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Participation of *Placental Opioid-Enhancing Factor* in Opioid-Modulated Events at Parturition

Endogenous opioids play a significant role in mammalian parturition as well as in the behaviors associated with delivery itself and toward the emerging young. Distention of the uterus, and distention and mechanical stimulation of the vaginal/cervical area are critical stimuli in the positive feedback loop associated with expulsion of the fetus, and in the onset of maternal behavior upon emergence of the fetus [9, 11, 25]. These stimuli are sufficiently "stressful", "painful", or "aversive" so that they occur during a period of elevated endogenous opioid levels and elevated pain threshold [8, and see 12 for review]. Furthermore, the elevation of endogenous opioids in certain brain areas at delivery, namely the ventral tegmental area, is associated with the rapid onset of appropriate maternal caretaking behavior at that time [24]. One might hypothesize that if endogenous opioids facilitate maternal behavior and delivery, that the level of opioid present would be the same for the optimization of both dimensions of parturition. In fact, increasing pain threshold beyond that observed immediately prior to delivery, in rats, by administration of exogenous opioid (morphine), **interferes** with the onset of proper maternal care [1, 10, 19, 21]. How then is antinociception increased, at parturition, in a way that does not interfere with the expression of proper maternal behavior?

Placenta and amniotic fluid contain a molecule(s) that potentiates the antinociceptive action of opioids. We refer to this substance as POEF, for Placental Opioid-Enhancing Factor. In an extensive series of experiments, we elucidated many of the features of POEF [see 12 for review]. The following is a summary of the findings of the early phase of POEF research:

- Ingestion of either placenta **or** amniotic fluid produces opioid-analgesia enhancement (amniotic fluid is available to the mother before expulsion of the fetus, whereas placenta is only available afterward)
- POEF enhances pregnancy-mediated analgesia, as well as that produced by footshock, morphine injection, pseudopregnancy, and vaginal/cervical probing

- POEF does not produce antinociception if administered in the absence of opioid-mediated analgesia, despite the opioid content of afterbirth materials
- Enhanced antinociception is detectable in rats using all assays tested (tail-flick, hot-water tail-immersion, formalin test, hot-plate test)
- POEF does **not** enhance aspirin-induced [13] or nicotine-induced [18] antinociception in naltrexone-treated rats, and is therefore apparently specific for opioid-mediated antinociception
- POEF must be ingested; it is apparently ineffective if injected
- The optimal amounts for enhancement of 3 mg/kg morphine in rats: 1 placenta (500 mg) or .25 ml amniotic fluid.
- Too high a dose of POEF may produce hyperalgesia
- Enhancement in rats is detectable within 5 min after ingestion and lasts 30 - 40 min
- Male rats show enhancement of morphine antinociception after ingestion of placenta
- Human, bovine, and dolphin placenta contain POEF activity (when tested in rats)
- Bovine amniotic fluid contains POEF activity when tested in cows [16]
- Pregnant-rat liver does **not** contain POEF activity
- Ingested POEF enhances the **central** antinociceptive action of morphine [5]
- POEF apparently works by activating gut vagal receptors
- Gastric vagotomy blocks the enhancing action of ingested POEF on morphine antinociception [22]
- Pretreatment with famotidine, an H₂ histamine-receptor antagonist, to block digestion, does not block the POEF effect [17]
- During delivery, early amniotic-fluid treatment affects tail-flick latency, contractions, and aggression, depending on time and dose of treatment
- Effects on other opioid-mediated phenomena:
 - morphine-induced hyperthermia is unaffected
 - contralateral circling after unilateral injection of morphine into the ventral tegmentum is inhibited
 - morphine analgesia is produced by a normally subthreshold dose in morphine tolerant rats
 - the effect of a low dose of morphine on amelioration of withdrawal symptoms is enhanced

