Enhancement of Opioid-Mediated Analgesia: A Solution to the Enigma of Placentophagia

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KRISTAL, M.B. Enhancement of opioid-mediated analgesia: A solution to the enigma of placentophagia. NEUROSCI BIOBEHAV REV 15(3) 425-435, 1991. -- Two major consequences of placentophagia, the ingestion of afterbirth materials that occurs usually during mammalian parturition, have been uncovered in the past several years. The first is that increased contact, associated with ingesting placenta and amniotic fluid from the surface of the young, causes an accelerated onset of maternal behavior toward those young. The second, which probably has importance for a broader range of mammalian taxa than the first, is that ingestion of afterbirth materials produces enhancement of ongoing opioid-mediated analgesia. The active substance in placenta and amniotic fluid has been named POEF, for Placental Opioid-Enhancing Factor. Recent research on both consequences is summarized, with particular attention to POEF, the generalizability of the enhancement phenomenon, its locus and mode of action, and its significance for new approaches to the management of pain and addiction.

Placenta	Placentophagia	Amniotic fluid	Parturition	Labor	Deliv
Analgesia	Opioids	Morphine	Addiction	Tolerance	Withdı
Maternal behavior	Mammals	POEF	Afterbirth	Pregnancy	Pai

PLACENTOPHAGIA, the term generally applied to ingestion of birth membranes and fluids, is a conspicuous feature of the delivery process in nearly all nonaquatic placental mammals, whether carnivorous or herbivorous, nesting or nonnesting, arboreal or terrestrial, monotocous or polytocous, primiparous or multiparous. No one who has carefully observed parturitional behavior in mammals in the laboratory or field, in pets, or even in zoo mammals, can doubt the avidity shown by the mothers in ingesting the afterbirth (43). In fact, rat mothers give up their newly-delivered pups to the intruding researcher more readily than they do the placentas (43, 52).

The literature prior to 1980 was fraught with untested speculations and unverified anecdotes about the proximal causes and the benefits of placentophagia. Among the causal hypotheses in the literature (which often did not distinguish between proximal and ultimate causality) were speculations invoking a general temporary shift to "voracious" carnivorousness; an attempt to keep the nest site clean to avoid attracting predators; general hunger resulting from prolonged discomfort and hypophagia during labor; a specific hunger based on the need to replenish substances (e.g., nutrients) lost during pregnancy or delivery; and a specific hunger for substances (e.g., hormones) that subsequently facilitate lactation or maternal behavior. In a review paper in 1980 (43), I attempted to organize the research that had actually been done on the subject,

and to dispel some misconceptions. Among the findings summarized in that paper were the following:

1) Placentophagia seems more properly conceptualized as an ingestive behavior that is characteristic of maternal females than as a maternal behavior, in the sense of a caretaking behavior directed toward the neonate, although elements of both classes of behavior are present. It can be eliminated with flavor-aversion conditioning procedures (18), and in the absence of previous experience can be eliminated with lateral hypothalamic lesions (42). Also, placentophagia (and nest building) can be dissociated from pup-directed caretaking behaviors at delivery with periventricular medial preoptic lesions (64). This latter finding was confirmed in a more recent study on the effects of knife cuts in the hypothalamus on maternal behavior in rats (23).

2) Placentophagia at delivery does not represent a general shift to carnivorousness in the monkey (76) or even in an omnivore like the rat (42), since other meats are refused at that time.

3) Delivery in the rat is not preceded by a period of hypophagia (54), which suggests that at least in the rat, general hunger is not the explanation of parturitional placentophagia.

4) Placentophagia at delivery in rats, except perhaps during adverse conditions, is not critical for normal maternal behavior, lactation, or postpartum estrus (18, 29, 30, 70) (although the authors of one obscure and ambiguous report of a human experiment have suggested that there is a beneficial effect on lactation of postnatal ingestion of freeze-dried human placenta [71]). It should be noted, however, that all the early studies examined the effects ingestion of placenta per se, not of amniotic fluid.

