Available online at www.sciencedirect.com

SCIENCE DIRECT

Brain Research 1014 (2004) 22–33

BRAIN RESEARCH

www.elsevier.com/locate/brainres

Research report

Placenta ingestion by rats enhances δ- and κ-opioid antinociception, but suppresses μ-opioid antinociception

Jean M. DiPirro*, Mark B. Kristal

Behavioral Neuroscience Program, Department of Psychology, University at Buffalo, Buffalo, NY 14260, USA

Accepted 1 April 2004
Available online 28 May 2004

Abstract

Ingestion of placenta or amniotic fluid produces a dramatic enhancement of centrally mediated opioid antinociception in the rat. The present experiments investigated the role of each opioid receptor type (μ, δ, κ) in the antinociception-modulating effects of Placental Opioid-Enhancing Factor (POEF—presumably the active substance). Antinociception was measured on a 52 °C hotplate in adult, female rats after they ingested placenta or control substance (1.0 g) and after they received an intracerebroventricular injection of a δ-specific ([D-Pen2,D-Pen5]enkephalin (DPDPE); 0, 30, 50, 62, or 70 nmol), μ-specific ([D-Ala2,*N*-MePhe4,Gly5-ol]enkephalin (DAMGO); 0, 0.21, 0.29, or 0.39 nmol), or κ-specific (U-62066; spiradoline; 0, 100, 150, or 200 nmol) opioid receptor agonist. The results showed that ingestion of placenta potentiated δ- and κ-opioid antinociception, but attenuated μ-opioid antinociception. This finding of POEF action as both opioid receptor-specific and complex provides an important basis for understanding the intrinsic pain-suppression mechanisms that are activated during parturition and modified by placentophagia, and important information for the possible use of POEF as an adjunct to opioids in pain management.

Theme: Neurotransmitters, modulators, transporters and receptors
Topic: Opioid receptors

Keywords: Placentophagia; POEF; Antinociception; Opioid; Parturition; DPDPE; DAMGO; Spiradoline; Rat; Opioid receptor

1. Introduction

Ingestion of placenta or amniotic fluid enhances opioid-mediated antinociception [39]. The active substance(s) in placenta and amniotic fluid has been termed Placental Opioid-Enhancing Factor (POEF) [43]. The antinociception-enhancing effect of POEF has been well documented in rats of both sexes, in different reproductive states (in virgin and parturient females), and in several algesiometric tests (radiant heat tail-flick test, hot water tail-immersion test, formalin test, and hotplate test) [1,39,41,44,45,73]. In addition, antinociception enhancement has been observed in rats that eat placenta or amniotic fluid of all other species tested to date, including that of humans, dolphins [1], and cows [12], and has been observed in cows after ingestion of bovine amniotic fluid [71]. At parturition, changing levels of ovarian sex steroids and uterine afferent activity produce ''pregnancy-mediated analgesia'', an opioid-mediated increase in maternal pain threshold that is particularly pronounced in the periparturitional period [11,23,31,33,95]. A likely benefit derived from ingestion of afterbirth materials—placentophagia—is the augmentation of this parturitional antinociception [39].

As yet, little is known of the intervening steps by which placentophagia ultimately modifies pain suppression. The data indicate that the effect of ingested placenta or amniotic fluid is strictly modulatory; ingested POEF does not generate antinociception, but rather potentiates antinociception that is already present [39]. Furthermore, this modulatory influence appears to be specific to opioid mechanisms. In the rat, the ingestion of POEF as either placenta or amniotic fluid produces significant elevation of antinociception resulting from a number of opioid-mediated or at least partly opioid-mediated mechanisms,

* Corresponding author. Department of Psychology, State University College at Buffalo, 1300 Elmwood Avenue, Buffalo, NY 14222, USA. Tel.: +1-716-878-4317; fax: +1-716-878-6228.
E-mail address: dipirrjm@buffalostate.edu (J.M. DiPirro).

0006-8993/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2004.04.006

including those induced by the physiology of late pregnancy, peripheral or central injection of morphine, vaginal/cervical stimulation, footshock, or endogenous opioid release [19,20,42,45,87]. POEF does not, however, modify antinociception that results from the non-opioid analgesics aspirin [44] or nicotine [73]. Injection of opioid receptor antagonists such as naloxone or naltrexone blocks POEF enhancement, presumably by removing the opioid activity upon which POEF acts [41]. These data suggest that the modulatory influence of POEF ingestion on pain suppression is limited to opioid-mediated mechanisms. However, the effects of POEF do not extend equally to all opioid-mediated phenomena. POEF ingestion does not affect morphine-mediated hyperthermia [1], and it suppresses contralateral circling produced by unilateral morphine injection into the ventral tegmental area (VTA) [90].

The different effects of POEF action on different opioid-induced phenomena are most likely mediated by different opioid receptor systems (receptor specificity), at different anatomical sites (location specificity), or both. Previous work has shown that POEF acts on the central, rather than peripheral action of morphine [19], but it does not appear to work directly in the CNS. Gastric vagotomy blocks the effect of ingested afterbirth material on morphine antinociception [87], and this block seems to be due to the disruption of gastric vagal afferent fibers, rather than to the disruption of the efferent fibers that influence digestion [72]. POEF ingestion enhances antinociception produced by morphine [1,12,19,20,43,88], a nonselective μ-opioid agonist that has activity at all of the opioid receptors (i.e., $\mu \gg \delta > \kappa$) [25]. The strategic location of μ-, δ-, and κ-opioid receptors at different points of the opioid-antinociception system in brain and spinal cord [50,51], where each is involved in the mediation of antinociception [4,6,9,13,27,30,31,66,82,96], makes each receptor type a potential candidate for involvement in the POEF effect. Direct tests of receptor specificity, however, have yet to be performed.

The present series of experiments was designed to examine the contribution of each receptor separately by investigating the modulatory influence of ingested POEF on antinociception produced by independent central pharmacological activation of each of the three principal opioid receptor types. In each of the three experiments, placenta ingestion was combined with the intracerebroventricular (i.c.v.) injection of one of three different opioid receptor selective agonists: δ-preferring [D-Pen2,D-Pen5]enkephalin (DPDPE) [24,32,61]; μ-preferring [D-Ala2,*N*-MePhe4,Gly5-ol]enkephalin (DAMGO) [24,28,32]; or κ-preferring spiradoline [46,93]. In the present study, we hypothesized that placenta ingestion would enhance antinociception selectively induced at the δ-opioid receptor by DPDPE injection (Experiment 1), the μ-opioid receptor by DAMGO injection (Experiment 2), and the κ-opioid receptor by spiradoline injection (Experiment 3).

2. Materials and methods: general

2.1. Subjects

Subjects were 282 experimentally naive, virgin female Long–Evans (hooded, Blue Spruce) rats, 3–5 months old, weighing 250–350 g. All subjects were born and raised in our laboratory in the Psychology Department's Behavioral Neuroscience Complex at the University at Buffalo and were the first- or second-generation offspring of rats purchased from Harlan Sprague Dawley. All procedures were approved by the University at Buffalo Institutional Animal Care and Use Committee.

Rats were maintained in a controlled environment with an ambient temperature of 22 ± 1 °C, a relative humidity of 40–60%, and a 14-h on/10-h off light–dark cycle (lights on at 0500 h, EST). Rats were housed individually in 32 × 20 × 20 cm, standing, clear plastic cages, and were allowed ad lib access to food (Agway Prolab Rat/Mouse/Hamster Formula 3000) and water, except where otherwise stated.

From the age of 2 months, each rat was monitored daily for estrous cycle stage by vaginal smear; rats were considered to be reproductively mature when they exhibited normal estrous cyclicity (i.e., two consecutive cycles of 4–5 days). After reaching maturity, each rat underwent stereotaxic cannula implantation.

2.2. Stereotaxic surgery

All rats received a single, permanent, indwelling guide cannula through which opioid agonists could be injected directly into the right lateral ventricle. In Experiments 1 and 2, surgery was performed while rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) after they had been food deprived for 8 h. Atropine sulfate (4 mg/kg, s.c.) to suppress mucus secretion was administered shortly after the sodium pentobarbital had taken effect. In Experiment 3, rats were anesthetized with ketamine hydrochloride (21.8 mg/kg, i.p.) and xylazine (26 mg/kg, i.p.) because sodium pentobarbital was no longer available. Rats that were anesthetized with ketamine–xylazine (Experiment 3) were not food-deprived during the pre-surgery period. All rats were injected with Combiotic (0.05 ml, i.m.—Experiments 1 and 2) or Baytril (0.04 ml, i.m.—Experiment 3) to prevent infection.