The question posed above, namely "How then is antinociception increased, at parturition, in a way that does not interfere with the expression of proper maternal behavior?" can be answered with our knowledge of POEF. POEF provides for a potentiation of those aspects of opioid function that enhance pain relief at delivery, but not those aspects that would interfere with proper maternal care of the young. To confirm that hypothesis, we [21] administered different doses of morphine systemically to postpartum rats, in conjunction with either amniotic fluid (orogastrically) or saline. We then examined both changes in pain threshold and changes in the quality of maternal care. Fig. 1 shows that an injection of 2.0 mg/kg morphine, in conjunction with an orogastric infusion of amniotic fluid, produces a significant elevation of pain threshold, a level not significantly different from the level produced by 3.0 mg/kg morphine (without amniotic fluid). Fig. 2 shows though, that whereas an injection of 2.0 mg/kg morphine (either with or without concurrent amniotic fluid) does not interfere with maternal behavior, a dose of 3 mg/kg morphine (without amniotic fluid) dramatically suppresses retrieval of the young by the mother. Retrieval is a key element of appropriate early maternal care in rats, as it is in most altricial species.

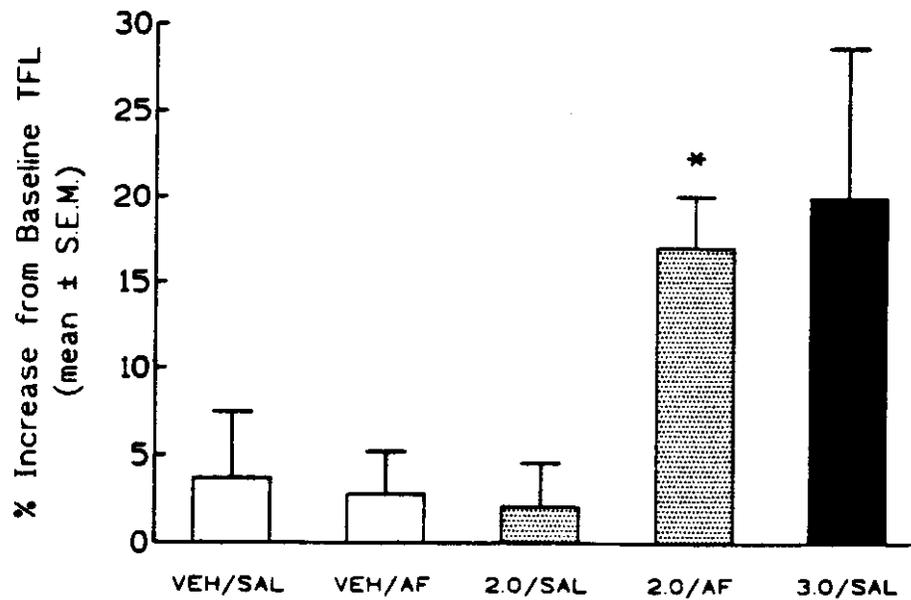


FIG. 1. Mean percent increase from baseline tail-flick latency (\pm SEM) of rats on postpartum Day 3. VEH/SAL = 1 ml/kg vehicle injection + 0.25 ml saline infusion ($n = 9$); VEH/AF = 1 ml/kg vehicle injection + 0.25 ml amniotic fluid infusion ($n = 9$); 2.0/SAL = 2 mg/kg morphine injection + 0.25 ml saline infusion ($n = 9$); 2.0/AF = 2 mg/kg morphine injection + 0.25 ml amniotic fluid infusion ($n = 9$); 3.0/SAL = 3 mg/kg morphine injection + 0.25 ml saline infusion ($n = 10$). *2.0/AF > 2.0/SAL, VEH/AF, VEH/SAL; $p < 0.01$. 2.0/AF \cong 3.0/AF, $p > 0.05$.

