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Enhanced antinociception with repeated microinjections of apomorphine into the periaqueductal gray of male and female rats

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Abstract

Dopamine neurons in the ventrolateral periaqueductal gray (PAG) have been reported to contribute to antinociception. The objective of this manuscript was to determine how this dopamine mediated antinociception differs from what is known about morphine-induced antinociception.

Microinjection of the dopamine receptor agonist apomorphine into the PAG produced a dose dependent increase in hot plate latency and a decrease in open field activity that was greater in male than female rats. The peak antinociceptive effect occurred 5 min after apomorphine administration. Surprisingly, the antinociceptive potency of apomorphine was enhanced following systemic administration of the opioid receptor antagonist naloxone in male, but not female rats. The antinociceptive potency of microinjecting apomorphine into the ventrolateral PAG in male and female rats was also enhanced following twice-daily injections for two days. The characteristics of apomorphine-induced antinociception differ from previous reports of morphine antinociception following PAG microinjections, in that morphine antinociception peaks at 15 min, is blocked by naloxone, and is susceptible to tolerance with repeated administration. These results indicate that apomorphine-induced antinociception is distinct from opioid-induced antinociception, and that dopamine receptor agonists may provide a novel approach to pain modulation.

Keywords

pain modulation; analgesia; dopamine; naloxone; sex differences; rat

Introduction

The periaqueductal gray (PAG) is an anatomically and pharmacologically complex structure involved in a wide range of behaviors (Carrive and Morgan, 2012). One of these behaviors is the modulation of nociception. Microinjection of morphine into the ventrolateral PAG produces antinociception (Jacquet and Lajtha, 1974, Jensen and Yaksh, 1986, Bobeck et al.,

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2009) that shows tolerance with repeated administration (Tortorici et al., 1999, Morgan et al., 2006) and is associated with defensive immobility (Morgan et al., 1998). The antinociceptive effects of PAG opioids appear to be mediated by direct inhibition of GABAergic neurons (Vaughan and Christie, 1997, Vaughan et al., 1997).

In contrast to the vast knowledge about PAG opioids, relatively little is known about the contribution of PAG dopamine neurons to antinociception. Systemic administration of dopamine receptor agonists such as apomorphine produces antinociception (Michael-Titus et al., 1990, Morgan and Franklin, 1991, Pelissier et al., 2006), and these effects are mediated in part by the PAG. Microinjection of apomorphine into the ventrolateral PAG produces antinociception (Meyer et al., 2009) and disruption of dopamine signaling in the PAG attenuates morphine antinociception (Flores et al., 2004, Meyer et al., 2009). Although apomorphine is an agonist at D1 and D2 receptors, the antinociception mediated by microinjection of apomorphine into the PAG is mediated by D2 receptors (Flores et al., 2004, Meyer et al., 2009). Selective chemogenic activation of tyrosine hydroxylase neurons (i.e., catecholaminergic neurons) in the PAG has been shown to produce a mild antinociceptive effect in mice (Li et al., 2016). Neurons containing the dopamine synthesis enzyme tyrosine hydroxylase have been reported throughout the rostrocaudal extent of the ventrolateral PAG (Grzanna and Molliver, 1980, Arsenault et al., 1988, Hasue and Shammah-Lagnado, 2002, Flores et al., 2004), but this synthetic pathway appears to lead to norepinephrine in the larger more posterior neurons. The smaller dopamine neurons are located in more medial and anterior parts of the PAG (Suckow et al., 2013), suggesting that antinociception may be site specific.

The degree to which PAG dopamine and opioids interact is less clear. Disrupting dopamine signaling in the PAG disrupts the antinociceptive effect of microinjecting morphine into the PAG (Flores et al., 2004, Meyer et al., 2009), but whether this signaling is bidirectional — such that blocking opioid signaling blocks dopamine mediated antinociception — is not known. In addition, given that tolerance to the antinociceptive effects of morphine develops rapidly with PAG administration (Morgan et al., 2006, Bobeck et al., 2012), we hypothesized that tolerance would also occur to repeated apomorphine injections. In contrast to morphine, the limited distribution of dopamine neurons in the PAG suggests that the antinociceptive effects of apomorphine would be greatest at more anterior sites. These hypotheses were tested by measuring the antinociceptive effect of microinjecting the non-selective dopamine receptor agonist apomorphine into the PAG of male and female rats.

