 2001a; Li, et al., 1997)) and mechanical (Li, et al., 1997) nociceptive latencies, implying an analgesic profile. Despite screening on over 100 enzymes, receptors and ion channels (Hough, et al., 2001a), impropgan's biochemical target remains unknown. The drug does not require activity at supraspinal opioid (Hough, et al., 2000b), histamine (Mobarakeh, et al., 2003; Zhu, et al., 2001), adrenergic α_2 (Svokos, et al., 2001), nicotinic cholinergic, muscarinic cholinergic, 5HT₃ (Nalwalk, et al., 2005) or GABA_A receptors (Cannon, et al., 2004). Extensive in vivo pharmacology has shown a non-opioid, cannabinoid-like antinociceptive profile, but impropgan has no affinity for known cannabinoid receptors

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(Gehani, et al., 2007). For example, impropion antinociception is blocked by the cannabinoid antagonist rimonabant and shows cross-tolerance with cannabinoid agonists (Gehani, et al., 2007). Thermal nociceptive testing, combined with either intracerebral (ic) microinjections (Nalwalk, et al., 2004), or with in vivo electrophysiology (Heinricher, et al., 2010) has shown that impropion acts in the rostral ventromedial medulla (RVM) to stimulate descending analgesic circuits. However, the efficacy of impropion has only been evaluated in models of acute nociception.

Unlike acute pain, which is generated from local nociceptor activity, neuropathic pain is a chronic condition which arises from damage, disease, or other dysfunctions of the nervous system. The etiologies of neuropathic pain are manifold, but most often involve trauma, disease, or infection (Campbell and Meyer, 2006; Rowbotham, 2005). Neuropathic pain is often present in the absence of overt tissue damage or inflammation. Because neuropathic pain is often refractory to analgesics that work well in acute pain, clinicians struggle to find effective, alternative and/or combination therapies for human neuropathic pain (Argoff, et al., 2009). Currently, an estimated 4–5.5 million people in the United States have some form of chronic neuropathic pain, with a projected increase moving forward (Rowbotham, 2005). Thus, there is a significant unmet need for new, effective medications to treat these painful, often irreversible disorders. Although impropion has documented antinociceptive activity on several acute pain tests, the possibility that this drug might attenuate neuropathic pain symptoms has not been described in the literature. Presently, we report the efficacy of the experimental analgesic impropion on mechanical allodynia in a rat model of neuropathic pain. We also show that this drug acts directly in the RVM to produce its anti-allodynic activity.

2. Results

2.1. Anti-allodynic effects of icv-administered impropion

All subjects displayed mechanical allodynia when tested for baseline responses prior to drug treatment (thresholds were 3 – 5 g, not different between the treatment groups, see zero time points in Fig. 1). Impropion (40 μ g, icv) produced a small, short-lived, but statistically significant attenuation of allodynia 10 min after administration (Fig. 1); this effect completely dissipated by the later test times. After a larger dose of drug (80 μ g), a robust, maximal reversal of allodynia was seen which persisted for 30 – 60 min following drug treatment (Fig. 1). Thresholds returned to pre-drug values 120 min later, indicating a reversible drug effect. ANOVA (between groups [factor #1]: dose of impropion; within groups [factor #2, repeated measures]: time 0 – 60 min) found significant main effects of dose ($F_{2,15}=35.9$, $P<0.0001$), time ($F_{4,60}=18.3$, $P<0.0001$), with a significant dose by time interaction term ($F_{8,60}=11.8$, $P<0.0001$). Saline-treated subjects showed consistent, allodynic thresholds throughout the experiment (Fig. 1). Close observation of the subjects during and after infusion of drug or vehicle revealed no apparent abnormalities in posture, movement, or general behavior.

2.2. Anti-allodynic effects of intra-RVM administered impropion

Microinjections of several doses of impropion into the RVM mimicked the effects of icv administration. Highest doses (15 – 30 μ g) produced anti-allodynic effects which were maximal within 10 min after injection, and detectable for two hr (Fig. 2A). A lower dose (5 μ g) produced intermediate effects. ANOVA (between groups [factor #1]: dose of impropion; within groups [factor #2, repeated measures]: time) found significant main effects of dose ($F_{3,15}=25.9$, $P<0.0001$) and time ($F_{4,60}=27.8$, $P<0.0001$), with a significant dose by time interaction term ($F_{12,60}=4.7$, $P<0.0001$). No behavioral or motor effects were noted in any of the subjects receiving intra-RVM injections.