During surgery, rats were secured in a Kopf stereotaxic apparatus and permanently implanted with a 22-ga stainless-steel cannula (Plastic Products) inserted into the right lateral ventricle at the following coordinates: A–P = 0.0 mm (bregma); L = −2.0 mm (center of midsagittal sinus); D–V = −2.8 mm (measured from dura), with the incisor bar positioned 5 mm above the interaural line. The coordinates were modified from the stereotaxic atlas of Pellegrino, Pellegrino, and Cushman [67]. The guide cannula was anchored to the skull with dental cement affixed to three

0-80 stainless-steel screws. At the end of surgery, a stainless–steel obturator, cut flush with the guide cannula, was inserted into the guide cannula. The internal cannula, used to deliver drug to the lateral ventricle, was cut 1 mm longer than the tip of the guide cannula, so that the site of drug injection would be beyond any scar-tissue formation or gliosis that may have occurred between surgery and testing.

2.3. Drug injections

Intracerebroventricular drugs were injected with a Harvard microinfusion pump (Model 944) at a rate of approximately 1 μl/min. The volume ranged from 4.0 to 4.5 μl, but remained constant within a particular experiment. During injection, the rat was handheld. After injection, the internal cannula was left in position in the ventricle for a period of 30 s to allow for drug dispersal from the cannula tip. The internal cannula was then replaced with the obturator in order to minimize backflow of drug into the guide cannula. Each rat was tested with only one opioid agonist, although cross-tolerance to different opioid receptor-preferential agonists is minimal [69,99], to avoid the possibility of a repeated-testing effect. Agonist doses were determined in pilot studies by their ability to generate a small amount of antinociception (10–40% increase from baseline response latency) so that POEF-induced effects, if present, could be distinguished from simple drug-induced effects, within the 55-s test.

2.4. Placenta collection

Placenta was harvested on Day 21 of pregnancy (presence of sperm = Day 1) from females killed with CO_2. Placenta was immediately frozen (− 40 °C) and stored for later use. During testing, frozen placenta was warmed for 15 min to 37 °C in a heating block, weighed, and immediately presented to the subjects. This procedure is standard for our laboratory (e.g., Ref. [43]).

2.5. Behavior assays

2.5.1. Antinociception assay

The detection of antinociception produced by opioid receptor-selective agonists depends on the type of antinociception test and the route of drug administration. The present study used a hotplate (Life Science Instruments, model 39D), set at 52 °C, to measure nociceptive threshold, because this particular assay is sensitive to the antinociception induced by i.c.v. injection of all three of the opioid agonists used. The hotplate assay was adapted from that originally described by Woolfe and McDonald [97]. A rectangular Plexiglas chamber (28 cm high) with removable top was used to confine the rats to a 28.8 × 26.6 cm hotplate surface during testing. Nociception (pain or discomfort) threshold was quantified as latency (in seconds) to lick the hindpaw or jump vertically (all paws simultaneously leaving the plate surface) after placement of the rat on the hotplate. Immediately after nociceptive threshold (response latency) determination, rats were removed from the hotplate. If no response was observed, hotplate exposure was terminated at 55 s in order to avoid tissue damage. Each rat was tested only once so that learning effects would not influence hotplate performance. All testing was performed by a tester who was blind to experimental conditions.

2.5.2. Motor activity assay

The behavioral effects of opioids are not limited to antinociception, and include a pronounced effect on motor activity, which might confound pain-threshold determination in a hotplate assay. This motor effect varies as a function of opioid receptor type and the degree of activation (i.e., dose and type of agonist used to stimulate the receptors). Pilot data for the present study indicated that no apparent motor effects were produced by agonist injection, except in placenta-fed rats treated with the highest dose of the δ agonist (Experiment 1); those rats displayed circling contralateral to the site of drug injection. In order to separate any treatment-induced motor effects from antinociceptive effects, if possible, circling and nociceptive threshold were measured in the same rat for all rats in Experiment 1. Also, an additional (i.e., 5th) dose of the δ agonist, slightly lower than the highest dose, was tested to dissociate δ antinociception from circling. Therefore, to assess δ receptor motor effects, the presence or absence of contralateral circling (tight, head-to-tail turns) was determined for all rats given δ agonist injections. Circling was assessed at the time of the hotplate test (i.e., 10 min after agonist injection).

2.6. Testing procedures

2.6.1. Pretesting procedures

To minimize stress (and endogenous opioid effects) during testing, each rat was given a postsurgical recovery period of 7–10 days before being habituated to all testing procedures: (1) experimenter (each rat was handheld for 3–5 min/day for 7 days); (2) removal of obturator (cannula was unscrewed 1 time/day for 7 days); (3) hotplate (rats were exposed to room-temperature hotplate for 5 min/day for 2 days); and (4) feeding procedures (fed testing substance once/day for 5 days).

Habituation to feeding procedures over a 5-day period was designed to ensure that subjects would reliably eat placenta or control substance (ground beef) within the 5-min access, because very few virgin Long–Evans rats are spontaneous placentophages and many exhibit neophobic reactions to novel food [38–40]. During this time, once per day, each rat was proffered 1 g of novel food (ground beef, placenta, or a mix), presented in a glass dish (2.5 cm diameter), in addition to standard rat chow. The novel food remained in the cage until it was eaten; if this did not occur within the first 1–2 h of presentation, rat chow was then removed until the novel food was eaten. Each rat was

proffered the material according to the following schedule: Days 1–3: 1 g lean ground beef; Day 4: 0.5 g ground beef + 1 placenta (approximately 0.5 g); and Day 5: 2 placentas. Rats that failed to complete the feeding schedule successfully were dropped from the study (2 rats of 284 [< 1%] were excluded).

The habituation period lasted approximately 1 week. The interval between surgery and testing was therefore 14–18 days.

2.6.2. Testing timeline

Before the start of the test, rats were denied access to food for a 2-h period to decrease the likelihood that stomach contents would affect the action of ingested placenta (and presumably, therefore, POEF). After this period, each rat was fed 1.0 g of placenta or ground beef control in its home cage. Ten minutes later, it received an i.c.v. injection of opioid agonist or vehicle in a separate testing room, and was placed back in its home cage. Hotplate response latency was assessed at the time of peak antinociceptive drug effect, which occurred at 10, 20, and 30 min post-injection for DPDPE, spiradoline, and DAMGO, respectively. All algesiometric tests were conducted in a room other than the one in which subjects were housed. To minimize circadian fluctuations in endogenous opioid levels, all testing was conducted between 0830 and 1130 h, EST.

2.7. Histological examination

At the conclusion of the study, each rat was overdosed with sodium pentobarbital (0.6 ml, i.p.), and injected though the i.c.v. cannula with 0.1 μl methyl blue dye. The brain was then removed, frozen (−20 °C), and cut into 40-μm sections. Every 4th or 5th section that showed cannula track was mounted, stained with Cresyl violet, and saved. Placements were considered to be accurate if: (1) methyl blue dye was observed in the ventricular system, or (2) the cannula track could be traced into, but not past, the right lateral ventricle. In most cases, both criteria were met. Data from any rat with an inaccurate cannula placement were excluded from statistical analysis.

2.8. Statistical analysis

2.8.1. Nociceptive threshold

Median tests were used to analyze the differences in median response latencies between placenta-fed and beef-fed groups at each dose of each agonist. Nonparametric analyses were used because the data contained latency scores that were "at ceiling", (i.e., 55 s), which truncated the distribution of scores and led to a violation of the assumption of interval measurement necessary for parametric analysis [80]. For Experiment 1, in which $n < 20$ for each comparison, the probability of the observed values was calculated by the Fisher exact probability test. For Experiments 2 and 3, in which $n > 20$ for each comparison, the probability of the observed values was obtained by chi-square. The alpha level for all experiments was set at $p = 0.05$.

2.8.2. Motor integrity

The Fisher exact probability test was used to analyze the difference in the proportion of rats that exhibited contralateral circling in placenta-fed and control-fed groups at each dose of the δ agonist DPDPE.

3. Experiment 1: δ-opioid receptors

The effect of placenta ingestion on the antinociception produced by the i.c.v. injection of each of five doses of DPDPE, a δ-opioid receptor agonist, was measured.

3.1. Method

3.1.1. Subjects

Seventy-nine virgin female rats, demonstrating normal estrous cyclicity, were used.

3.1.2. Drug

The δ-receptor-selective agonist DPDPE (a gift from NIDA) was used. DPDPE was chosen because it displays a binding affinity for the δ-opioid receptor that is 100 times greater than its affinity for μ and κ receptors [24,32,61]. DPDPE was dissolved in 0.9% physiological saline and injected within 1 h of entering solution.