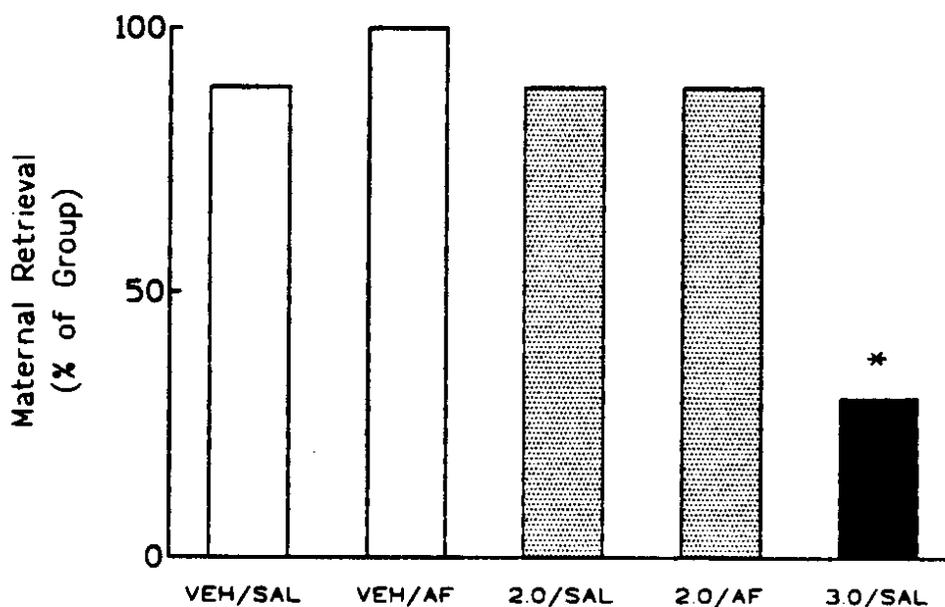
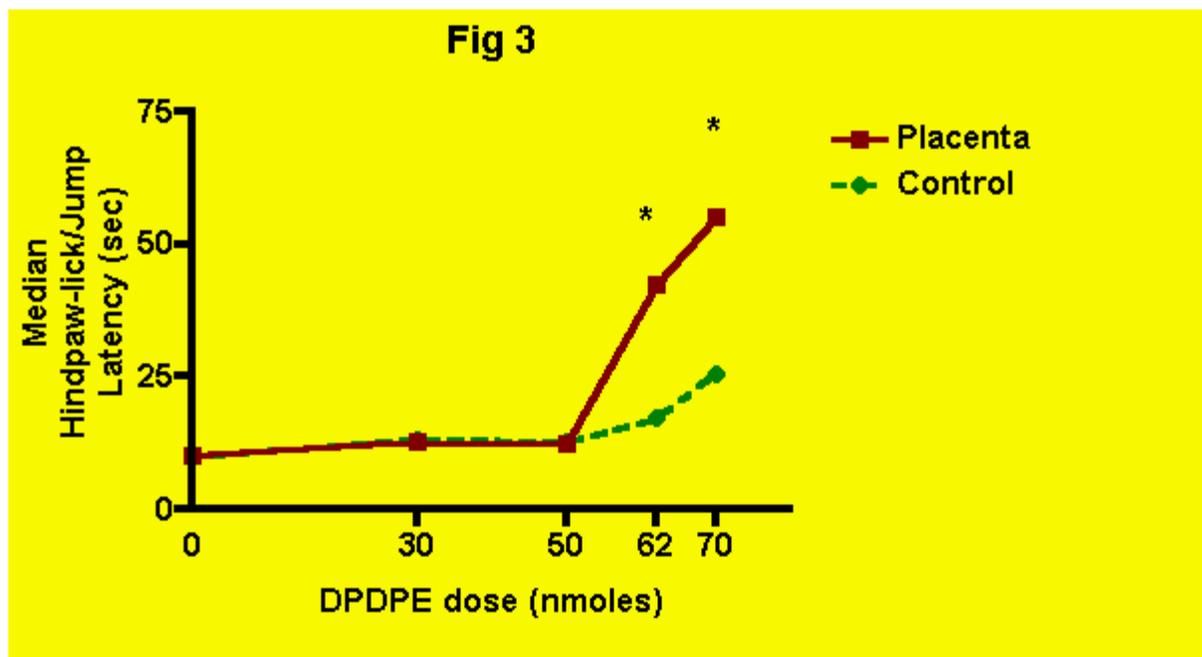