5) Nulliparous nonpregnant rats and mice show a dichotomous response to donor placenta: either they eat it immediately and in great quantity, or they react as if they are afraid of it, and attempt to escape from it (43, 46, 56, 63). Although about 40% of the virgin rats that are initially repelled by donor placenta become enthusiastic eaters during the last week of pregnancy, the same proportion of nonpregnant rats become placentophagic after other types of "stressful" events. The major shift toward the avid placentophagia that is characteristic of the delivery period (virtually 100% eat most, if not all, the placentas delivered) is not observed until only a few hours before delivery in normal rats (47). The tendency of pregnant females to become attracted to afterbirth materials only in the last few hours before delivery was documented subsequently in ewes (59). That a substantial proportion of virgin rats are attracted to placenta and ingest it enthusiastically argues against the notion that placentophagia is caused primarily by a specific hunger for substances depleted during pregnancy or delivery.

6) There seem not to be any human cultures on record that routinely practice, or practiced, placentophagia. Placentophagia was defined, however, as ingestion of the placenta, in particular; ingestion of amniotic fluid during delivery was not considered. (More on this later.)

BIRTH MATERIALS AND ATTRACTIVENESS OF NEONATES

Some theorists have speculated that different taxonomic groups of mammals derive different benefits from placentophagia (32). In contrast, our approach has been to search for one or a few general consequences that could serve as an adaptive advantage to a wide range of taxa. However, attempts to document beneficial consequences, either to mother or young, of placentophagia at parturition were at first frustrating. Initially, an immunological benefit was hypothesized: it was suggested that placentophagia might reduce the tendency of the mother's system to produce antibodies to the offspring's paternal antigens, i.e., to become immune to those antigens as a consequence of the first parturition, as in erythroblastosis fetalis (43). The possibility was also suggested that placentophagia stimulated the mother to develop antibodies against placental tissue remaining

in utero; the resulting immunity (leading to tissue rejection) might, in turn, reduce the possibility of choriocarcinoma (43). To date these hypotheses have been neither confirmed nor rejected.

The first important benefit of placentophagia that we found related to an idea originally suggested by Birch (5): ingestion of placenta and amniotic fluid on the skin of rat pups by virgin female rats screened for spontaneous attraction to placenta, increased contact between the adult and pups, and hastened the induction of maternal behavior toward those pups (55). This facilitation of the onset of maternal behavior by placenta and amniotic fluid on the neonate has also been documented in dogs (17) and in sheep (57, 58).

We asked two questions about this benefit of placentophagia. The first was whether afterbirth materials on the neonate function simply as powerful contact-generating attractants no different in kind from other attractants (55, 63) that also hasten the appearance of maternal behavior. [In our 1981 paper (55), we reported that pups smeared with rat liver or with a mash of chocolate-chip cookies and milk elicited maternal behavior more rapidly than a control group comprising pups smeared with saline, saccharin, or nothing.] If ingestion of afterbirth materials has some effect in addition to its contact-promoting one, then their ingestion in circumstances that do not generate such contact should nevertheless hasten the onset of maternal behavior. However, what we found was that placenta eaten from a dish placed next to untreated pups did not shorten the latency for virgins to become maternal toward the pups. We concluded, therefore, that placenta and birth fluids do not contain qualitatively special attractants or maternal-behavior inducers, but rather that any material on the skin of the pups that the adult female found extremely attractive would facilitate the onset of maternal behavior toward those pups by inducing proximity and contact, thereby providing the virgin with more stimulation from the pups themselves (55). Terkel and Rosenblatt had demonstrated a similar effect on the rapidity of induction of maternal behavior in rats by reducing the size of cages in which pups and a virgin adult female were co-housed (74).