Methods

Subjects

Experiments were performed on adult male (252 – 436 g) and female (212 – 364 g) Sprague-Dawley rats bred in captivity at Washington State University Vancouver. All protocols were approved by the Institutional Animal Care and Use Committee at Washington State University and followed the National Institute of Health Guide for the Care and use of Laboratory Animals. Within-subjects designs and cumulative dosing procedures were used when possible to reduce the number of animals used. Distress was

kept to a minimum by measuring the threshold to detect a thermal stimulus (see Behavioural Assessment section below).

Surgery

Rats were injected with atropine (0.08 ml, s.c.; Patterson Veterinary, Everett Washington) and anesthetized with pentobarbital (60 mg/kg, i.p., Sigma Aldrich, St. Louis, Missouri). A 9 mm, 26-gauge guide cannula aimed at the ventrolateral PAG was implanted using stereotaxic procedures (AP: +1.7 mm, ML: ± 0.6 mm, DV: -4.6 mm for males and -4.5 mm for females from lambda). The cannula was secured to two screws in the skull using dental cement (Patterson Dental, Spokane Washington). A 9 mm stylet was inserted into the guide cannula to maintain patency. Following surgery, antibiotic cream was applied to the incision, and the non-steroidal anti-inflammatory analgesic Meloxicam (1 mg/kg, s.c.; Patterson Veterinary, Everett Washington) was administered. Rats were maintained under a heat lamp until awake and then transferred to a cage where they were housed individually. Each rat was monitored for health status, handled, and the stylet checked daily during the week between surgery and testing.

Drug Administration

One day prior to testing, an injection cannula extending 2 mm beyond the tip of the guide cannula was lowered into the PAG to create a path for the injection on the test day. Apomorphine hydrochloride (Sigma Aldrich, St. Louis Missouri) was microinjected into the PAG in a volume of 0.4 μ l using a 1 μ l Hamilton syringe (Reno, Nevada). The injection required 40 s and the cannula was removed 20 s later. Low doses of apomorphine (2.2 and 4.6 μ g) were dissolved in saline, and higher doses (10, 22, and 46 μ g) were dissolved in 20% DMSO and saline. Matching vehicle injections were used for the control group.

Behavioural Assessment

Nociception was assessed using the hot plate test (Columbus Instruments, Columbus Ohio). Rats were placed on a 52.5 °C plate and the latency to lick the hindpaw was measured. The hot plate test was used because it can be applied repeatedly to generate morphine dose response curves (Morgan et al., 2006). The rat was removed from the plate if no response occurred within 50 s. No additional injections or testing occurred once the rat reached this cutoff latency.

Open field activity was assessed to determine whether microinjection of apomorphine, a psychostimulant, into the ventrolateral PAG would increase activity or cause immobility as occurs following morphine microinjection (Morgan et al., 1998). Rats were placed in an open field (0.6 $\times$ 1 m) and the number of 15 cm squares crossed in 30 s was counted. Rats were returned to their home cage immediately after testing.

Experiment 1: PAG Location and Antinociceptive Time Course

The distribution of noradrenergic neurons in the posterior PAG and dopamine neurons in more anterior regions (Suckow et al., 2013) suggests that microinjecting apomorphine into these more anterior PAG regions would have a greater antinociceptive effect than posterior injections. This hypothesis was tested by microinjecting apomorphine into posterior (0.28 –

1.00 mm anterior to the interaural line) and more anterior aspects (1.36 – 2.20 mm) of the ventrolateral PAG in male (n = 8/location) and female (n = 9 – 11) rats (Figure 1). Baseline hot plate latency and open field activity were assessed 15 min before administration of apomorphine. Apomorphine was administered using a cumulative dosing procedure that resulted in third log doses of 2.2, 4.6, 10, 22, and 46 µg/0.4 µl. Our previous research showed that nociception is not altered by repeated microinjections of vehicle into the PAG (Morgan et al., 2006). Injections were separated by 7 min, and hot plate latency and open field activity were assessed 5 min following each injection. Rats were returned to their home cage following each test. One week later, the time course for antinociception in male (n = 12) and female (n = 20) rats was assessed following microinjection of apomorphine (24 µg/0.4 µl) into the PAG.