FIG. 2. Percentage of each group of rats completing a maternal retrieval task on postpartum Day 3. VEH/SAL = 1 ml/kg vehicle injection + 0.25 ml saline infusion ($n = 9$); VEH/AF = 1 ml/kg vehicle injection + 0.25 ml amniotic fluid infusion ($n = 9$); 2.0/SAL = 2 mg/kg morphine injection + 0.25 ml saline infusion ($n = 9$); 2.0/AF = 2 mg/kg morphine injection + 0.25 ml amniotic fluid infusion ($n = 9$); 3.0/SAL = 3 mg/kg morphine injection + 0.25 ml saline infusion ($n = 10$). *3.0/SAL < 2.0/AF, 2.0/SAL, VEH/AF, VEH/SAL; $p < 0.05$.

More recent research has focused on the differential effects of POEF administration on various opioid-mediated phenomena (e.g., hyperthermia, locomotor activation, antinociception, maternal retrieval). These differential effects have led us to speculate that either POEF differentially modifies different classes of opioid receptors, or has location-specific effects, or both.

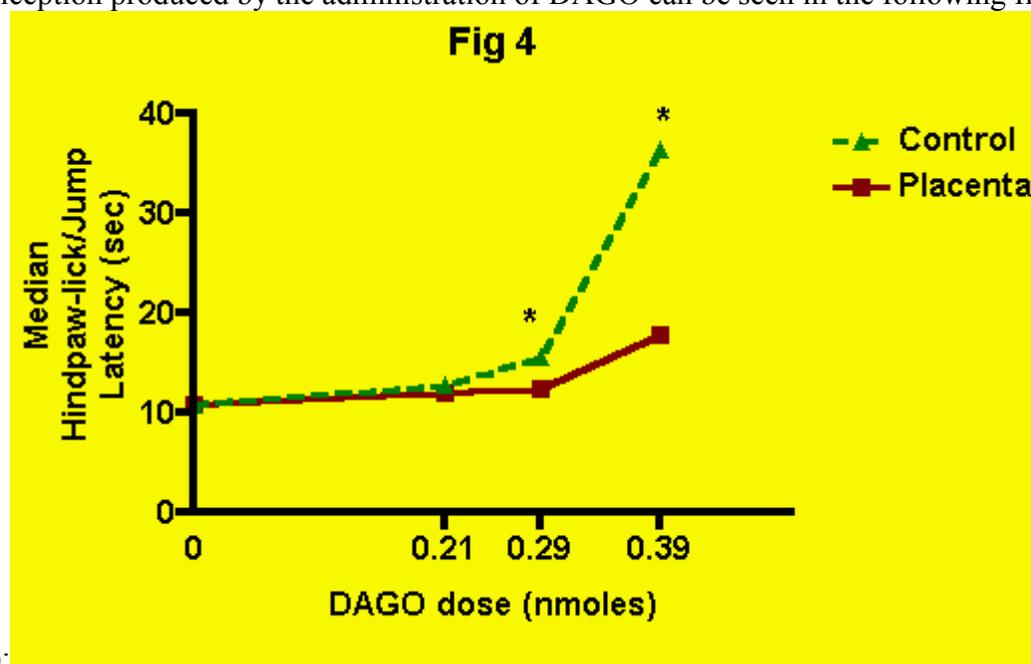
In an attempt to determine the extent to which POEF is opioid-receptor specific, DiPirro has conducted a series of studies in which the effect of ingested placenta was tested on rats receiving an intraventricular injection of a specific opioid-receptor agonist [2, 3, 6]. The agonists used were DPDPE for delta-opioid receptors; DAGO for mu-opioid receptors; and U-62066 (spiradoline) for kappa-opioid receptors. Different doses of agonist were injected in a constant volume through indwelling intracerebroventricular cannulae, and pain thresholds were assessed at peak agonist effect, using a 52° C hotplate, after ingestion of 1g of placenta or meat control. No repeated measures were used.