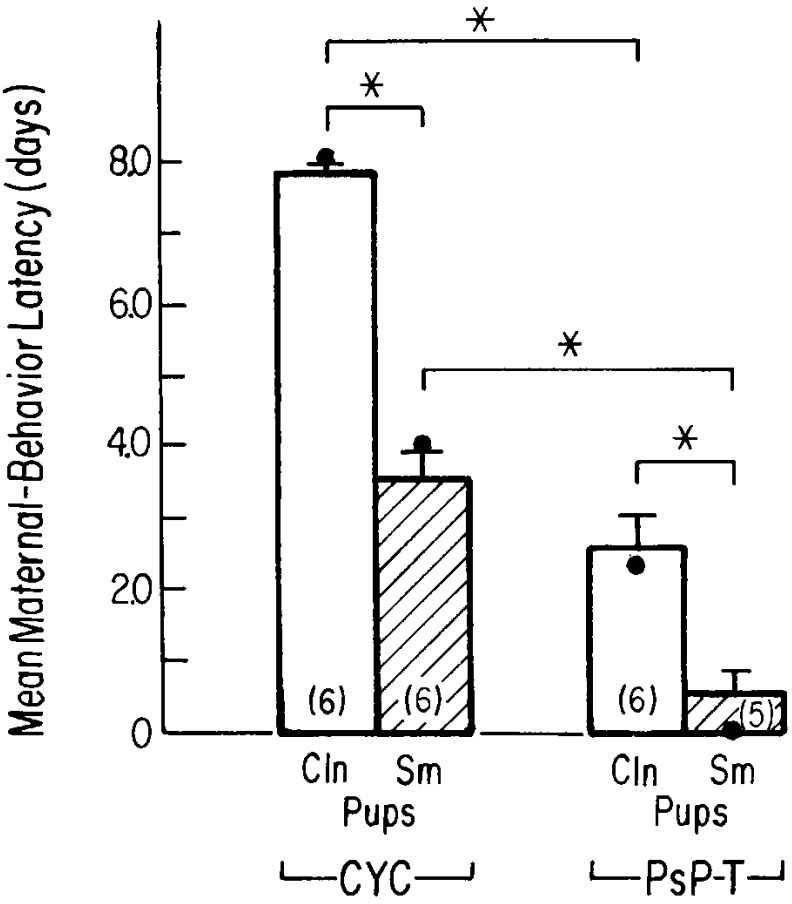

FIG. 1. Mean number of days (± S.E.M.) to the onset of maternal behavior in rats showing estrous cyclicity (CYC) or undergoing natural termination of pseudopregnancy (PsP-T) that were presented with either clean (Cln) pups or pups smeared with placenta and amniotic fluid (Sm). (Number in parentheses = n; • = median. * = $p < 0.05$.) [from (73) with permission. © 1987 APA].

The second question was whether the hormonal state of parturient rats and birth materials on the skin of pups were additive factors in inducing the onset of maternal behavior. We approached this question using a pseudopregnancy-termination model (73), because the hormonal changes occurring during the termination of pseudopregnancy are qualitatively very similar to those occurring during the natural termination of pregnancy (31). We found that rats with terminating pseudopregnancy that were presented with placenta-smeared pups were maternal sooner than were either cycling rats presented with placenta-smeared pups or terminating-pseudopregnancy rats presented with clean pups (see Fig. 1). We concluded that the hormonal state of the adult female at delivery increases the salience and attractiveness of the stimuli emanating from the pups, and from amniotic fluid and placenta, and helps to bring about almost immediate pup-directed maternal behavior at that time (73).

A study conducted in sheep had also addressed the issue of attractiveness to amniotic fluid on neonates and the emergence of maternal behavior at parturition (57). Washed lambs were not mothered as readily as unwashed lambs. Interpretation of this result is however rendered ambiguous by the possibility that the washing introduced an aversive quality. It might have been useful for the investigators to have washed all the lambs and then to have coated some of them with amniotic fluid or other attractive substances.

PLACENTAL OPIOID-ENHANCING FACTOR (POEF): THE BASIC PHENOMENON

The second major benefit of placentophagia we have been investigating concerns the effect of placenta ingestion on opioid-mediated processes, and was first documented in a study we conducted in 1985. The impetus for this study derived from a general interest that had been building up on the part of many researchers, beginning in about 1980, in the role of endogenous opioids and changes in pain threshold and other opioid-mediated phenomena during pregnancy and parturition. Although not unequivocal, evidence from both rat and human studies suggested that plasma and CNS levels of endogenous opioids rise during pregnancy and peak at or near delivery (19, 21, 28, 33, 40, 66, 67, 78). Further, pain threshold rises at the end of pregnancy, peaks at or around delivery, and returns to the nonpregnant level by 9-12 hours postpartum (3, 25, 27); Gintzler has dubbed this naltrexone-reversible change in pain threshold "pregnancy-induced analgesia". Although the findings, in rats, of Gintzler and his colleagues, have sparked some controversy (4, 12), our own research has confirmed them (51), and other research has extended the concept of "pregnancy-induced analgesia" to humans (10) and to pseudopregnant rats (26).