Experiment 2: Naloxone

Electrophysiological evidence suggests that PAG opioids disinhibit dopamine neurons (Flores et al., 2004, Li et al., 2016). If dopamine neurons are downstream of opioid neurons as these findings suggest, then administration of the opioid receptor antagonist naloxone should have no effect on the antinociceptive effect of microinjecting apomorphine into the PAG. This hypothesis was tested by injecting male (n = 12/condition) and female (n = 6 – 8 rats/condition) rats with naloxone and measuring changes in the antinociceptive potency of cumulative doses of apomorphine microinjected into the ventrolateral PAG. Hot plate latency was assessed 5 min following each injection. A large dose of naloxone (2 mg/kg, s.c.) was administered 1 min prior to the first microinjection of apomorphine (6 minutes before the first test) to insure potency throughout the 34 minutes of testing. Saline was injected as a control. Most, but not all of the rats, were tested in both naloxone and saline conditions with at least 3 days between tests.

Experiment 3: Repeated Apomorphine Microinjections

Tolerance to the antinociceptive effect of morphine occurs rapidly with repeated administration (Bobeck et al., 2012). The present experiment tested the hypothesis that tolerance would also occur to antinociception following repeated administration of apomorphine. Apomorphine (20 ug) was microinjected into the ventrolateral PAG of male (n = 7 – 9) and female (n = 6/condition) rats twice a day for 2 days. This procedure produces pronounced tolerance to opioids microinjected into the PAG (Bobeck et al., 2012). Control rats received twice daily injections of 20% DMSO in saline. Rats were given cumulative doses of apomorphine into the PAG 16 h later to determine whether the prior repeated apomorphine microinjections had altered antinociceptive potency.

Histology

Rats were euthanized with isoflurane immediately following testing. Once dead, the brain was removed and placed in 10% formalin. At least two days later, a vibratome (The Vibratome Company, St. Louis Missouri) was used to make 100 µm coronal sections through the PAG. The brain sections were mounted on a slide and viewed under a microscope to localize the injection site (Paxinos and Watson, 2005). Only cannula tips terminating in or immediately adjacent to the ventrolateral PAG were included in data analysis (Fig. 1).

Data Analysis

Mean hot plate data were used to generate dose response curves and calculate the antinociceptive potency of apomorphine (GraphPad Prism, San Diego, CA). Potency was defined as the dose (ED_{50}) of half maximal antinociception (Tallarida, 2000). The main effect of differences in potency between two groups were assessed with ANOVA using GraphPad Prism. The time course for apomorphine antinociception and dose dependent decreases in open field activity were analyzed with a repeated measures ANOVA. Statistical significance was defined as a probability less than .05.

Results

Experiment 1: PAG Location and Antinociceptive Time Course

Microinjection of apomorphine into the PAG produced a dose-dependent antinociception in both male and female rats. There was no difference in mean baseline hot plate latency between male (16.2 ± 1.1 s, $N = 16$) and female (15.6 ± 0.9 s, $N = 20$) rats ($t(34) = 0.41$, NS). Antinociceptive potency did not differ between anterior ($ED_{50} = 18.7$ μ g) and posterior ($ED_{50} = 14.0$ μ g) microinjections in male rats ($F(1,92) = 2.59$; NS; Figure 2). In contrast, the antinociceptive potency of apomorphine was greater following microinjection into the posterior ($ED_{50} = 18.3$ μ g) compared to the anterior ($ED_{50} = 32.1$ μ g) PAG in female rats ($F(1,116) = 11.16$, $p < .001$; Figure 2). Microinjection of apomorphine produced a dose-dependent decrease in open field activity in both male and female rats. There was no significant difference in activity between anterior and posterior ventrolateral PAG microinjections in male ($F(1,14) = 0.08$, NS) or female ($F(1,18) = 1.18$, NS) rats. Thus, the effect of injections in anterior and posterior regions were combined for presentation in Figure 3. The mean $\pm$ SEM number of open field squares crossed in 30 s during baseline assessment was 19.0 ± 1.3 for male rats and 20.8 ± 1.4 for female rats. The dose-dependent decrease in activity was statistically significant for both male ($F(4,60) = 16.65$, $p < .001$) and female ($F(4,76) = 13.22$, $p < .001$) rats. The ED_{50} for decreased open field activity was 4.1 μ g for male rats and 4.6 μ g for female rats.

Subsequent assessment of the duration of apomorphine-induced antinociception in these rats revealed peak antinociception 5 min following administration in both male and female rats (Figure 4). The increase in hot plate latency was significantly different from the baseline latency ($F(1,30) = 22.96$, $p < .0001$). Although male rats had a higher baseline hot plate latency (see first data point in Figure 4), the increase in latency following apomorphine administration did not differ between male and female rats, as indicated by a lack of a significant sex by trial interaction ($F(1,30) = 0.99$, NS). The greater antinociception in male compared to female rats was maintained throughout the 90 min test period ($F(1,30) = 26.75$, $p < .0001$).

