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Ingested bovine amniotic fluid enhances morphine antinociception in rats

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Abstract

Ingestion by rats of rat placenta or amniotic fluid enhances opioid-mediated, or partly opioid-mediated, antinociception produced by morphine injection, vaginal or cervical stimulation, late pregnancy, and foot shock. This phenomenon is believed to be produced by a placental opioid-enhancing factor (POEF). Ingestion by rats of human or dolphin placenta has also been shown to enhance opioid antinociception, suggesting that POEF may be common to many mammalian species. We tested bovine amniotic fluid (BAF) for its capacity to enhance morphine antinociception in female Long–Evans rats, as determined by percentage change from baseline tail-flick latency in response to radiant heat, and we report that 0.50 mL BAF effectively enhanced morphine antinociception but did not by itself produce antinociception. The efficacy of POEF across species suggests that POEF may have been functionally (and structurally) conserved during evolution. Furthermore, the availability of POEF at parturition, as well as its ability to enhance pregnancy-mediated antinociception without disrupting maternal behavior, offers a tenable explanation for the long-debated ultimate causality of placentophagia.

Keywords: POEF; Antinociception; Morphine; Opioids; Afterbirth; Amniotic fluid; Placenta; Bovine; Pain; Tail-flick; Rats

1. Introduction

Throughout mammalian evolution, many behavioral and physiological characteristics presumably have been maintained (albeit with modification) or have independently converged, the result of which has been behavioral and morphological parallelism among mammalian species. One characteristic that is recognized in all pregnant eutherian mammals is the presence of placenta as a secretory substrate and as a pathway for selective physiological exchange between parent and offspring [20]. Of particular interest in the comparative study of mammalian behavior is the fact that at parturition, females of almost all eutherian species engage in placentophagia—ingestion of placenta and amniotic fluid (AF) [9].

The desire to understand the proximate and ultimate causes of placentophagia has inspired a variety of explanations for the phenomenon. Although these hypotheses may include evolutionarily sound consequences of the behavior, such as satisfaction of specific hunger, maintenance of nest hygiene, and facilitation or induction of maternal behavior [9,18,24], they are typically too narrow or species specific in their scope. Alternatively, there is ample empirical evidence to suggest that enhancement of opioid-mediated antinociception at parturition (i.e., pregnancy-mediated analgesia [8]) is the most likely, and possibly most valuable, consequence of placentophagia [10]. Ingestion of placenta or AF enhances the opioid, or partly opioid, antinociception produced by the central action [4] of morphine sulfate (MS) injected peripherally [7,11,14,16,25], foot shock [16], vaginal or cervical stimulation [1,6,17,28], and late pregnancy [15], and does so via gastric vagal afferents [22,26]. More recent evidence indicates that placenta ingestion preferentially enhances antinociception produced by activation of δ or κ opioid receptors, but inhibits antinociception produced by activation of μ opioid receptors [3,5,11]. Ingestion of placenta or AF does not, however, affect morphine-induced hyperthermia [1] or nonopioid antinociception [13,23], nor does it produce antinociception by itself [4,12–14,16,17,23, 25]. Because opioid action is a prerequisite for the enhancing activity of ingested placenta and AF, Kristal et al. [14] have named the enhancing factor POEF—placental opioid-enhancing factor.

The enhancement of opioid antinociception by ingestion of POEF is well documented [10], but that research has predominantly made use of rat placenta and AF to demonstrate enhancement in rats. Interestingly, there is evidence that POEF is present in bovine amniotic fluid (BAF) and that it enhances opioid analgesia in cows [21], and there is evidence that POEF shows allopatric efficacy in its enhancement; specifically, both bottlenose dolphin placenta and human placenta enhance opioid antinociception in rats [1]. Demonstrations of POEF activity in rat, cow, dolphin, and

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human afterbirth materials are significant because they reveal POEF activity in each of four mammalian orders: Rodentia, Artiodactyla, Cetacea, and Primates, respectively. Similarly, the demonstrations of allopatric efficacy are important because they support the suggestion that POEF, as well as the system that mediates it, is consistent across species. In this study, we further explored POEF's allopatric opioid-enhancing quality by determining whether ingestion by rats of BAF enhances opioid antinociception. Whereas the POEF research on bottlenose dolphin placenta and human placenta revealed the cross-species effectiveness of carnivore and omnivore afterbirth material, respectively, testing BAF offers the opportunity to reveal cross-species effectiveness of herbivore afterbirth material. We expected that BAF would show POEF activity, yet would do so only in rats already experiencing opioid antinociception. Such interaction has been reported many times in the research on POEF and is one of the defining characteristics of POEF.