Our initial idea was that ingestion of placenta during delivery contributed to the return of pain thresholds to the lower, nonpregnant levels, which were reinstated by 9-12 hours after delivery. To test this possibility, we conducted a pilot study in which nonpregnant rats, with pain thresholds elevated by morphine injection, were fed donor rat placenta. We anticipated that after ingestion of donor placenta, pain threshold, measured as tail-flick latency, would fall more rapidly than in rats fed a control meat (ground beef). The results were quite the reverse of what we had anticipated: the morphine-injected rats ingesting rat placenta had significantly higher pain thresholds than did those ingesting ground beef, and the morphine analgesia lasted longer in the placenta-fed rats than it did in the beef-fed rats. We recognized that enhanced analgesia might be a far more important consequence of placentophagia than the one we had originally hypothesized, and immediately set about designing a formal study in which to begin testing a new hypothesis, namely, that placentophagia is involved in the elevation of pain threshold.

The first published study showing an enhancing effect of placenta ingestion on opioid-mediated analgesia (52) demonstrated that (a) nonpregnant rats ingesting donor placenta showed significantly more analgesia after intraperitoneal injection of a threshold dose (3 mg/kg) of morphine sulfate (see Fig. 2), or after inescapable footshock (2.5 mA for 90 or 120 sec), than did nonpregnant rats ingesting ground beef, or nothing. However, (b) placenta ingestion did not produce analgesia in rats not receiving morphine or footshock. (c) Treatment with the opiate antagonist naltrexone blocked the opioid component of hindpaw-shock-induced analgesia (79) and also rendered the treated rats refractory to the enhancing effect of ingested placenta. Finally, (d) small amounts of placenta enhanced analgesia whereas large amounts did not. We concluded that whereas ingestion of donor placenta does not produce analgesia in nonpregnant female rats, ingestion of small amounts enhances existing opioid-mediated analgesia.

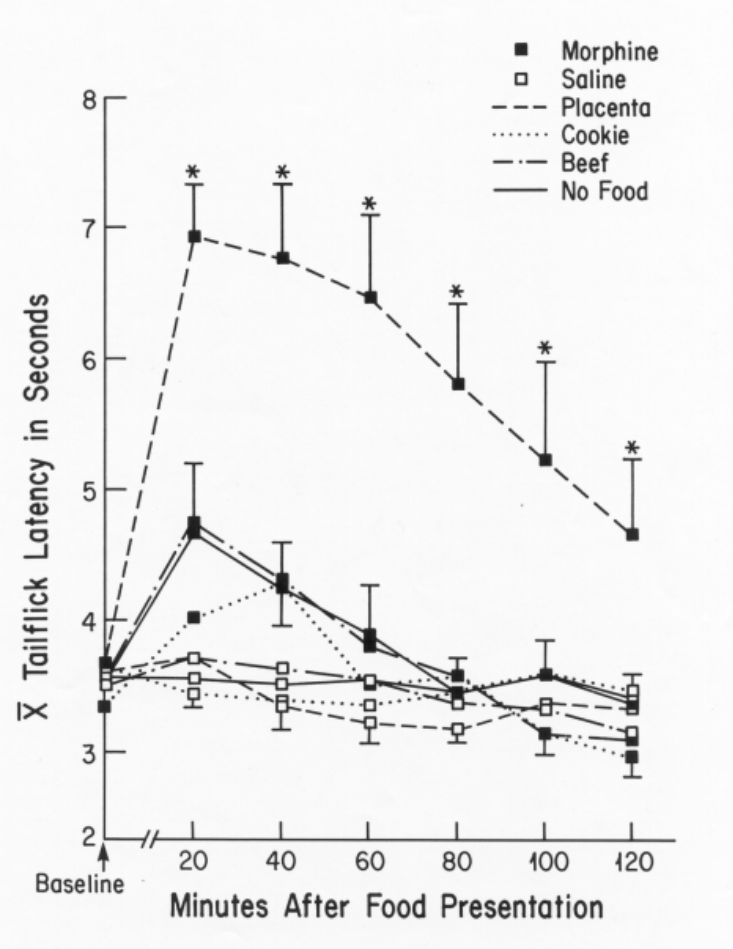

FIG. 2. Mean tail-flick latencies (± S.E.M.) of virgin rats that received either a morphine (threshold dose, 3 mg/kg) or saline injection, then a 20-min exposure to either placenta, cookie mash, ground beef, or an empty dish. The Morphine + Placenta Group, which had the same mean baseline latency as the other groups, showed significantly longer latencies than all other groups on all trials (*$p < 0.05$). [from (52) with permission].