2.3 Effects of ic improgan injected outside of the RVM

Improgan (15 μ g) and saline were also injected into medullary sites outside the RVM; results were compared with those from intra-RVM injections (Fig. 2B). Locations for all ic injections are depicted in Fig. 3. ANOVA of the data in Fig. 2B (between groups [factor #1]: injection site; between groups [factor #2]: drug; within groups [factor #3, repeated measures]: time) found significant main effects of drug ($F_{1,19}=51.1$, $P<0.0001$) and time ($F_{4,76}=25.9$, $P<0.0001$), with significant ($P<0.02$) site by drug, site by time, and drug by time interactions. The absence of a significant 3-way interaction term (site by drug by time, $P=0.36$) prevented specific post-hoc comparisons in Fig. 2B. In order to understand the main drug effect and the drug by site interaction in Fig 2B, the effects of improgan in the two medullary sites were broken down by simpler two-factor ANOVAs. These analyses showed that improgan (vs. saline) significantly reduced allodynia when injected both within (drug by time, $P<0.0001$) and outside of (drug by time, $P=0.03$) the RVM. A separate 2-factor ANOVA of improgan treatments (i.e. RVM vs. non-RVM) showed that the improgan effect was significantly less when injected outside the RVM (main effect of region, $P<0.05$). Also noteworthy was the short-term effect (mostly at 10 min, Fig. 2B) of saline injected into non-RVM areas; no such effects were observed after icv (Fig. 1) or intra-RVM (Fig. 2) treatments.

3. Discussion

Improgan has the preclinical profile of a highly effective analgesic drug. Following icv injection, the compound maximally suppresses spinally-mediated and supraspinally-organized reflexes elicited by high temperature thermal and mechanical noxious stimuli (Hough, et al., 2006). Measurements of locomotor activity and rotorod behavior show that improgan antinociception is not explained by reductions in motor behavior or motor coordination (Li, et al., 1997). Unlike results with thermal tests employing warm and cold stimuli, suppression of high temperature tail flick responses in rats is a good predictor of human analgesia (Le Bars, et al., 2001). Thus, improgan-like drugs should have morphine-like, analgesic efficacy in humans. However, opioids are incompletely effective in human neuropathic pain (Argoff, et al., 2009), and the mechanisms that attenuate neuropathic allodynia are not the same as those that suppress acute nociception. Thus, the present experiments were performed to assess the potential utility of improgan-like drugs in the treatment of neuropathic pain.

Dramatic, dose-dependent, anti-allodynic effects of improgan were observed presently after icv (Fig. 1) and ic (Fig. 2) administration. Fig. 1 shows intermediate and maximal effects of 40 μ g and 80 μ g of improgan, respectively, similar to improgan's potency in thermal tests (Hough, et al., 2006). When injected into the RVM, 30 μ g of improgan maximally suppresses mechanical allodynia (Fig. 2A) and thermal nociception (Nalwalk, et al., 2004). Fig. 2A suggests an anti-allodynic ED_{50} of approximately 10 μ g of improgan in the RVM (Fig. 2A). Against thermal nociception, intra-RVM improgan has a very steep dose-response curve, with 30 μ g being fully active, and 10 μ g having no activity (Nalwalk, et al., 2004). Present results suggest that improgan's anti-allodynic activity lasts longer than its antinociceptive actions after icv (up to 60 min, Fig. 1) or ic (up to 2 hr, Fig. 2A) injections; thermal antinociception produced by this drug typically lasts 5 – 10 min after either route of administration (Nalwalk, et al., 2004; Hough et al., 2006).

Although the improgan receptor has not been identified, considerable information has been gained about its antinociceptive mechanism. Previous studies with acute thermal nociceptive assays have shown that ic improgan acts in the PAG and RVM to stimulate descending analgesic circuits (Nalwalk, et al., 2004). The analgesic mechanism includes inhibition of supraspinal GABAergic transmission, activation of supraspinal cannabinoid, supraspinal

epoxygenase and spinal noradrenergic mechanisms (Hough et al., 2011). The RVM is critically important for impropgan's antinociceptive activity. For example, pharmacological silencing of RVM neurons completely blocked the antinociceptive activity of icv-administered impropgan (Nalwalk, et al., 2004). Consistent with the hypothesis that RVM neuronal activity is required for impropgan antinociception, recent experiments in lightly-anesthetized rats found that icv-administered impropgan activates RVM pain-inhibiting OFF-cells, while blocking the activity of pain-facilitating ON-cells (Heinricher, et al., 2010). Similar to the proposed mechanisms for the analgesic actions of opioids and cannabinoids (Heinricher and Ingram, 2008), these findings suggest that impropgan's activation of RVM OFF-cells is the neural basis for the attenuation of acute nociceptive responses by this drug (Heinricher, et al., 2010).