3.1.3. Design

The design of this experiment was a 2 × 5 between-subjects factorial: Enhancer (1.0 g placenta or 1.0 g beef control) × Dose of δ agonist (0, 30, 50, 62, or 70 nmol DPDPE in 4.5 μl, i.c.v.). Rats were randomly assigned to 1 of 10 experimental conditions and tested only once.

3.2. Results and discussion

Of the 79 rats tested in Experiment 1, three rats failed to eat the proffered enhancer (placenta or beef control) during testing, and six had inaccurate cannula placements. Only the data from the remaining 70 rats were used for analysis.

3.2.1. POEF and δ-receptor-mediated antinociception

The effect of placenta ingestion (and therefore, presumably POEF ingestion) on δ-mediated antinociception is depicted in Fig. 1. Placenta ingestion significantly enhanced the antinociception produced by both the 62- and 70-nmol doses of DPDPE ($p \leq 0.025$, median test). At those doses, placenta-fed rats exhibited response latencies that were more than 200% of those of their control-fed counterparts (62 nmol DPDPE: placenta Mdn = 55.0 s, control Mdn = 13.9 s; 70 nmol DPDPE: placenta Mdn = 55.0 s, control Mdn = 25.6 s). Placenta ingestion did not alter response latency in rats injected with vehicle or with the

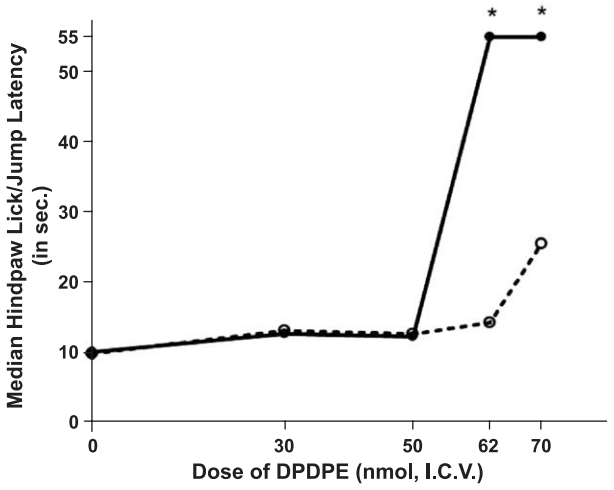

Fig. 1. Enhancement by placenta ingestion of δ-opioid receptor-mediated antinociception. Female rats were fed 1.0 g placenta (●) or control substance (○) 10 min before they were injected with DPDPE (0, 30, 50, 62, or 70 nmol, i.c.v.). Pain threshold is represented by median response latency (in seconds) on a 52 °C hotplate test 10 min after DPDPE injection ($n = 5$–8 rats/group). *Significantly different from control-fed treatment group at the corresponding DPDPE dose ($p < 0.05$, median test).

two lowest doses (30 and 50 nmol) of DPDPE ($p > 0.05$, median test).

The results of Experiment 1 clearly demonstrate that POEF ingestion (as placenta) enhances δ-mediated antinociception in the hotplate test in a dose-dependent fashion. Because this assay measures purposeful antinociceptive responses that are coordinated at a level of the neuraxis above the spinal cord [65], these results indicate that ingested POEF modulates supraspinally organized antinociception. Given the route of DPDPE injection (i.c.v.) and the post-injection interval of antinociception determination (10 min), the DPDPE effect in the present study is likely due to an action at supraspinal receptors. This points to the conclusion that POEF enhances antinociception at supraspinal δ_1 sites [76]. However, the present study did not test whether POEF can also affect δ_2 activity; in the rat, at some brain sites, agonists selective for the δ_2 receptor are more effective than are δ_1 agonists in the production of antinociception [62,75].

3.2.2. POEF and δ-receptor-mediated circling

The effect of DPDPE on contralateral circling is depicted in Table 1. At all doses, DPDPE injection alone produced no apparent motor effects in rats at the time of the hotplate test, suggesting that doses used in the present study were relatively low. In contrast, when combined with placenta ingestion, DPDPE injection induced circling in a significant proportion of rats (86%), but only at the highest dose of DPDPE ($p < 0.05$, Fisher exact probability test).

These results indicate that POEF ingestion can modulate motor components of δ-opioid activity as well as the antinociceptive component. This is consistent with a previous report that placenta ingestion (1.0 g) roughly doubles the potency of DPDPE in the induction of circling [18]. It is important that the results of Experiment 1 show that at different doses of DPDPE, the motor-enhancing effects of POEF can be dissociated from the antinociception-enhancing effects. At the highest dose of DPDPE (70 nmol), placenta ingestion enhanced both antinociception and circling in the same rats. In contrast, at 62 nmol DPDPE, placenta ingestion enhanced antinociception, but had no effect on circling. The finding that POEF induced an elevation in paw-lick/jump latency—both with and without the simultaneous induction of circling, depending on dose of DPDPE—suggests that the elevated response latencies manifested in the hotplate test represent a potentiation of opioid antinociception, and not a confounding motor effect. In addition, because the mechanism underlying locomotor activation induced by i.c.v. δ agonists is thought to involve enhanced dopamine release in the nigrostriatal pathway [14], these data suggest that POEF ingestion might modulate opioid activity in that system.

4. Experiment 2: μ-opioid receptors

The effect of placenta ingestion on the antinociception produced by the i.c.v. injection of each of four doses of the μ-opioid-preferential agonist DAMGO was measured.

4.1. Method

4.1.1. Subjects

One hundred one virgin rats, demonstrating normal estrous cyclicity, were used.

4.1.2. Design

The design was a 2 × 4 between-subjects factorial: Enhancer (1.0 g placenta or 1.0 g control substance) × Dose of μ agonist (0.00, 0.21, 0.29, or 0.39 nmol DAMGO in 4.0 μl, i.c.v.). Rats were randomly assigned to one of eight experimental conditions and tested only once.

4.1.3. Drug

The μ-receptor-selective agonist DAMGO (a gift from NIDA) was used. DAMGO was chosen because it displays a

Table 1
Proportion of group showing stereotypic circling at the time of antinociception measurement (Experiment 1)

DPDPE dose (nmol)	Enhancer	
	Placenta	Control
0	0/7	0/8
30	0/8	0/8
50	0/8	0/7
62	0/5	0/6
70	6/7[a]	2/6

[a] Significantly different from control-fed treatment group at the corresponding DPDPE dose ($p < 0.05$, Fisher exact probability test).

binding affinity for the μ-opioid receptor that is 100 times greater than its affinity for δ and κ receptors [24,28,36]. DAMGO was dissolved in 0.9% physiological saline and injected within 1 h of entering solution.

4.2. Results and discussion

Of the 101 rats tested in Experiment 2, three rats failed to eat placenta or beef control during testing, and two rats had inaccurate cannula placements. Only the data from the remaining 96 rats were used for analysis.

4.2.1. POEF and μ-receptor-mediated antinociception

The effect of POEF on μ-mediated antinociception is depicted in Fig. 2. POEF, ingested as placenta, significantly attenuated the antinociception induced by DAMGO at both the high (0.39 nmol) dose ($\chi^2[1, n=24]=4.0, p<0.05$), and moderate (0.29 nmol) dose ($\chi^2[1, n=24]=4.2, p<0.05$). Placenta ingestion did not affect response latency in rats injected with the low (0.21 nmol) dose of DAMGO ($\chi^2[1, n=24]=0, p>0.05$), or with vehicle ($\chi^2[1, n=24]=0, p>0.05$). These data indicate that the μ-opioid receptor is not involved in the antinociception-enhancing effect of POEF, and instead may be involved in an antinociception-inhibiting effect observed after treatment with large amounts of placenta or amniotic fluid.

The finding that POEF does not potentiate the μ antinociception produced by DAMGO was somewhat surprising in light of previous evidence showing that POEF induces a marked potentiation of antinociception produced by i.c.v. morphine [19], an opiate agonist that acts predominantly at μ receptors [25,52,84]. Several explanations for this apparent discrepancy are possible. The simplest and most likely is that the enhancing mechanism of POEF does not involve the μ receptor. Morphine is considered to be a non-selective μ-preferring agonist, with binding affinity at each of the three types of opioid receptor [25]. Although antinociception generated by this drug is attributed primarily to the activation of μ receptors [52,84], it can also result from stimulation of either δ [36,94] or κ receptors [86]. These findings, together with data from Experiment 2, suggest that the dramatic potentiation of non-receptor-specific morphine antinociception by POEF represents the modulation of opioid activity at the δ receptor, κ receptor, or both. Whether POEF ingestion affects morphine activity simultaneously at all three opioid receptors is unknown, but if it does, then enhancement occurs because the elevation of δ or κ activity is robust enough to outweigh a coincident and functionally opposite inhibition of μ activity. The results of Experiments 1 and 2 are clearly consistent with such a proposition. Therefore, the differential effect of POEF on antinociception generated by DAMGO and morphine can likely be attributed to the fact that the μ receptor is not a substrate for the enhancement effect.