Our next step was to confirm the enhancement of opioid-mediated analgesia by placenta ingestion in another laboratory, using a different strain of rat and yet another type of opioid-mediated analgesia. Komisaruk invited us to attempt the replication in his laboratory, where we could use his paradigm of vaginal/cervical stimulation-induced analgesia, the powerful, reliable, stimulus-bound, partly-opioid-mediated form of analgesia he had been studying extensively (11, 41, 72). That experiment did confirm our initial finding of enhancement of morphine-mediated analgesia with placenta ingestion, and also extended the phenomenon to include enhancement of analgesia produced by vaginal/cervical probing (53). As before, we found that ingestion of placenta in the absence of ongoing analgesia did not produce analgesia. It is important to note that mechanical stimulation of the receptor field of the hypogastric nerve, which is a characteristic of vaginal/cervical stimulation-induced analgesia, is also characteristic of the mechanical processes occurring during descent and expulsion of the fetus during delivery (27, 65). The finding that analgesia produced by vaginal/cervical stimulation is enhanced by ingestion of placenta was the first piece of evidence linking placentophagia to analgesia enhancement in the periparturitional period.

These results precipitated many questions that would have to be addressed. Among the more pressing were: If placenta is often ingested long after delivery of the neonate, what is the significance of our finding to events occurring before and during delivery? What are the dose-response characteristics? Is the effect of placenta ingestion on analgesia limited to opioid-mediated analgesia? Is it limited to female rats? Is it limited to rats?

Is the enhancing effect specific for certain types of opioid-mediated analgesia? Is the effect specific to the tail-flick-latency pain assay? What substance in placenta is responsible for enhancement? Are opioid-mediated processes other than analgesia affected by the enhancing substance? What is the mechanism for enhancement?

Placenta vs. Amniotic Fluid

One aspect of the logic of our hypothesis, that placentophagia elevates periparturitional pain threshold, i.e., enhances pregnancy-induced analgesia, remained perplexing. The snag was that the placenta is always delivered *after* the fetus in normal deliveries. Therefore, any enhancement of pregnancy-induced analgesia derived from placentophagia would not affect pain threshold until after delivery of the first neonate in polytocous species, and not until the postpartum period in monotocous species. However, it occurred to us that if ingestion of amniotic fluid also enhances opioid-mediated analgesia, the hypothesis would still be tenable; amniotic fluid is always available to the mother *during* or shortly *before* the delivery of a neonate. We tested the effect of amniotic fluid ingestion on morphine-induced analgesia in a series of studies that allowed nonpregnant rats to eat amniotic-fluid-soaked ground beef or receive amniotic fluid as an orogastric infusion (49). In both cases, the amniotic fluid ingestion significantly elevated morphine-induced analgesia (see Fig. 3); in fact, the effect of amniotic fluid ingestion was more dramatic than that of placenta ingestion.

