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Research Report

Non-opioid antinociception produced by brain stem injections of improgan: Significance of local, but not cross-regional, cannabinoid mechanisms

Lindsay B. Hough, Konstantina Svokos, Julia W. Nalwalk*

Center for Neuropharmacology and Neuroscience, Albany Medical College MC-136, 47 New Scotland Avenue, Albany, NY 12208, USA

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ABSTRACT

Improgan, a cimetidine derivative which lacks activity at known histamine, opioid or cannabinoid receptors, acts by an unknown mechanism in the periaqueductal gray (PAG) and raphe magnus (RM) to stimulate descending, analgesic circuits. These circuits may utilize cannabinoid mechanisms. To characterize further the nature of these circuits, the effects of intracerebral (i.c.) microinjections of rimonabant (a CB_1 receptor inverse agonist) were studied on antinociceptive responses following i.c. microinjections of improgan and the cannabinoid agonist WIN 55,212 (WIN) in rats. Separate intra-RM injections of improgan (30 μg) and WIN (8 μg) produced near-maximal antinociception on both the hot plate (HP) and tail flick (TF) nociceptive tests. Pretreatment with intra-RM rimonabant (20 μg) antagonized the antinociception produced by both intra-RM improgan and intra-RM WIN, but had no effects when given alone. Similar studies with improgan demonstrated rimonabant-sensitive sites within the dorsal and ventrolateral PAG. However, intra-RM pretreatment with rimonabant had no effect on antinociceptive responses following intra-PAG improgan. These studies show that improgan activates pain-relieving mechanisms in the PAG and the RM, both of which may utilize local cannabinoid mechanisms.

1. Introduction

Improgan, a compound derived from the H_2 antagonist cimetidine, produces strong antinociception in rodents following intracerebroventricular (icv) administration (Li et al., 1996). Extensive testing with tail-pinch, tail flick, hot plate, and neuropathic pain assays (Hough, 2004 and in preparation) shows a broad antinociceptive efficacy; a lack of activity by improgan on locomotor and rotorod tests suggest a true analgesic (vs. motor impairment) action (Li et al., 1997). Several congeners of improgan with considerably higher potency and/or brain-penetrating properties have been recently discovered (Hough et al., 2005, 2006, 2007).

More is known about the anatomical sites of improgan action than is known about its mechanism. CNS mapping studies have shown that improgan, like cannabinoids and opioids, acts in the dorsal PAG (DPAG), the ventrolateral PAG (VLPAG), and RM (Nalwalk et al., 2004). Unlike these other analgesics, however, improgan has no direct activity in the spinal cord (Nalwalk et al., 2004). Descending circuits

* *Corresponding author*. Fax: +1 518 262 5799.
E-mail address: houghl@mail.amc.edu (L.B. Hough).

Abbreviations: ANOVA, analysis of variance; DPAG, dorsal periaqueductal gray; EMB, explosive motor behavior; HP, hot plate; i.c., intracerebral; icv, intracerebroventricular; PAG, periaqueductal gray; RM, raphe magnus; TF, tail flick; VLPAG, ventrolateral periaqueductal gray; WIN, WIN 55,212

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connecting the PAG, RM, and spinal cord are thought to mediate improgan antinociception (Hough et al., 2001). The antinociceptive mechanism is not mediated by opioids (Hough et al., 2000), and improgan lacks activity on known receptors for histamine (Mobarakeh et al., 2003), opioids (Hough et al., 2000), cannabinoids (Hough et al., 2006), and over 100 other receptors (Hough, 2004). Thus, improgan produces non-opioid antinociception by actions in the PAG and RM, but the improgan receptor has not been found.

Evidence is accumulating that a cannabinoid mechanism may be relevant for improgan analgesia. Cannabinoid-blocking doses of rimonabant (SR141716A, the CB_1 antagonist/inverse agonist) antagonize improgan antinociception in rats and mice (Hough et al., 2002). However, radioligand binding assays and GTP-γ-S functional assays have confirmed that improgan does not directly bind to, block or activate CB_1 or CB_2 receptors (Hough et al., 2002, 2006). These findings imply that improgan might indirectly activate CB_1 receptors (e.g. by releasing endocannabinoids). Improgan studies with germline CB_1 null mice gave equivocal results which neither support nor refute the relevance of CB_1 receptors (Hough et al., 2002). In a recently-reported study, acute improgan antinociception was reduced by chronic cannabinoid pretreatment, also in support of some type of improgan–cannabinoid interaction (Nalwalk et al., 2006). Improgan was also found to produce mild hypothermia in rats, a response which was attenuated by rimonabant (Salussolia et al., 2007). Very recently, Gehani et al. (2007) described the existence of rimonabant congeners with very low CB_1 affinities which retained improgan-blocking properties, suggesting the possible relevance of non-CB_1, non-CB_2 cannabinoid receptors linked to improgan antinociception. Because the rimonabant-induced antagonism of improgan is an important clue to improgan's mechanism of action, the present study investigated the rimonabant sensitivity of improgan antinociception following microinjections into three brain stem areas relevant to analgesic circuits.