Experiment 2: Naloxone

Administration of cumulative doses of apomorphine into the ventrolateral PAG caused a dose-dependent increase in hot plate latency for both male and female rats consistent with the data in Experiment 1. There was no significant difference in baseline hot plate latency for the two male (13.4 ± 1.0 vs. 13.1 ± 1.3 s; $t(22) = 0.19$, NS) or two female (13.0 ± 1.4 vs.

14.1 $\pm$ 1.0 s; $t(12) = 0.64$, NS) groups prior to drug administration. Systemic administration of naloxone enhanced the antinociceptive effect of apomorphine in male, but not female rats (Figure 5). The antinociceptive potency of apomorphine in male rats increased from a ED_{50} value of 26.0 μ g in control rats to an ED_{50} of 11.1 μ g in rats treated with naloxone ($F(1,140) = 20.47$, $p < .001$). The antinociceptive potency of apomorphine in female rats did not differ between rats treated with saline or naloxone ($F(1,80) = 2.36$, NS; $ED_{50} = 34.7$ vs. 26.9 μ g).

Experiment 3: Repeated Apomorphine Microinjections

There was no significant difference in baseline hot plate latency following two days of repeated apomorphine vs. vehicle microinjections in male (12.7 $\pm$ 0.9 vs. 13.7 $\pm$ 2.4 s; $t(14) = 0.10$, NS) or female (15.6 $\pm$ 3.5 vs. 10.8 $\pm$ 1.1 s; $t(10) = 1.29$, NS) rats. In contrast, repeated microinjections of apomorphine into the ventrolateral PAG enhanced antinociception to subsequent apomorphine injections in both male and female rats (Figure 6). Male rats showed an increase in potency from 34.5 to 18.0 μ g ($F(1,92) = 7.90$, $p < .01$) and female rats showed an increase in potency from 42.7 to 20.8 μ g ($F(1,68) = 6.43$, $p < .01$).

Discussion

The present data confirm that microinjection of the dopamine receptor agonist apomorphine into the PAG produces antinociception (Meyer et al., 2009). Certain aspects of these data, such as the greater antinociception in male compared to female rats and the suppression of open field activity, are consistent with the effects of morphine when microinjected into the ventrolateral PAG. Other data, such as the enhancement of antinociceptive potency following naloxone administration or repeated apomorphine administration are distinct from the effects of morphine.

The present study extends previous research by showing that microinjection of apomorphine into the PAG of both male and female rats produces antinociception. The magnitude of apomorphine-induced antinociception differed between male and female rats because of reduced apomorphine antinociception with microinjections into anterior regions of the ventrolateral PAG in female rats. The antinociceptive potency of apomorphine injections into the posterior PAG of female rats was comparable to that produced by apomorphine injections into anterior and posterior regions of the ventrolateral PAG of male rats (see Figure 2). Although previous studies in male rats show that the magnitude of morphine antinociception is comparable regardless of the PAG injection site (Jensen and Yaksh, 1986), morphine is less potent in female compared to male rats when injected into the ventrolateral PAG (Bobeck et al., 2009). Given that dopamine neurons do not appear to be present in the posterior PAG (Suckow et al., 2013), the antinociceptive effect produced by apomorphine injections into the posterior PAG indicates that dopamine receptors have a broader distribution than the neurons. Presumably these receptors receive input from PAG dopamine neurons located within the PAG (Hasue and Shammah-Lagnado, 2002, Dong and Swanson, 2006).

Microinjection of apomorphine into the ventrolateral PAG also produced a dose-dependent decrease in open field activity, similar to that which occurs following morphine

microinjections (Morgan et al., 1998). This was a surprising finding given that apomorphine and other dopamine agonists cause hyperactivity following systemic administration (Lassen, 1976). However, a decrease in activity is consistent with the role of the ventrolateral PAG in mediating defensive immobility, which occurs whether output neurons in the ventrolateral PAG are activated by morphine, glutamate receptor agonists, electrical stimulation (Fardin et al., 1984, Morgan et al., 1998), or, as the present data show, by the dopamine receptor agonist apomorphine. These data, along with the lack of antinociception following injections outside the PAG (Meyer et al., 2009) indicate that PAG neurons mediate the antinociceptive effects of apomorphine.