2. Method

All procedures used in this research were approved by the Institutional Animal Care and Use Committee (IACUC) of the University at Buffalo. Ninety-six female Long–Evans rats were used, all of which were born and maintained in the Behavioral Neuroscience Research Facility of the University at Buffalo Department of Psychology. Rats were housed individually under a 14:10-h light:dark cycle (lights on at 0500 h Eastern Standard Time) in 24.5 × 18 × 18–cm wire mesh cages with food (Agway Prolab Rat/Mouse/Hamster Formula 3000, Syracuse, NY) and water available ad lib. All rats exhibited normal estrous cyclicity prior to testing, had a mean body weight of 250 g, and were 75–100 days of age.

A tail-flick latency (TFL) test was used to determine antinociceptive threshold to radiant heat applied to the tail [16]. Morphine sulfate (Sigma Chemical Co., St. Louis, MO) was administered through the i.p. route at 3.0 mg/mL per kilogram in isotonic saline vehicle through a 25-gauge hypodermic needle. Orogastric infusions of BAF were performed with a blunted, 16-gauge hypodermic needle fitted with an 11.5-cm length of PE-160 tubing (Clay Adams, Franklin Lakes, NJ). During testing, rats were restrained in opaque polyvinyl restraining/testing tubes (5 × 21 cm). Bovine amniotic fluid (a gift of Dr. Carlos Pinheiro Machado) and saline were maintained at −20°C until being warmed to 37°C for 20 min immediately prior to infusion.

Rats were habituated to the testing procedure on 5 consecutive days prior to testing by daily intubation without infusion and by daily restraint for 1 h in restraining/testing tubes. Prior to and during testing, rats were food- and water-deprived for 3–4 h to reduce stomach contents. During testing, rats received either MS or saline injection and received orogastric infusion of either BAF or saline in either a 0.25- or 0.50-mL dose (12 per group; 2 × 2 × 2 design: drug [morphine, saline] × enhancer [BAF, saline] × volume of enhancer [0.25 mL, 0.5 mL]; no repeated measures). TFL was determined by averaging the latency on the last three of four TFL trials. TFL trials were separated by 30 s, and an 8-s ceiling was imposed on each trial to avoid tissue damage.

After baseline TFL (TFL_B) was determined, rats were returned to their cages. Ten minutes after TFL_B ($TFL_B + 10$), rats were injected with either MS or saline, and at $TFL_B + 25$, rats were infused with either BAF or saline in one of two doses. A postmanipulation TFL (TFL_P) was determined 20 min after infusion ($TFL_B + 45$), and antinociception was measured as percentage change from TFL_B.

3. Results

Ingestion of 0.50 mL BAF significantly enhanced MS-mediated antinociception. Figure 1 depicts the mean (±SEM) percentage increase from TFL_B for each of the eight groups of rats. A three-way ANOVA revealed a significant main effect of each of the three factors ($\alpha = 0.05$) [drug: $F(1, 88) = 39.607$, $p < 0.001$; enhancer: $F(1, 88) = 7.237$, $p < 0.01$; volume: $F(1, 88) = 7.890$, $p < 0.01$] and also revealed significant drug × enhancer [$F(1, 88) = 5.432$, $p < 0.05$] and drug × volume [$F(1,88) = 6.025$, $p < 0.05$] interactions. In the presence of interactions, however, main effects are easily misleading and should be evaluated with caution. Consequently, we relied on the planned, simple analyses described below (two-tailed, $\alpha = 0.05$) to confirm the significant differences between conditions.

In rats injected with MS, 0.50 mL BAF produced a mean increase in TFL of 60.73%, a level of antinociception significantly greater than that seen both in rats infused with 0.50 mL saline (25.43%), $t(22) = 3.93$, $p < 0.01$ and in rats infused with 0.25 mL BAF (24.34%), $t(22) = 4.05$, $p < 0.01$. MS-injected rats infused with 0.50 mL BAF also showed a greater mean increase in TFL than did saline-injected rats infused with 0.50 mL BAF (4.32%), $t(22) = 6.28$, $p < 0.01$. The increase in TFL seen in MS-injected rats infused with 0.25 mL BAF did not differ from that seen