2. Results

Various combinations of vehicle, improgan, WIN, and rimonabant were administered by i.c. injections into the RM, VLPAG, or DPAG (see Fig. 1 for placements). Microinjections of improgan into the RM and DPAG had no observable untoward effects. Injections of improgan into the VLPAG occasionally produced a motor syndrome previously described as "explosive motor behavior" (EMB), consisting of uncontrolled jumping, stereotyped circling, excessive running, sometimes accompanied by vocalization (Nalwalk et al., 2004). Improgan-induced EMB is further discussed below.

The effects of i.c. rimonabant or vehicle (administered as a pretreatment into the RM) were studied on nociceptive responses following intra-RM administration of improgan, WIN, or vehicle (Fig. 2). In vehicle-pretreated subjects, both improgan (30 μg) and WIN (8 μg) elicited strong reductions in HP and TF nociceptive responding when administered into the RM. Rimonabant pretreatment (20 μg) produced complete or nearly-complete antagonism of both improgan and WIN antinociception (Fig. 2). ANOVA of the HP data of Fig. 2 (between group #1: rimonabant pretreatment; between group #2: antinociceptive treatments; within groups [repeated measures]: time) found highly significant ($P<0.001$) main effects of rimonabant pretreatment, antinociceptive treatments, and time, with a significant ($P<0.001$) pretreatment by treatment by time interaction. Identical results were obtained when a comparable ANOVA was performed on the TF data of Fig. 2. Rimonabant given alone did not change nociceptive thresholds (Fig. 2).

Additional single-cannula experiments were performed with drug injections made into two areas of the PAG (Fig. 3). In these experiments, however, three doses of rimonabant (5, 20 and 40 μg) were studied. ANOVA of the HP data of Fig. 3 (between group #1: cannula placement [DPAG vs. VLPAG]; between group #2: rimonabant pretreatment; within groups [repeated measures]: time) found significant main effects of rimonabant ($P<0.001$) and time ($P<0.001$), with a significant ($P<0.001$) rimonabant by time interaction. The same results were obtained from the comparable ANOVA of the TF data of Fig. 3 (for main effect of rimonabant, $P=0.017$). Because there were no significant main effects or interaction terms involving PAG region in either the HP or TF data sets (indicating no differences between the two areas), results from these two regions were pooled (Fig. 3). Following vehicle pretreatments, intra-PAG improgan produced large increases in both HP and TF nociceptive latencies. Intra-PAG pretreatment with the lowest dose of rimonabant (5 μg) was largely without effect (Fig. 3). Pretreatment with the larger dose (20 μg) reduced improgan antinociception by about 50% on both tests at 5 and 10 min after administration (Fig. 3). The largest dose of rimonabant (40 μg) tended to further reduce the antinociception on the TF, but not on the HP test (Fig. 3). A dose-related antagonism of improgan was most evident on the TF test at the 10 min point (Fig. 3).

Presently, a total of 50 intra-PAG injections of improgan (DPAG=11, VLPAG=39) produced no EMB in the DPAG, and severe EMB in 5 of the VLPAG injections; data from the latter subjects were not collected. Six other VLPAG subjects showed mild motor changes that did not prevent testing. One of these six had a placement outside of the VLPAG target area and was not used. Data from the other five were included in Fig. 3, (total $n=44$); location of injections in these subjects are shown in the left PAG in Fig. 1. Rimonabant pretreatment did not affect the incidence of EMB at either dose level (data not shown).

Because of the known significance of PAG-RM circuitry in analgesic mechanisms, the possibility that improgan antinociception elicited from the PAG might have a cannabinoid component in the RM was investigated in double-cannulated subjects (Figs. 4 and 5). Improgan microinjections into either the VLPAG (Fig. 4) or DPAG (Fig. 5) increased both HP and TF nociceptive latencies. ANOVA of HP data (Fig. 4) from vehicle-RM treated subjects (between groups: VLPAG improgan vs. VLPAG vehicle; within groups [repeated measures]: time) found highly significant ($P<0.001$) main effects of improgan, time and a significant ($P<0.01$) improgan by time interaction term. Identical results were obtained with TF data of Fig. 4. However, pretreatment with rimonabant into the RM had no effect on the antinociception elicited by intra-VLPAG (Fig. 4) or DPAG (Fig. 5) improgan injections. For both sets of data (Figs. 4 and 5), this was substantiated by separate ANOVAs of data