DPDPE -- The effect of placenta (and presumably POEF) ingestion on delta-opioid antinociception produced by an intracerebroventricular injection of DPDPE is presented in the next figure (Fig. 3):



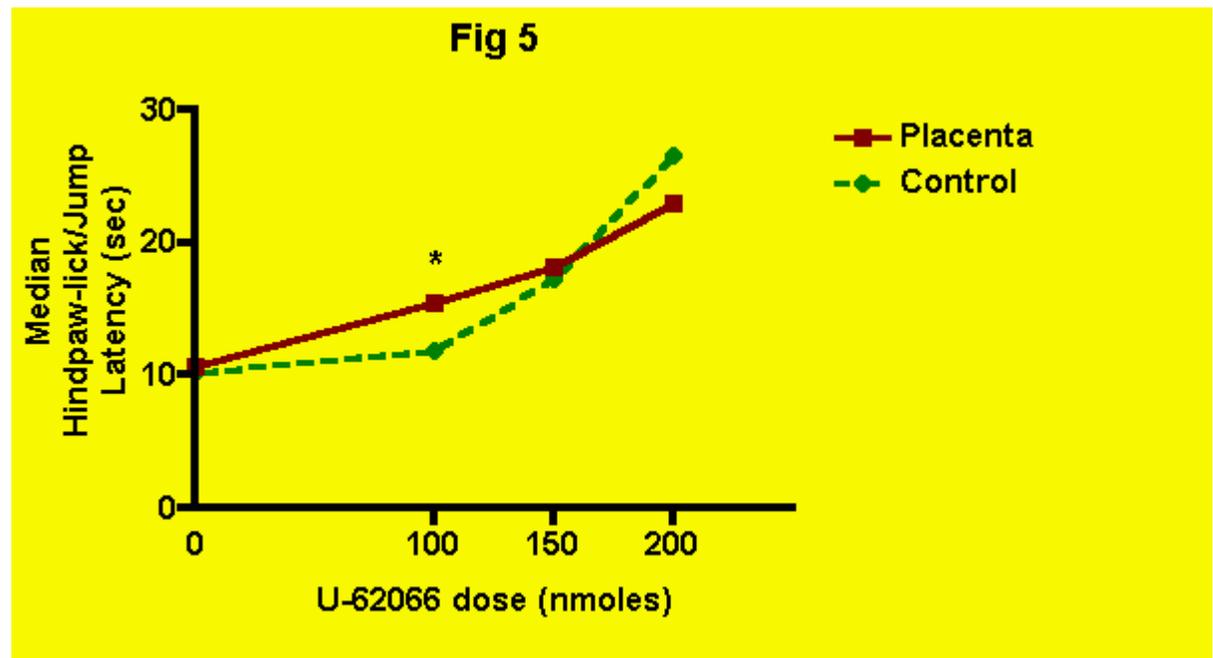
Clearly, placenta ingestion potentiated the effect of delta-opioid mediated antinociception produced by DPDPE. Without placenta, DPDPE did not produce a significant elevation of pain threshold at a dose of 62 nmoles. Even at 70 nmoles, the increase in pain threshold was minor. With placenta ingestion, however, DPDPE produced a 250% increase in pain threshold at a dose of 62 nmoles.

DAGO -- The effect of placenta (and presumably POEF) ingestion on mu-opioid-mediated antinociception produced by the administration of DAGO can be seen in the following figure