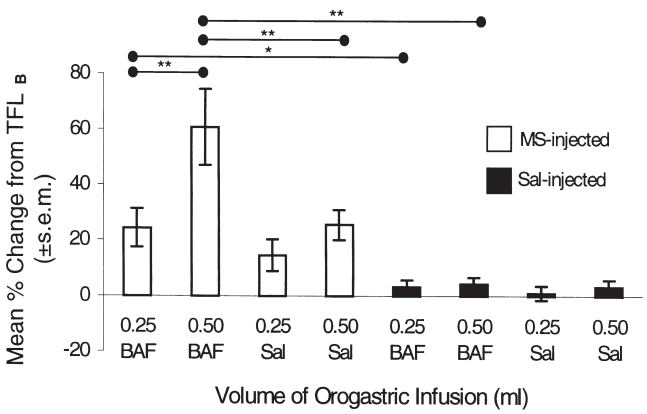

Fig. 1. Mean (±) SEM percentage increase from baseline tail-flick latency (TFL_B) after injection of either 3.0 mg/mL per kilogram morphine sulfate (MS) or saline (Sal), and orogastric infusion of either 0.25 or 0.50 mL saline or bovine amniotic fluid (BAF). Asterisks indicate significant differences between groups (*, $p < 0.05$; **, $p < 0.01$).

in MS-injected rats infused with 0.25 mL saline (14.54%), $t(22) = 1.09$, although it was greater than the increase in TFL seen in saline-injected rats infused with 0.25 mL BAF (3.29%), $t(22) = 2.34$, $p < 0.05$. A one-way ANOVA revealed no differences in TFL_B among any of the eight groups of rats, $F(7, 88) < 1.0$. Similarly, there were no differences in percentage change from TFL_B among any of the four groups of saline-injected rats, $F(3, 44) < 1.0$.

4. Discussion

Our data confirm that BAF possesses POEF activity and demonstrate that ingested bovine POEF enhances opioid-mediated antinociception in Long–Evans rats. Injection of morphine through the intraperitoneal route, coupled with ingestion of 0.50 mL saline, produced antinociception that was 25.43% above baseline, but morphine coupled with ingestion of 0.50 mL BAF enhanced antinociception to a level that was 60.73% above baseline (see Fig. 1). This enhancement represents a 138% increase in morphine efficacy.

Infusion of 0.50 mL BAF produced greater enhancement of morphine antinociception than did infusion of 0.25 mL BAF, but 0.25 mL BAF was not different from 0.25 mL saline, indicating that 0.50 mL BAF was the only effective dose tested. Rat AF, however, has previously been found to enhance morphine antinociception optimally when ingested at 0.25 mL, yet it is also effective at 0.50 and 1.00 mL [12]. The present data, therefore, suggest different dose-response characteristics for bovine and rat AF when tested in rats. Finally, as predicted, neither 0.50 nor 0.25 mL BAF in the absence of morphine treatment produced a significant increase from TFL_B (Fig. 1, saline-injected groups). Both the dose-dependent efficacy and the inability of BAF to produce antinociception independent of opioid antinociception are consistent with previous findings [4,12–14,16,17,23,25].

POEF activity has previously been found in rat AF [4,6,7,12–15] and placenta [3,5,11,12,16,17] when tested in rats and in BAF [21] when tested in cows. These results, however, were obtained in assays in which the subjects tested and the POEF donors were conspecifics. Abbott et al. were the first to reveal the ability of POEF to enhance opioid antinociception across species when they demonstrated that both bottlenose dolphin and human placenta, when ingested by Long–Evans rats, enhance opioid antinociception [1]. The present data confirm the existence of POEF activity in BAF and demonstrate that ingested BAF can act allopatrically to enhance opioid antinociception in female Long–Evans rats. Although the number of mammalian species in which POEF is potentially detectable far exceeds that tested to date, the research on POEF continues to return consistent results, and the expanding number of reports identifying POEF across species, even in the afterbirth of species that typically do not engage in placentophagia (i.e., bottlenose dolphin, human), suggest that POEF is ubiquitous among mammalian taxa.

Beyond the laboratory and human environments, morphine antinociception is unknown. Endogenous opioid antinociception, however, occurs in several behavioral contexts, one of which is parturition [8]. Although recent work has revealed that opioid application to the midbrain facilitates the onset of maternal behavior [27], there is significant evidence that high opioid levels disrupt maternal behavior [2,19]. An antagonism potentially occurs, then, when a parturient female benefits from opioid antinociception (resulting from pregnancy and vaginal stimulation of delivery) but risks disruption of maternal behavior because of high opioid levels. This being the case, placentophagia at parturition provides an excellent opportunity for natural enhancement of endogenous opioid antinociception without disrupting maternal behavior [10,25], and it is in this context that opioid enhancement could easily provide both proximate and ultimate benefits to a parturient female [10]. This concept is particularly intriguing considering that AF is available and ingested prior to delivery of the first neonate and that parturient females of even herbivorous species ingest their afterbirth. The research on POEF, then, reinforces the notion of POEF as a ubiquitous component of mammalian afterbirth and suggests that the proposed function of POEF as a non-disruptive, natural enhancer of endogenous opioid antinociception at parturition may be just as ubiquitous.

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