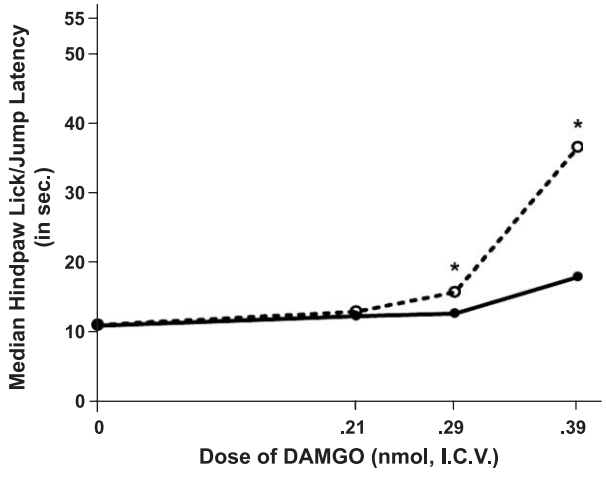

Fig. 2. Attenuation by placenta ingestion of μ-opioid receptor-mediated antinociception. Female rats were fed 1.0 g placenta (●) or control substance (○) 10 min before they were injected with DAMGO (0, 0.21, 0.29, or 0.39 nmol, i.c.v.). Pain threshold is represented by median response latency (in seconds) on a 52 °C hotplate test 30 min after DAMGO injection ($n=11-13$ rats/group). *Significantly different from control-fed treatment group at the corresponding DAMGO dose ($p<0.05$, median test).

Alternatively, the effect of ingested POEF on antinociception induced by selective (DAMGO) and non-selective (morphine) μ receptor agonism may reflect an enhancing mechanism that depends on combined activation of two or more opioid receptors [3,57,83]. If such an interaction were necessary for an enhancement of μ antinociception by POEF ingestion, then antinociception produced by morphine or other nonspecific opioid receptor agonists should be enhanced, but not that produced by the μ-receptor-specific agonist DAMGO. However, the results of Experiment 2 show that selectively induced μ activity is actually attenuated by POEF ingestion. Therefore, again, it seems unlikely that the μ receptor is involved in the POEF enhancement effect.

Last, it is possible that the different effects of POEF ingestion on DAMGO- and morphine-induced antinociception reflect differences between the agonists themselves rather than reflect the effect of POEF on μ-mediated phenomena [64]. If morphine and DAMGO activate distinct μ-receptor-containing antinociception pathways, as appears to be true of morphine and β-endorphin [59,91], then it is conceivable that an enhancing action of POEF on μ-opioid activity might have been obscured in the present study by the selection of DAMGO as the μ agonist.

This experiment represents the first systematic documentation of the antinociception-attenuating ability of POEF ingestion. It is not, however, the first evidence that POEF can exert a negative modulatory effect on opioid-mediated processes. Amniotic fluid ingestion attenuates contralateral circling induced by unilateral injection of morphine into the VTA [90]. Because increases in forward locomotion after intra-VTA opioid injection are thought to be mediated by μ or δ receptor activation of dopaminergic projections from the VTA to nucleus accumbens [10,34,47] and because

morphine has affinity for both μ and δ receptors [25], it is likely that POEF suppression of morphine-induced circling reflects the inhibition of opioid activity at VTA μ or δ sites. The attenuation of DAMGO antinociception by POEF ingestion, then, represents a second case in which POEF inhibits behavior produced by activation of opioid (probably μ) receptors. It is clear, however, that POEF does not inhibit all μ-related behavior; amniotic fluid ingestion has no effect on opioid-induced hyperthermia [1], which is likely mediated at medial preoptic μ receptors [98].

An important question is whether POEF (during placentophagia) modulates the μ-opioid component of non-antinociceptive processes that are involved in parturitional behavior. Maternal care of the young, for example, is disrupted by μ-opioid activity at certain brain sites. In rats on postpartum day 5, i.c.v. injection of the μ agonist DAMGO, but not the δ agonist DPDPE or the κ agonist U-50,488, disrupted ongoing maternal behavior (as defined by latency to retrieve, group, and crouch over pups) [49]. The data of Experiment 2 demonstrate an inhibitory role for central opioids in the regulation of maternal behavior that is specific to the μ receptor. In light of these data, it possible to hypothesize that POEF, through anti-μ-opioid action (Experiment 2), may contribute to the facilitation of maternal behavior by attenuating a disruptive opioid influence, perhaps in the medial preoptic area [49], while simultaneously enhancing the positive motivational aspect of the stimuli by enhancing opioid activity of non-μ-receptors in the VTA [88,89].

5. Experiment 3: κ-opioid receptors

The effect of placenta ingestion on the antinociception produced by the i.c.v. injection of each of four doses of the κ opioid-preferential agonist spiradoline was tested.

5.1. Method

5.1.1. Subjects

One hundred two virgin female rats served as subjects. In contrast to Experiments 1 and 2, where rats in all stages of the estrous cycle were used, in Experiment 3 only rats in Day 1 or 2 of diestrus on the day of testing were used. Pilot studies showed that spiradoline effects were very sensitive to changes in the estrous cycle. This sensitivity has been demonstrated with other opioids, such as morphine [35], but was not present in the current series of studies with either DAMGO or DPDPE, based on a post hoc examination of the data.

5.1.2. Design

The design of this experiment was a 2×4 between-subjects factorial: Enhancer (1.0 g placenta or 1.0 g control substance) × Dose of κ agonist (0, 100, 150, or 200 nmol spiradoline in 4.0 μl, i.c.v.). Rats were randomly assigned to one of eight experimental conditions and tested only once.

5.1.3. Drug

The κ-receptor-selective agonist used was (±)-(5α,7α,8β)-3,4-dicloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-(oxaspiro-[4,5]dec-8-yl]benzeneacetamide monohydrochloride (spiradoline or U-62,066—purchased from Research Biochemicals International). This drug displays a binding affinity for the κ-opioid receptor that is 84 times greater than its affinity for μ and δ receptors [6,92]. Spiradoline was dissolved in 0.02% ascorbic acid and injected within 48 h of entering solution.

5.2. Results and discussion

Of the 102 rats tested in Experiment 3, two rats failed to eat placenta or beef control during testing, and four rats had inaccurate cannula placement. Only the data from the remaining 96 rats were used for further analysis.

5.2.1. POEF and κ-mediated antinociception

The effect of POEF ingestion on κ-mediated antinociception is depicted in Fig. 3. Placenta ingestion significantly enhanced the response latency produced by the low (100 nmol) dose of spiradoline, lengthening the median response latency by approximately 30% (placenta Mdn = 15.7 s; control Mdn = 12.2 s) ($\chi^2[1, n=26] = 3.84$, $p < 0.05$). At the low dose, spiradoline, alone, seems not to have produced measurable antinociception (subthreshold dose), whereas spiradoline, in conjunction with placenta ingestion, did produce measurable antinociception. Placenta ingestion was without effect on the hotplate latency of rats injected

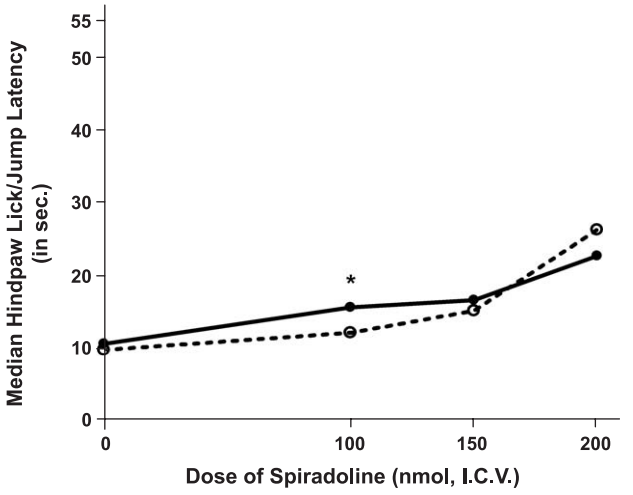

Fig. 3. Enhancement by placenta ingestion of κ-opioid receptor-mediated antinociception. Female rats in diestrus (Day 1 or 2) were fed 1.0 g placenta (●) or control substance (○) 10 min before they were injected with spiradoline (0, 100, 150, or 200 nmol, i.c.v.). Pain threshold is represented by median response latency (in seconds) on a 52 °C hotplate test 20 min after spiradoline injection ($n = 11-13$ rats/group). *Significantly different from control-fed treatment group at the corresponding spiradoline dose ($p < 0.05$, median test).

with vehicle ($\chi^2[1, n=24]=0$, $p>0.05$), on the very slight level of antinociception produced by the moderate (150 nmol) spiradoline dose ($\chi^2[1, n=22]=0.09$, $p>0.05$), or on the modest antinociception produced by the high (200 nmol) spiradoline dose ($\chi^2[1, n=22]=0.18$, $p>0.05$). Spiradoline is considered to be a selective agonist for the κ_1 receptor subtype [67]. Therefore, these data indicate that the opioid-enhancing action of POEF, already demonstrated at the δ_1 receptor, extends to the κ_1 receptor.