Disruption of dopamine transmission in the PAG attenuates morphine antinociception (Flores et al., 2004, Meyer et al., 2009) indicating that dopamine neurons are downstream of opioid actions on mu-opioid receptors. This hypothesis is consistent with *in vitro* recordings showing that opioids disinhibit dopamine neurons (Li et al., 2016). According to this model, blocking opioid receptors would increase GABAergic inhibition onto dopamine neurons. Our finding that administration of the opioid receptor antagonist naloxone enhances apomorphine-induced antinociception suggests that apomorphine may produce antinociception via autoreceptor inhibition of dopamine neurons. That is, both blockade of opioid inhibition of GABAergic neurons and autoreceptor activation would reduce the activity of PAG dopamine neurons projecting to outside targets. This hypothesis is consistent with the finding that blockade of D2 receptors attenuates the antinociceptive effect of apomorphine microinjected into the PAG (Meyer et al., 2009). Such inhibition would reduce dopamine mediated inhibition of targets such as the basal nucleus of the stria terminalis (Li et al., 2016).

A similar enhancement of apomorphine-induced antinociception occurred with repeated administration. This enhanced apomorphine potency occurred in both male and female rats and is in direct contrast to the rapid decrease in morphine potency with repeated administration (Morgan et al., 2006, Bobeck et al., 2012). Although the neural mechanism for antinociceptive sensitization to apomorphine is not known, it is consistent with the behavioral sensitization that can occur with repeated administration of psychostimulants (Kalivas et al., 1993, Braga et al., 2009). Sensitization to apomorphine-induced antinociception could potentially be exploited therapeutically if dopamine receptors can be targeted specifically and in the absence of side effects.

In conclusion, the present study shows that activation of dopamine receptors in the PAG is sufficient to produce antinociception, but that this antinociception is distinct from, but interacts with, opioid mechanisms of antinociception. The enhanced antinociception with repeated apomorphine administration suggests there may be clinical benefits from dopamine-based treatments that are distinct from opioid analgesia.

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Figure 1.

Location of apomorphine microinjection sites. Apomorphine was injected into or adjacent to the ventrolateral PAG in male (left) and female (right) rats. Data from injections in posterior and anterior aspects of the PAG relative to the interaural line (Paxinos & Watson, 2005) were compared. All injections were on the right side of the PAG, but the injection sites for male rats are displayed on the left for clarity.

Figure 2.

Microinjection of apomorphine into the anterior or posterior ventrolateral PAG produced a dose-dependent antinociception. There was no significant difference in the antinociceptive potency of apomorphine between anterior and posterior PAG injections in male rats ($n = 8$ /condition), but anterior injections were less effective than posterior injections in female rats ($n = 9-11$ /condition). The location of the injection sites is shown in Figure 1. Nociception was assessed with the hot plate (HP) test and mean $\pm$ SEM hot plate latency is plotted

Figure 3. Microinjection of apomorphine into the ventrolateral PAG produced a dose-dependent decrease in open field activity in both male ($n = 16$) and female ($n = 20$) rats. The mean $\pm$ SEM number of squares crossed in 30 s is shown on the vertical axis.

Figure 4.

The peak antinociceptive effect of apomorphine was 5 min after microinjection into the PAG. Rats received a single injection of apomorphine (24 μ g/0.4 μ l) into the ventrolateral PAG. Mean $\pm$ SEM hot plate (HP) latency was higher from baseline throughout testing in male ($n = 12$) compared to female ($n = 20$) rats.

Figure 5. Administration of the opioid receptor antagonist naloxone enhanced the antinociceptive effect of apomorphine in male rats (left; $n = 12/\text{condition}$), but not in female rats (right; $n = 6\text{--}8/\text{condition}$). Naloxone (2 mg/kg, s.c.) was administered 1 min prior to the first of five cumulative apomorphine injections into the PAG. Mean $\pm$ SEM hot plate (HP) latency is plotted.

Figure 6.

Repeated microinjection of apomorphine into the PAG enhanced subsequent apomorphine antinociception in male (left; $n = 7-9$ /condition) and female (right; $n = 6$ /condition) rats.

Rats were injected with apomorphine ($20 \mu\text{g}/0.4 \mu\text{l}$) or vehicle (saline in 20% DMSO) into the PAG twice a day for two days. 16 h later, all rats were injected with cumulative doses of apomorphine and tested using the hot plate test. Mean $\pm$ SEM hot plate (HP) latency is plotted.