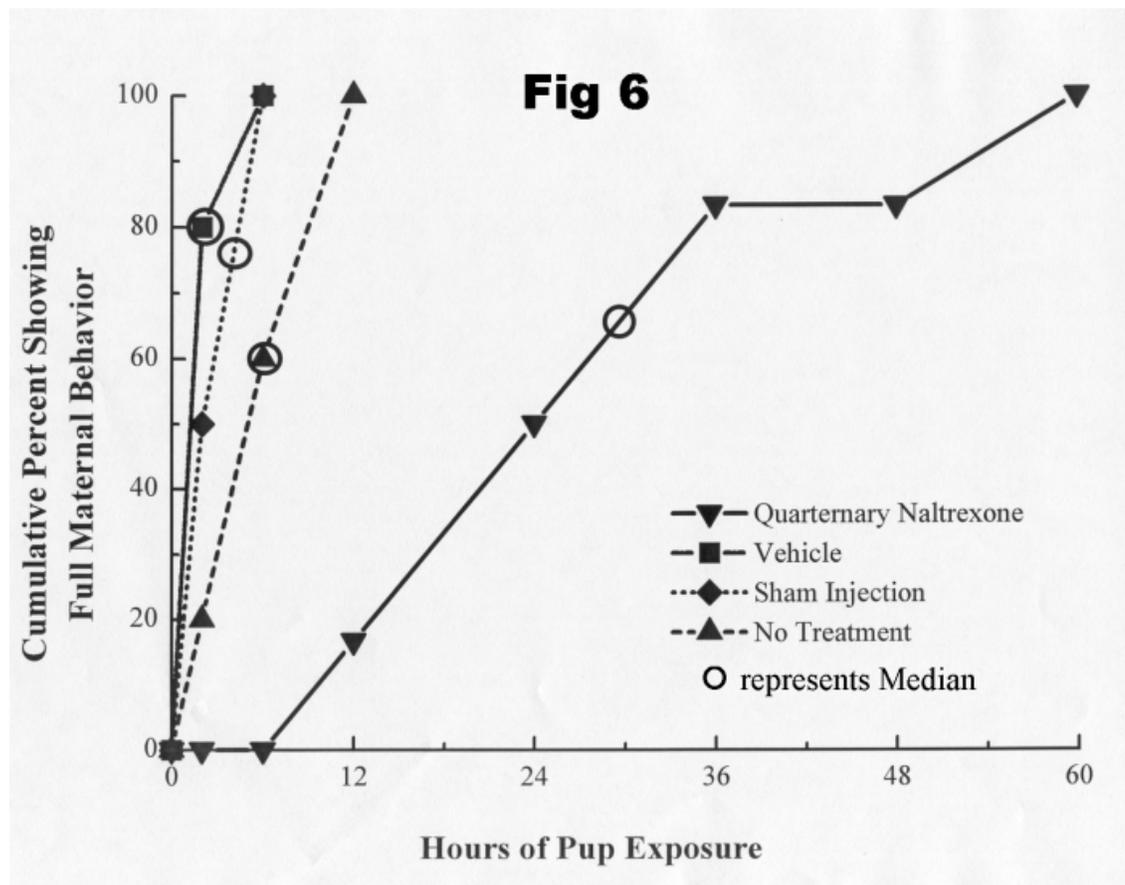
(Fig. 4): The antinociceptive effect of DAGO alone (along with ingestion of meat control) appears at the 0.29-nmole dose. At 0.39 nmoles, the antinociception is quite pronounced. However, in combination with placenta ingestion, a significant attenuation of DAGO-induced antinociception becomes apparent at the 0.29-nmole dose, and the elevation of pain threshold produced by 0.39 nmoles DAGO is all but eliminated. Therefore, placenta (and presumably, therefore, POEF) ingestion, blocks antinociception mediated by mu-opioid receptors.

U-62066 -- The effect of placenta (and presumably POEF) ingestion on kappa-opioid-mediated antinociception produced by central administration of U-62066 (spiradoline) is illustrated in the following figure (Fig. 5):



At the lowest dose of U-62 used, 100 nmoles, U-62 alone was clearly ineffective, whereas U-62 in conjunction with placenta ingestion, produced a significant elevation of pain threshold.