POEF enhancement, although to a small degree, is clearly evident statistically at the low dose of spiradoline, but disappears at the high dose. The reason for the dose selectivity may be that spiradoline contains both (+) and (−) enantiomers, and the (+) enantiomer shows some weak μ-agonist activity [54,92]. Therefore, the effect of POEF on spiradoline antinociception may represent a narrow range of dose effectiveness: enhancement of κ activity only at low doses (100 nmol).

Prior to the execution of Experiment 3, pilot studies were performed using a κ agonist that is more selective than spiradoline: U-50,488 [46,93]. However, hotplate-test results indicated that this κ ligand does not produce antinociception when injected i.c.v. This finding, together with reports that spiradoline, although slightly less selective than U-50,488, displays greater antinociceptive potency in the rat warmplate (49.5 °C) and hotplate (55.0 °C) assays, after systemic injection [60,92], and in the mouse hotplate test, after intracranial injection [70], led to the decision to use spiradoline in Experiment 3.

That μ agonism does not contribute appreciably to spiradoline antinociception is an important consideration in the interpretation of the present results, and in the conclusion that POEF enhances κ receptor activity. Note that spiradoline, although less selective than U-50,488, exhibits considerable κ receptor binding preference, as mentioned [46]. Furthermore, the κ-receptor-specific nature of spiradoline antinociception can be asserted on the basis of its reversibility by κ-selective antagonism. This has been demonstrated both with conventional pharmacological strategies and with molecular (antisense) techniques [4,8,60]. These results indicate that the antinociceptive effects of spiradoline are mediated exclusively at the κ receptor, and together with evidence that POEF decreases the μ antinociception generated by DAMGO (Experiment 2), make it very likely that the enhancing action of POEF on antinociception induced by the low dose of spiradoline represents a κ, and not a μ, effect.

6. General discussion

The present studies provide evidence that POEF, ingested as a component of placenta, exerts a complex modulatory influence on antinociception produced by the central pharmacological activation of different opioid receptors. POEF ingestion enhanced the antinociceptive efficacy of i.c.v. δ-opioid receptor agonist DPDPE and i.c.v. κ-opioid receptor agonist spiradoline, but decreased the antinociceptive efficacy of i.c.v. μ-opioid receptor agonist DAMGO. These results strongly suggest that the antinociception-modulating properties of POEF are positive for δ and/or κ activity, and negative for μ activity. Furthermore, the absence of a POEF effect on nociceptive threshold when no exogenous opioid was given, in each of the three experiments reported here, confirms all previous reports [39] that POEF acts, not as an analgesic itself, but rather as a potent modulator of opioid antinociceptive activity.

Previous observations indicate that the modulatory nature of POEF on nonspecific opioid receptor-induced antinociception is biphasic: antinociception-enhancing at low opiate doses, and antinociception-inhibiting at high opiate doses (Kristal laboratory, unpublished observations). The present data, which indicate a receptor-specific mode of action of POEF, suggest that such a biphasic effect may be due to differential action at different opioid receptors. In the present study, in no case was POEF ingestion linked to both an elevation and a reduction of nociceptive-response levels at different doses of the same agonist.

The results of this series of studies indicate a role for both δ and κ receptors in the antinociception-enhancing effects of ingested POEF. However, the data also suggest that the degree of enhancement, presumably a reflection of the effectiveness of POEF to modify activity at a particular opioid site, is different for these two receptors. It is likely that potency differences in enhancement observed at these receptors reflect, in part, the different maximal analgesic effect of each agonist and the different physicochemical properties of each agonist (which determine, for example, rate and distance of drug diffusion), and that these differences might not be apparent if POEF ingestion were tested with other selective agonists.

The present strategy, the use of individual receptor-preferring agonists, allowed for a straightforward test of the ability of POEF, as ingested placenta, to modulate antinociceptive activity induced at each individual receptor type. However, this strategy does not rule out the small possibility of a non-receptor-selective action by each agonist. A more definitive test might be to measure the effect of POEF on antinociception induced by an opioid receptor-selective agonist during simultaneous blockade of alternative receptors (i.e., other opioid receptors). Furthermore, the strategy we used does not provide information about the influence of POEF on antinociception induced by simultaneous activation of more than one receptor, which may be different—quantitatively or qualitatively—from the simple sum of antinociceptive activity at each receptor type [63,75,77,81]. Such information could be obtained by testing POEF with combinations of opioid receptor-selective agonists.

It seems unlikely that non-specific explanations (e.g., thermoregulatory or motoric effects) can account for the POEF modulation of hotplate paw-lick/jump latency

reported here. In dose ranges comparable to those used in the present study, opioid agonists administered i.c.v. affect thermoregulatory processes in a receptor-specific way, leading to a pronounced effect on body core temperature (i.e., μ: hyperthermia; κ: hypothermia; δ: no clear effect) [5,29]. However, it is improbable that the POEF-induced changes in response latency reported here after DAMGO or spiradoline injection reflect such a temperature alteration. (1) There is indirect evidence that hotplate latencies are not affected by changes in body temperature (e.g., see Ref. [2]). (2) DAMGO-induced alterations in body temperature are insignificant or still slight at post-injection times that correspond to the time of antinociception measurement in the present study [85]. Although spiradoline has been reported to be hyperthermic [5], the effects are not characterized well enough to make such a comparison.

Motor effects induced by opioids or POEF represent a potential confound in the assessment of nociceptive threshold in the present study [55,56]. However, in no instance were motor effects readily apparent after opioid treatment, except in Experiment 1. Here, rats treated with the highest dose of DPDPE and POEF showed circling and elevations in paw-lick/jump latency. However, POEF-induced elevations in response latency were also evident at a dose of DPDPE (62 nmol) that had no effect on circling.

At this point, any conclusion about the involvement of a particular brain site in the POEF effect is purely conjectural. However, because the effect of ingested POEF apparently affects the CNS via vagal afferent information, the CNS sites of action are likely limited to the areas supplied by input from the nucleus tractus solitarius (NTS). There is indirect evidence [18,90] that POEF can influence non-antinociceptive opioid activity at multiple sites: the VTA, at which POEF inhibits morphine-induced contralateral circling [90], and the caudate nucleus [14], at which, presumably, POEF enhances DPDPE-induced circling (Experiment 1). In a similar fashion, POEF might influence antinociceptive opioid activity at more than one brain locus.

During late pregnancy and parturition, changes in hormonal and sensory aspects of maternal physiology induce a significant increase in pain threshold [11,15,33,53]. Opioid mechanisms play a pivotal role in the mediation of this effect [23,78], and provide, presumably, the endogenous analgesic substrate(s) for ingested POEF at parturition [45]. Pregnancy is associated with elevated levels of all three classes of opioid peptides (i.e., enkephalin, β-endorphin, and dynorphin) and with increased numbers of opioid receptors (i.e., μ receptor) in several brain sites [7,21,26,68,79,95]. However, to date, little is known of opioid changes in many specific brain areas, especially antinociception-processing sites (where neural input from ingested POEF likely acts).

At the spinal level, opioid changes in late pregnancy have been investigated more extensively and the results indicate that spinal-opioid mediation of pregnancy-mediated analgesia is specific to δ and κ systems [16,17]. Consistent with this pattern of opioid receptor involvement are data showing that elevated pain-response thresholds evident during pregnancy are associated with enhancement in the activity of endogenous ligands of the δ (enkephalin) and κ (dynorphin) receptors [33,37,74]. However, because the opioid receptor-specific nature of POEF action has only been studied at the supraspinal level, the significance of this relationship is not yet clear.