The importance of RVM circuits in impropgan's activity and in neuropathic pain in general, led to the present assessment of impropgan's anti-allodynic activity following intra-RVM administration. The finding (Fig. 2) that impropgan acts in the RVM to reverse mechanical allodynia and to fully mimic the icv activity of this drug identifies this brain stem area as an important site for impropgan's anti-allodynic activity. The finding (Fig. 2B) that impropgan also had detectable activity when injected into dorsolateral medulla (chosen to be a control site, Fig. 3) was unexpected. The effect, significantly less than that seen with this dose in the RVM, may be partially due to effects of saline injections at this site (Fig. 2B), and partially due to diffusion of the drug to the RVM. Impropgan (30 μ g) was inactive on thermal nociception when it was injected into the ventrolateral medulla (Nalwalk et al., 2004).

As mentioned, neuronal circuits within the RVM are linked to the modulation of both thermal nociception in normal animals and mechanical allodynia following nerve injury, but the modulatory mechanisms are distinct. Thus, in healthy subjects, pharmacological inactivation of RVM neurons has no effect on baseline tail flick responses (Hough, et al., 2001b), but such treatments reverse nerve injury-induced mechanical allodynia, indicating that RVM neuronal activity is required to maintain the allodynic state (Bee and Dickenson, 2008; Burgess, et al., 2002). Sustained firing of spinally-projecting, pain-enhancing ON-cells in the RVM has been shown to follow spinal nerve injury, and chemical lesioning of these cells shows their key participation in the maintenance of the persistent allodynic state (Bee and Dickenson, 2008; Burgess, et al., 2002; Carlson, et al., 2007). The recent demonstration that icv impropgan inhibits ON-cell activity in the RVM (Heinricher, et al., 2010) is commensurate with the anti-allodynic profile of impropgan in this brain area (Fig. 2).

The present results, which show that impropgan suppresses mechanical allodynia following nerve injury, might suggest that impropgan-like drugs could be effective medications for the treatment of human neuropathic pain. However, reversal of neuropathic allodynia in animal models is not always a reliable predictor of efficacy in human neuropathic pain. Recent studies of conditioned place preference in SNL rats strongly suggest that rodents with nerve injury experience spontaneous pain which can be differentiated from allodynic responses (Qu et al., 2011). It was shown that in the absence of evoked responses, intra-RVM lidocaine created a positive place preference (i.e. was rewarding) in SNL rats, and this effect was prevented by lesions of the anterior cingulate cortex. Such lesions did not reverse tactile allodynia, indicating that "evoked pain is insufficient to account for the aversive state of the animals" (Qu et al., 2011). This important finding means that drugs which attenuate SNL-induced allodynia will not necessarily relieve spontaneous neuropathic pain in humans, consistent with several failed clinical trials of drugs which attenuated evoked responses in animal models (King et al., 2009). On the other hand, the fact that intra-RVM lidocaine appears to relieve spontaneous pain in neuropathic rats shows that pharmacological attenuation of RVM neuronal activity (likely to be RVM ON-cell activity), could relieve both spontaneous and evoked pain. Since impropgan is known to silence RVM ON-cells

(Heinricher et al., 2010), then brain-penetrating congeners of impropion (e.g. Hough et al., 2005) may indeed be capable of attenuating both spontaneous and evoked pain in human neuropathic conditions. Additional studies are needed to assess these possibilities.

4. Experimental procedures

4.1. Animals

Sprague-Dawley rats (male, 200–250 g, Taconic Farms, Germantown, NY) were maintained on a 12-h light/dark cycle and provided food and water ad libitum. They were housed in groups of three or four until surgery and individually thereafter. All animal experiments were approved by the Institutional Animal Care and Use Committee of Albany Medical College.

4.2. Spinal Nerve Ligation (SNL)

Unilateral tight ligations of the L5 and L6 spinal nerves were performed following the methods of Kim and Chung (1992), as modified by Lee et al. (2003). Animals were anesthetized with isoflurane, a midline incision was made over the lumbar region and the back musculature opened to reveal the L6 transverse process. The process was removed and the L4, L5, and L6 spinal nerves were exposed using a pulled glass hook. The L5 and L6 spinal nerves were tightly ligated, while the L4 nerve root was gently irritated by light rubbing and stretching using the glass hook. The deep back musculature was then sutured, the wound closed with clips, and antibacterial ointment applied. Animals were monitored daily for general health and allowed to recover for 3 days prior to nociceptive testing. Animals displaying mechanical allodynia (i.e. scores less than 6 g) on days 4–5 post-SNL were subjected to a second surgery for insertion of either an icv or ic guide cannula; a minimum of 5 days elapsed between the original SNL surgery and surgical placement of the cannula.