The effect of POEF on central opioid processes is almost certainly location specific as well as receptor specific; very recent data indicate that antinociception produced by morphine injected directly into the periaqueductal gray matter is unaffected by placenta ingestion [4]. Opioid receptors are differentially distributed, and clearly show different effects at different locations. Bridges' work, for instance, has demonstrated that increased opioid levels in the medial preoptic area interfere with maternal behavior [1, 10, 19]. In contrast, Thompson's recent work has shown that increasing the opioid activity of the ventral tegmental area (by injecting morphine), which also increases motivated behavior, facilitated the onset of maternal behavior in inexperienced, nonpregnant rats. Conversely, blocking the effect of morphine injection in the ventral tegmental area, by pretreating the rats with the opiate antagonist naltrexone, blocked the facilitative effect on maternal behavior of the intra-tegmental morphine injection. Furthermore, interfering with the effect of **endogenous** opioids in the ventral tegmental area **at the end of parturition** by intra-tegmental injection of naltrexone methobromide, severely inhibited the naturally-occurring onset of maternal behavior at that time [24]. The following figure (Fig 6) shows the deleterious effect on the rate of onset of maternal behavior, at parturition, of naltrexone methobromide (quaternary naltrexone) injected into the ventral tegmental area.



Although POEF ingestion can modify the effects of intra-tegmental morphine on locomotor behavior [24], we do not yet know if POEF ingestion potentiates the effect of intra-VTA morphine on maternal behavior; POEF, ingested in amniotic fluid, however, did not increase the effect of a subthreshold systemic dose of morphine on maternal retrieval to the point where it disrupted the retrieval of young by mother rats (see Fig. 2).

The mechanical stimulation and distention of the vaginal/cervical area during expulsion of the fetus is well above the minimum amount necessary to trigger pseudopregnancy in nonpregnant females experiencing elevated estrogen levels. Yet the parturient rat does not enter pseudopregnancy, but rather experiences a postpartum estrus and, if inseminated, a postpartum pregnancy. We hypothesized, based on some supportive pilot data, that afterbirth ingestion would decrease the likelihood that relatively intense vaginal/cervical stimulation would induce pseudopregnancy. We tested this hypothesis in a study [23] in which groups of nonpregnant rats received vaginal/cervical stimulation of pressures of either 75, 125, 175 or 225 g. The stimulation applied to the vaginal/cervical area, as expected [14], produced a dose-dependent level of antinociception (measured as lengthening of tail-flick latency or hot-water tail-withdrawal latency). In addition, orogastric infusion of amniotic fluid, shortly before the application of vaginal/cervical stimulation, enhanced the level of antinociception produced by 125 g pressure. Interestingly, the 225-g level of stimulation induced pseudopregnancy in 44% of the rats receiving an orogastric infusion of saline, but only in 10% of the rats infused with amniotic fluid. A detailed series of follow-up studies on the effect of amniotic-fluid ingestion on pseudopregnancy induction is currently being conducted by Patricia Abbott.

Mechanical stimulation during delivery, resulting from expulsion and activity of the fetus,

produces major activation of the pelvic, hypogastric [15], and pudendal nerves. This afferent information, including nociceptive, results in a central release of endogenous opioids. The opioids, in turn, orchestrate a variety of parturitional phenomena including an elevation of pain threshold, the onset of maternal caretaking behaviors, and even perhaps a reduction in the likelihood that this vaginal/cervical stimulation will induce pseudopregnancy, a condition that would eliminate the postpartum estrus. Paradoxically, a higher level of endogenous opioids, despite producing an apparently advantageous additional rise in pain threshold, would counteract some other beneficial effects of opioids, especially those relating to the emergence or performance of the complex of behaviors necessary for caretaking of the young. However, it is apparent that a substance is available in afterbirth material (POEF) that, when ingested by the mother, potentiates the **antinociceptive** actions of endogenous opioids (raising pain threshold above that level produced solely by the opioids), but **not** the actions that would, if increased, have a deleterious effect on maternal behavior. This differential effect on certain opioid functions is the result of selective action on some classes of opioid receptor (as well as some degree of location specificity). Mu-opioid receptor activity, largely associated with negative "side" effects of opioid activity, is suppressed by POEF, whereas kappa- and delta-opioid-mediated phenomena are enhanced. This is particularly interesting, and important, in light of recent studies showing that kappa-opioid analgesics (and to a lesser extent delta-opioid analgesics) are particularly effective in females [e.g., 7, 20].

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