At the present time, the standard analgesic for the treatment of chronic pain is morphine, an only slightly selective (μ) opioid agonist [64]. Although narcotics such as morphine typically generate analgesia that is both robust and lasting, they also induce a constellation of undesirable side effects, including the potential for abuse, decreased gastrointestinal motility, sedation, nausea, vomiting, and potentially life-threatening respiratory depression [48,58]. Given the potent opioid-enhancing properties of POEF, it is reasonable to suggest that using POEF as an opioid-analgesic adjuvant might be an effective pain-management strategy, and might offer advantages over standard narcotic treatment. For example, because POEF would presumably enhance the potency of a simultaneously administered opiate drug, the dose necessary to obtain the desired level of pain relief would be lower if POEF were given in combination with the opiate than if the opiate were given alone [20]. Therefore, because the severity and number of side effects are related to the dose of opiate, among other factors, POEF, if used as an analgesia enhancer, would likely minimize the side effects that are normally associated with a particular level of opiate analgesia. This model has already been demonstrated: placenta ingestion in conjunction with a subthreshold dose of morphine was shown to produce the same amount of antinociception as an optimum dose of morphine in rats. However, the optimum dose of morphine, when administered alone, disrupted ongoing maternal behavior, whereas the subthreshold dose in conjunction with placenta ingestion did not [88].

The complex nature of POEF action on antinociception produced at different opioid receptor types makes it possible that POEF induces an optimal combination of receptor-specific effects: the inhibition of μ-mediated side effects and the facilitation of intended δ and κ receptor analgesic effects. That POEF enhances antinociception produced by the activation of κ-opioid receptors may be particularly relevant to the treatment of pain in women [22]. As analgesics, μ-acting opioids are generally considered to be far superior to κ- and δ-acting opioids. However, this conclusion is based largely on studies using males as subjects (because few comparable studies exist in women), so it may not apply equally well to both sexes.

7. Conclusion

The results of these experiments show that ingestion of POEF, a component of afterbirth tissues, exerts a

potent modulatory action on opioid-mediated antinociception that is both complex and receptor specific. Placenta ingestion enhanced antinociceptive activity at the δ- and κ-opioid receptor, and suppressed antinociceptive activity at the μ-opioid receptor. These data suggest that a similar profile of opioid receptor effects is likely induced by placentophagia during parturition, and point to a number of significant benefits that may be provided to the parturient organism by such a receptor-specific pattern, notably enhancement of pain relief without suppression of maternal care. Elucidation of the potentially novel mechanism of pain modulation involved in POEF action should provide valuable insight into endogenous systems of analgesia and aid ongoing efforts to develop improved therapeutic strategies to manage pain in both humans and nonhuman animals.

Acknowledgements

Funds used for this research were from NSF Grant IBN-9123748 awarded to M.B.K., and from funds allocated to M.B.K. by the Office of the Provost at the University at Buffalo.

References

[1] P. Abbott, A.C. Thompson, E.J. Ferguson, J.C. Doerr, J.A. Tarapacki, P.J. Kostyniak, J.A. Syracuse, D.M. Cartonia, M.B. Kristal, Placental opioid-enhancing factor (POEF): generalizability of effects, Physiol. Behav. 50 (1991) 933–940.

[2] J.U. Adams, J.L. Bussiere, E.B. Geller, M.W. Adler, Pyrogenic doses of intracerebroventricular interleukin-1 did not induce analgesia in the rat hot-plate or cold-water tail-flick tests, Life Sci. 53 (1993) 1401–1409.

[3] J.U. Adams, R.J. Tallarida, E.B. Geller, M.W. Adler, Isobolographic superadditivity between delta and mu opioid agonists in the rat depends on the ratio of compounds, the mu agonists and the analgesic assay used, J. Pharmacol. Exp. Ther. 266 (1993) 1261–1267.

[4] J.U. Adams, X. Chen, J.K. DeRiel, M.W. Adler, L.-Y. Liu-Chen, Intracerebroventricular treatment with antisense oligodeoxynucleotide to κ-opioid receptors inhibited κ-agonist-induced analgesia in rats, Brain Res. 667 (1994) 129–132.

[5] M.W. Adler, E.B. Geller, Physiological functions of opioids: temperature regulation, in: A. Herz, H. Akil, E.J. Simon (Eds.), Handbook of Experimental Pharmacology: Opioids I, vol. 104, Springer-Verlag, Berlin, 1993, pp. 205–238.

[6] R.J. Bodnar, C.L. Williams, S.J. Lee, G.W. Pasternak, Role of μ1-opiate receptors in supraspinal opiate analgesia: a microinjection study, Brain Res. 447 (1988) 25–34.

[7] R.S. Bridges, P.M. Ronsheim, Immunoreactive beta-endorphin concentrations in brain and plasma during pregnancy in rats: possible modulation by progesterone and estradiol, Neuroendocrinology 45 (1987) 381–388.

[8] S.L. Briggs, R.H. Rech, D.C. Sawyer, Kappa antinociceptive activity of spiradoline in the cold-water tail-flick assay in rats, Pharmacol. Biochem. Behav. 60 (1998) 467–472.

[9] D.J. Calcagnetti, S.G. Holtzman, Delta opioid antagonist, naltrindole, selectively blocks analgesia induced by DPDPE but not DAGO or morphine, Pharmacol. Biochem. Behav. 38 (1991) 185–190.

[10] G. Calenco-Choukroun, V. Dauge, G. Gacel, J. Feger, B.P. Roques, Opioid δ agonists and endogenous enkephalins induce different emotional reactivity than μ agonists after injection in the rat ventral tegmental area, Psychopharmacology 103 (1991) 493–502.

[11] R. Cogan, J.A. Spinnato, Pain discomfort thresholds in late pregnancy, Pain 27 (1986) 63–68.

[12] J.W. Corpening, J.C. Doerr, M.B. Kristal, Ingested bovine amniotic fluid enhances morphine antinociception in rats, Physiol. Behav. 70 (2000) 15–18.

[13] A. Czlonkowski, M.J. Millan, A. Herz, The selective kappa-opioid agonist, U-50,488H, produces antinociception in the rat via a supraspinal action, Eur. J. Pharmacol. 142 (1987) 183–184.

[14] V. Dauge, P. Rossignol, B.P. Roques, Blockade of dopamine receptors reverses the behavioral effects of endogenous enkephalins in the nucleus caudatus but not in the nucleus accumbens: differential involvement of δ and μ opioid receptors, Psychopharmacology 99 (1989) 168–175.

[15] M. Dawson-Basoa, A.R. Gintzler, 17-β-Estradiol and progesterone modulate an intrinsic opioid analgesic system, Brain Res. 601 (1993) 241–245.

[16] M. Dawson-Basoa, A.R. Gintzler, Estrogen and progesterone activate spinal kappa-opiate receptor analgesic mechanisms, Pain 64 (1996) 608–615.

[17] M. Dawson-Basoa, A.R. Gintzler, Involvement of spinal cord δ opiate receptors in the antinociception of gestation and its hormonal simulation, Brain Res. 757 (1997) 37–42.

[18] J.M. DiPirro, M.B. Kristal, Placenta ingestion facilitates locomotion and antinociception induced by activation of delta-opioid receptors in the rat, Abstr.-Soc. Neurosci. 23 (1997) 680.

[19] J.M. DiPirro, A.C. Thompson, M.B. Kristal, Amniotic-fluid ingestion enhances the central analgesic effect of morphine, Brain Res. Bull. 26 (1991) 851–855.

[20] J.C. Doerr, M.B. Kristal, Amniotic-fluid ingestion enhances morphine analgesia during morphine tolerance and withdrawal in rats, Physiol. Behav. 50 (1991) 633–635.

[21] D. Dondi, R. Maggi, A.E. Panerai, F. Piva, P. Limonata, Hypothalamic opiatergic tone during pregnancy, parturition and lactation in the rat, Neuroendocrinology 53 (1991) 460–466.

[22] R.W. Gear, C. Miakowski, N.C. Gordon, S. Paul, P.H. Heller, J.D. Levine, Kappa-opioids produce significantly greater analgesia in women than in men, Nat. Med. 2 (1996) 1248–1250.

[23] A.R. Gintzler, Endorphin-mediated increase in pain threshold during pregnancy, Science 210 (1980) 193–195.

[24] A. Goldstein, Binding selectivity profiles for ligands of multiple receptor types: focus on opioid receptors, Trends Pharmacol. Sci. 8 (1987) 456–459.

[25] A. Goldstein, A. Naidu, Multiple opioid receptors: ligand profiles and binding signatures, Mol. Pharmacol. 36 (1989) 265–272.