4.3. Icv and ic cannulation

To permit icv or ic drug administration in conscious subjects, rats previously subjected to SNL surgery were anesthetized with pentobarbital (50 mg/kg i.p., supplemented with isoflurane) and a chronic guide cannula containing a stylet was implanted into either the left lateral ventricle or the RVM and anchored to the skull (Crane and Glick, 1979). Stereotaxic coordinates (AP, ML and DV, mm from bregma [Paxinos and Watson, 1998]) for the lateral ventricle and the RVM were –0.8, 1.5, –3.3, and –11.0, 0.0, –7.5, respectively. After the cannulation surgery, animals were allowed to recover for at least 5 days prior to additional nociceptive testing. Thus, a minimum of ten days elapsed between SNL surgery and drug studies. Each animal was only used for a single experiment.

4.4. Nociceptive testing

Mechanical nociceptive thresholds (up to 15 g of force) were monitored with von Frey filaments (Stoelting, Inc., Wood Dale, IL) applied to the rat hind paw plantar surface following the up-down method (Chaplan, et al., 1994). Animals were placed on a raised wire mesh floor in a plexiglass cylinder, allowed to acclimate for five min, and tested. Subjects were tested following recovery from SNL surgery, and following recovery from cannulation surgery. Only subjects displaying mechanical allodynia (i.e. scores less than 6 g) after both surgeries were used.

4.5. Drug treatment and testing procedure

Impropion base was synthesized as described (Hough et al., 2000b), and dissolved in dilute HCl, neutralized to pH 5.5 to 6, and diluted with saline. On the day of drug testing, animals

were placed in the test chamber and allowed to acclimate for 5 min. They were then baseline tested, removed from the test chamber, and gently secured by wrapping with a laboratory pad. The cannula stylet was removed, and replaced by the injection cannula, which extended 1 mm beyond the guide to penetrate into the lateral ventricle. For ic microinjections, the injection cannula length was 2 mm beyond the tip of the guide cannula. Icv microinjections (total volume = 5 μ l) of either saline or improgan were performed manually over a 5 min period. Ic injections were made over a one min infusion period in a total volume of 0.5 μ l. Both the subject's drug group and the identities of the injected solutions were unknown to the experimenter. Sample sizes of n=4–7 were used as described in each figure. Successful injections were assured by following the movement of an air bubble in the tubing between the syringe and the cannula, and by the absence of leakage. One min after the end of the infusion, wire cutters were used to clip off and seal the injection cannula. Subjects were returned to the test chamber where they remained until re-testing ten min post-infusion, and were then returned to their home cages. Subjects were again placed in the test chamber and were re-tested at the indicated times. Once testing was complete, subjects were deeply anesthetized with Nembutal (100 mg/kg, i.p.), received an icv or ic injection of India Ink, and were euthanized. Successful placements were verified by proper distribution of ink in the cerebroventricular system (icv) or within the RVM after sectioning and staining as previously described (Nalwalk, et al., 2004). Data from animals with improper cannula placements were excluded.

4.6. Statistical analysis

Mechanical thresholds were subjected to ANOVA followed by Bonferroni comparisons (Prism 5.0, Graphpad Software, LaJolla, CA).

Highlights

> Improgan is an experimental analgesic with good pre-clinical activity on acute pain tests. > The efficacy of improgan in neuropathic pain testing has not been assessed. > We show that improgan attenuates the symptoms of neuropathic pain in rats. > The rostral ventromedial medulla may be an important site of improgan action. > Improgan – like drugs developed for human use may be effective in treating neuropathic pain.

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Figure 1.

Improgan-induced reversal of mechanical allodynia. SNL rats were baseline tested for mechanical thresholds (0 time), then received an icv injection of either improgan (40 or 80 µg) or saline vehicle, and were re-tested at the times shown after drug administration (abscissa, min). Thresholds (ordinate, g, mean ± SEM) are shown for the number of subjects given in parentheses. +, ++P < 0.05, 0.01 vs. saline, respectively, at the times indicated. #n=4 at this time point only (one subject not tested). Light dashed line indicates maximal possible effect (15 g).

Figure 2.

Anti-allodynic effects of ic-administered improgan. SNL rats were pre- and post-tested exactly as in Figure 1, but received an ic injection of either saline vehicle (0.5 μ l) or the specified dose of improgan (Imp). A) Effects of intra-RVM Imp (5 to 30 μ g as specified) are shown. B) Effects of Imp (15 μ g) or saline microinjected into or outside of the RVM (RVM and Non-RVM, respectively). The data from RVM saline and RVM Imp 15 μ g treatments shown in B) are taken from A). Placements for all ic injections are depicted in Figure 3. *, ** P < 0.05, 0.01 vs. Saline at the same time point.

Figure 3.

Ic injection sites from the experiments of Fig. 2 are shown at AP -10.52 mm from bregma (Paxinos and Watson, 1998). AP placements for most injections were located at -10.5 mm, but ranged from -10.3 to -11.3 mm.