[26] R.P. Hammer, R.S. Bridges, Preoptic area opioids and opiate receptors increase during pregnancy and decrease during lactation, Brain Res. 420 (1987) 48–56.

[27] D.L. Hammond, P.E. Stewart, L. Littell, Antinociception and delta-1 receptors in the rat spinal cord: studies with intrathecal 7-benzylidenenaltrexone, J. Pharmacol. Exp. Ther. 274 (1995) 1317–1324.

[28] B.K. Handa, A.C. Lane, J.A.H. Lord, B.A. Morgan, M.J. Rance, C.F.C. Smith, Analogues of beta-LPH61-64 possessing selective agonist activity at mu-opiate receptors, Eur. J. Pharmacol. 70 (1981) 531–540.

[29] C.M. Handler, E.B. Geller, M.W. Adler, Effects of mu-, kappa-, and delta-selective opioid agonists on thermoregulation in the rat, Pharmacol. Biochem. Behav. 43 (1992) 1209–1216.

[30] G. Improta, M. Broccardo, Spinal antinociceptive effects of [D-Ala2]-Deltorphin II, a novel and highly selective delta-opioid receptor agonist, Peptides 13 (1992) 1123–1126.

[31] H. Iwasaki, J.G. Collins, Y. Saito, A. Kerman-Hinds, Naloxone-sensitive, pregnancy-induced changes in behavioral responses to colorec-

tal distention: pregnancy-induced analgesia to visceral stimulation, Anesthesiology 74 (1991) 927–933.
[32] I.F. James, A. Goldstein, Site-directed alkylation of multiple opioid receptors: I. Binding selectivity, Mol. Pharmacol. 25 (1984) 337–342.
[33] A. Jayaram, P. Singh, H. Carp, SCH 32615, an enkephalinase inhibitor, enhances pregnancy-induced analgesia in mice, Anesth. Analg. 80 (1995) 944–948.
[34] F. Jenck, M. Bozarth, R.A. Wise, Contraversive circling induced by ventral tegmental microinjections of morphine and [D-Pen2,D-Pen5]enkephalin, Brain Res. 450 (1988) 382–386.
[35] K.L. Kepler, B. Kest, J.M. Kiefel, M.L. Cooper, R.J. Bodnar, Roles of gender, gonadectomy and estrous phase in the analgesic effects of intracerebroventricular morphine in rats, Pharmacol. Biochem. Behav. 34 (1989) 119–127.
[36] J.M. Kiefel, G.C. Rossi, R.J. Bodnar, Medullary mu and delta opioid receptors modulate mesencephalic morphine analgesia in rats, Brain Res. 624 (1993) 151–160.
[37] H.W. Kosterlitz, Opioid peptides and their receptors, Proc. R. Soc. Lond., B Biol. Sci. 225 (1985) 27–40.
[38] M.B. Kristal, Placentophagia: a biobehavioral enigma (or De gustibus non disputandum est), Neurosci. Biobehav. Rev. 4 (1980) 141–150.
[39] M.B. Kristal, Enhancement of opioid-mediated analgesia: a solution to the enigma of placentophagia, Neurosci. Biobehav. Rev. 15 (1991) 425–435.
[40] M.B. Kristal, J.F. Whitney, L.C. Peters, Placenta on pups' skin accelerates onset of maternal behavior in nonpregnant rats, Anim. Behav. 29 (1981) 81–85.
[41] M.B. Kristal, A.C. Thompson, P. Abbott, Ingestion of amniotic fluid enhances opiate analgesia in rats, Physiol. Behav. 38 (1986) 809–815.
[42] M.B. Kristal, A.C. Thompson, S.B. Heller, B.R. Komisaruk, Placenta ingestion enhances analgesia produced by vaginal/cervical stimulation in rats, Physiol. Behav. 36 (1986) 1017–1020.
[43] M.B. Kristal, P. Abbott, A.C. Thompson, Dose-dependent enhancement of morphine-induced analgesia by ingestion of amniotic fluid and placenta, Pharmacol. Biochem. Behav. 31 (1988) 351–356.
[44] M.B. Kristal, J.A. Tarapacki, D. Barton, Amniotic fluid ingestion enhances opioid-mediated but not nonopioid-mediated analgesia, Physiol. Behav. 47 (1990) 79–81.
[45] M.B. Kristal, A.C. Thompson, P. Abbott, J.M. DiPirro, E.F. Ferguson, J.C. Doerr, Amniotic-fluid ingestion by parturient rats enhances pregnancy-mediated analgesia, Life Sci. 46 (1990) 693–698.
[46] R.A. Lahti, M.M. Mickelson, J.M. McCall, P.F. Von Voightlander, [3H]U-69593 a highly selective ligand for the opioid kappa receptor, Eur. J. Pharmacol. 109 (1985) 281–284.
[47] L.G. Latimer, P. Duffy, P.W. Kalivas, Mu opioid receptor involvement in enkephalin activation of dopamine neurons in the ventral tegmental area, J. Pharmacol. Exp. Ther. 241 (1987) 328–337.
[48] R. Maldonado, J. Feger, M.C. Fournie-Zaluski, B.P. Roques, Differences in physical dependence induced by selective μ or δ opioid agonists and by endogenous enkephalins protected by peptidase inhibitors, Brain Res. 520 (1990) 247–254.
[49] P.E. Mann, C.H. Kinsley, R.S. Bridges, Opioid receptor-subtype involvement in maternal behavior in lactating rats, Neuroendocrinology 53 (1991) 487–492.
[50] A. Mansour, C.A. Fox, S. Burke, F. Meng, R.C. Thompson, H. Akil, S.J. Watson, Mu, delta, and kappa opioid receptor mRNA expression in the rat CNS: an in situ hybridization study, J. Comp. Neurol. 350 (1994) 412–438.
[51] A. Mansour, C.A. Fox, H. Akil, S.J. Watson, Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications, Trends Neurosci. 18 (1995) 22–29.
[52] H.W. Matthes, R. Maldonado, F. Simonin, O. Valverde, S. Slowe, I. Kitchen, K. Befort, A. Dierich, M. Le Meur, P. Dolle, E. Tzavara, J. Hanoune, B.P. Roques, B.L. Kieffer, Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene, Nature 383 (1996) 759–760.
[53] V.M. Medina, M.E. Dawson-Basoa, A.R. Gintzler, 17-β-Estradiol and progesterone positively modulate spinal cord dynorphin: relevance to the analgesia of pregnancy, Neuroendocrinology 58 (1993) 310–315.
[54] K.G. Meecham, S.J. Boyle, J.C. Hunter, J. Hughes, An in vitro profile of activity for the (+) and (−) enantiomers of spiradoline and PD117302, Eur. J. Pharmacol. 173 (1989) 151–157.
[55] M.E. Meyer, M.E. Meyer, Behavioral effects of μ-opioid peptide agonists DAMGO, DALDA, and PL017 on locomotor activities, Pharmacol. Biochem. Behav. 46 (1993) 391–395.
[56] M.E. Meyer, M.E. Meyer, Behavioral effects of opioid peptide agonists DAMGO, DPDPE, and DAKLI on locomotor activities, Pharmacol. Biochem. Behav. 45 (1993) 315–320.
[57] C. Miaskowski, Y.O. Taiwo, J.D. Levine, κ- and δ-opioid agonists synergize to produce potent analgesia, Brain Res. 509 (1990) 165–168.
[58] M.J. Millan, Kappa-opioid receptors and analgesia, Trends Pharmacol. Sci. 11 (1990) 70–76.
[59] P.J. Monroe, A.A. Hawranko, D.L. Smith, D.J. Smith, Biochemical and pharmacological characterization of multiple β-endorphinergic antinociceptive systems in the rat periaqueductal gray, J. Pharmacol. Exp. Ther. 276 (1995) 65–73.
[60] H.I. Mosberg, R. Hurst, V.J. Hruby, K. Gee, H.I. Yamamura, J.J. Galigan, T.F. Burks, Bis-penicillamine enkephalins possess highly improved specificity toward delta opioid receptors, Proc. Natl. Acad. Sci. U. S. A. 80 (1983) 5871–5874.
[61] M. Ohno, T.S. Yamamoto, S. Ueki, Analgesic and discriminative properties of U-62,066E, the selective kappa-opioid receptor agonist, in the rat, Psychopharmacology 106 (1992) 31–38.
[62] M.H. Ossipov, C.J. Kovelowski, M.L. Nichols, V.J. Hruby, F. Porreca, Characterization of supraspinal antinociceptive actions of opioid delta agonists in the rat, Pain 62 (1995) 287–293.
[63] Z.Z. Pan, S.A. Tershner, H.L. Fields, Cellular mechanism for anti-analgesic action of agonists of the κ-opioid receptor, Nature 389 (1997) 382–385.
[64] G.W. Pasternak, Pharmacological mechanisms of opioid analgesics, Clin. Neuropharmacol. 16 (1993) 1–18.
[65] L.N. Pastoriza, T.J. Morrow, K.L. Casey, Medial frontal cortex lesions selectively attenuate the hot plate response: possible nocifensive apraxia in the rat, Pain 64 (1996) 11–17.
[66] T. Pelissier, C. Paeile, R. Soto-Moyano, H. Saavedra, A. Hernandez, Analgesia produced by intrathecal administration of the kappa opioid agonist, U-50,488H, on formalin-evoked cutaneous pain in the rat, Eur. J. Pharmacol. 190 (1990) 287–293.
[67] L.J. Pellegrino, A.S. Pellegrino, A.J. Cushman, A Stereotaxic Atlas of the Rat Brain, Plenum, New York, 1979.
[68] F. Petraglia, M. Baraldi, G. Giarri, F. Facchinetti, M. Santi, A. Volpe, A.R. Genazzani, Opioid peptides of the pituitary and hypothalamus: changes in pregnant and lactating rats, J. Endocrinol. 105 (1985) 239–245.
[69] M.J. Picker, S.S. Negus, K.R. Powell, Differential cross-tolerance to mu and kappa opioid agonists in morphine-tolerant rats responding under a schedule of food presentation, Psychopharmacology 103 (1991) 129–135.
[70] M.F. Piercey, F.J. Einspaur, Spinal analgesic actions of kappa receptor agonists, U-50,488H and spiradoline (U-62066), J. Pharmacol. Exp. Ther. 251 (1989) 267–271.
[71] L.C. Pinheiro Machado F°, J.F. Hurnik, J.H. Burton, The effect of amniotic fluid ingestion on the nociception of cows, Physiol. Behav. 62 (1997) 1339–1344.
[72] T.M. Robinson, P. Abbott, M.B. Kristal, Blockade of digestion by famotidine pretreatment does not interfere with the opioid-enhancing effect of ingested amniotic fluid, Physiol. Behav. 57 (1995) 261–263.
[73] T.M. Robinson-Vanderwerf, J.M. DiPirro, A.R. Caggiula, M.B. Kristal, The analgesia-enhancing component of ingested amniotic fluid does not affect nicotine-induced antinociception in naltrexone-treated rats, Pharmacol. Biochem. Behav. 58 (1997) 147–151.
[74] B.P. Roques, F. Noble, V. Dauge, M.-C. Fournie-Zaluski, A. Beaumont, Neutral endopeptidase 24.11: structure, inhibition, and experimental and clinical pharmacology, Pharmacol. Rev. 45 (1993) 87–146.

[75] G.C. Rossi, G.W. Pasternak, R.J. Bodnar, μ and δ synergy between the periaqueductal gray and the rostro-ventral medulla, Brain Res. 665 (1994) 85–93.
[76] G.C. Rossi, W. Su, L. Levanthal, H. Su, G.W. Pasternak, Antisense mapping DOR-1 in mice: further support for δ receptor subtypes, Brain Res. 753 (1997) 176–179.
[77] R.B. Rothman, J.W. Holaday, F. Porecca, Allosteric coupling among opioid receptors: evidence for an opioid receptor complex, in: A. Herz, H. Akil, E.J. Simon (Eds.), Handbook of Experimental Pharmacology: Opioids, I vol. 104, Springer-Verlag, Berlin, 1993, pp. 217–237.
[78] H.W. Sander, A.R. Gintzler, Spinal cord mediation of the opioid analgesia of pregnancy, Brain Res. 408 (1987) 389–393.
[79] J.A. Schriefer, Diethylstilbesterol- and pregnancy-induced changes in rat neurointermediate lobe oxytocin, arginine vasopressin, methionine enkephalin and dynorphin, Neuroendocrinology 54 (1991) 185–191.
[80] S. Siegel, N.J. Castellan Jr., Nonparametric Statistics for the Behavioral Sciences, McGraw-Hill, New York, 1988.
[81] A.S. Smith, N.M. Lee, Opioid receptor interactions: local and nonlocal, symmetric and symmetric, physical and functional, Life Sci. 73 (2003) 1873–1893.
[82] D.J. Smith, J.M. Perrotti, T. Crisp, M.E.Y. Cabral, J.T. Long, J.M. Scalzitti, The mu receptor is responsible for descending pain inhibition originating in the periaqueductal gray region of the rat, Eur. J. Pharmacol. 156 (1988) 47–54.
[83] I. Sora, M. Funada, G.R. Uhl, The μ-opioid receptor is necessary for [D-Pen2,D-Pen5]enkephalin-induced analgesia, Eur. J. Pharmacol. 324 (1997) R1–R2.
[84] I. Sora, N. Takahashi, M. Funada, H. Ujike, R.S. Revay, D.M. Donovan, L.L. Miner, G.R. Uhl, Opiate receptor knockout mice define μ receptor roles in endogenous nociceptive responses and morphine-induced analgesia, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 1544–1549.
[85] R.L. Spencer, V.J. Hruby, T.F. Burks, Body temperature response profiles for selective mu, delta and kappa opioid agonists in restrained and unrestrained rats, J. Pharmacol. Exp. Ther. 246 (1988) 92–101.
[86] A.E. Takemori, P.S. Portoghese, Evidence for the interaction of morphine with kappa and delta opioid receptors to induce analgesia in β-Funaltrexamine-treated mice, J. Pharmacol. Exp. Ther. 243 (1987) 91–94.
[87] J.A. Tarapacki, A.C. Thompson, M.B. Kristal, Gastric vagotomy blocks opioid analgesia enhancement produced by placenta ingestion, Physiol. Behav. 52 (1992) 179–182.
[88] J.A. Tarapacki, M. Piech, M.B. Kristal, Ingestion of amniotic fluid by postpartum rats enhances morphine analgesia without liability to maternal behavior, Physiol. Behav. 57 (1995) 209–212.
[89] A.C. Thompson, M.B. Kristal, Opioid stimulation in the ventral tegmental area stimulates maternal behavior in rats, Brain Res. 743 (1996) 184–201.
[90] A.C. Thompson, J.M. DiPirro, C.D. Dickerson, M.B. Kristal, Amniotic-fluid ingestion inhibits contralateral circling after unilateral morphine injection into the ventral tegmental area, Abstr.-Soc. Neurosci. 17 (1991) 1539.
[91] L.L. Tseng, R. Tang, R. Stackman, A. Camara, J.M. Fujimoto, Brainstem sites differentially sensitive to beta-endorphin and morphine for analgesia and release of met-enkephalin in anesthetized rats, J. Pharmacol. Exp. Ther. 253 (1990) 930–937.
[92] P.F. Von Voigtlander, R.A. Lewis, Analgesic and mechanistic evaluation of spiradoline, a potent kappa opioid, J. Pharmacol. Exp. Ther. 246 (1988) 259–262.
[93] P.F. Von Voigtlander, R.A. Lahti, J.H. Ludens, U-50,488: a selective and structurally novel non-mu (kappa) opioid agonist, J. Pharmacol. Exp. Ther. 224 (1983) 7–12.
[94] H.Q. Wang, J.P. Kampine, L.F. Tseng, Antisense oligodeoxynucleotides to delta-opioid receptor messenger RNA selectively blocks the antinociception induced by intracerebroventricularly administered delta-, but not mu-, epsilon-, or kappa-opioid receptor agonists in the mouse, Neuroscience 7 (1996) 445–452.
[95] L.R. Wardlaw, A.G. Frantz, Brain beta-endorphin during pregnancy, parturition and the postpartum period, Endocrinology 113 (1983) 1664–1668.
[96] L.R. Watkins, E.P. Wiertelak, S.F. Maier, Kappa opiate receptors mediate tail-shock induced antinociception at spinal levels, Brain Res. 582 (1992) 1–9.
[97] G. Woolfe, A.D. MacDonald, The evaluation of analgesic action of pethidine hydrochloride (Demerol), J. Pharmacol. Exp. Ther. 80 (1944) 300–307.
[98] L. Xin, E.B. Geller, M.W. Adler, Body temperature and analgesic effects of selective mu and kappa opioid receptors microdialyzed into rat brain, J. Pharmacol. Exp. Ther. 281 (1997) 499–507.
[99] G.A. Young, N. Khazan, Differential tolerance and cross-tolerance to repeated daily injections of mu and kappa agonists in the rat, Neuropharmacology 23 (1984) 505–509.