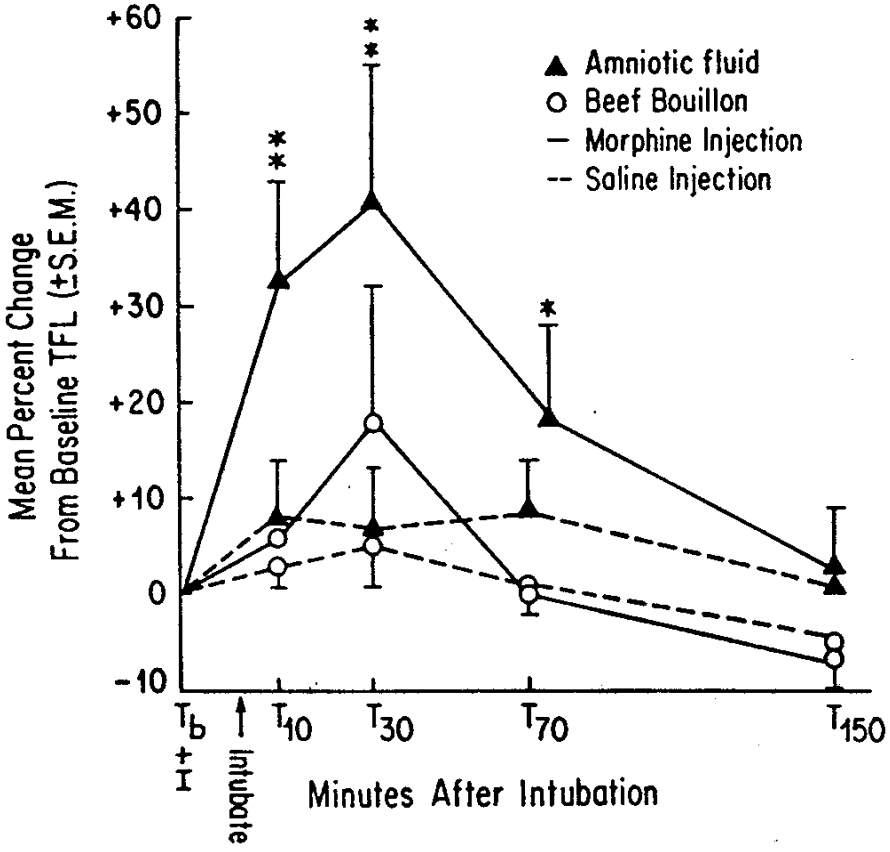

FIG 3. Mean percent change from baseline (± S.E.M.) tail-flick latency (TFL) of virgin female rats that received either amniotic fluid or beef bouillon by orogastric tube after receiving an injection of either morphine (3 mg/kg, IP) or saline (1 ml/kg, IP) (n = 8/group). I = injection; **significantly different from all other groups, $p < 0.01$; *significantly different from the morphine/beef bouillon group, $p < 0.01$. [from (49) with permission].

Furthermore, we confirmed that actual ingestion, and not merely seeing and smelling amniotic fluid, was

necessary to produce the enhancement of analgesia.
At this point we began referring to the putative enhancing substance as POEF, for *p*lacental *o*pioid-*e*nhancing *f*actor.

Dose and Time Course

The next step in developing the case that placentophagia, i.e., ingestion of either placenta or amniotic fluid, serves to enhance periparturitional analgesia was to examine the dose-response relationship and time course of the effect, to see whether these were within the range of parameters imposed by the delivery process. The dose-response characteristics of placenta and amniotic fluid were tested first (44). In this series of studies, on nonpregnant rats, we found that the optimal dose of placenta for enhancement of a 3-mg/kg (threshold) dose of morphine was about one whole placenta (500 mg), with doses of 0.25, 0.50, 2 and 4 placentas also producing measurable enhancement. The optimal dose of amniotic fluid (delivered by orogastric infusion) for enhancement of the same dose of injected morphine was 0.25 ml, with 0.50 and 1.00 also producing measurable enhancement. Therefore, at least in regard to enhancement of analgesia produced by injection of 3 mg/kg morphine, the optimal doses of placenta (one) and of amniotic fluid (0.25 ml), are approximately the amounts that we have found are available to the rat mother with delivery of each neonate.

We have recently completed a study on the effect of a given dose of amniotic fluid (0.25 ml) on enhancing the analgesia produced by various doses of vaginal/cervical stimulation, i.e., produced by various amounts of pressure applied to the vaginal cervix. In nonpregnant rats in diestrus, 0.25 ml infused amniotic fluid enhanced the analgesia produced by 125 g vaginal/cervical pressure, but not that produced by 75, 175, or 225 g of pressure (75). This finding, coupled with the results of an ongoing study in our laboratory on the enhancing effects of a given dose of amniotic fluid on analgesia produced by various doses of morphine, and results from a study on the effects of amniotic-fluid ingestion on morphine-induced hyperthermia (1, 45), suggests an interaction between the dose of enhancer and dose of opioid substrate.

The time course of POEF activity was determined by testing for the enhancing effect of 0.25 ml orogastrically infused amniotic fluid on vaginal/cervical stimulation-induced analgesia. Nonpregnant rats were again used so that we might avoid confounding the effect of POEF with physiological changes occurring during pregnancy. Vaginal/cervical stimulation-induced analgesia was used because it not only has an abrupt onset and offset, and is therefore useful in studies involving time course, but it also probably most accurately represents the form of opioid-mediated (actually partly opioid-mediated) analgesia that occurs during delivery. We found that enhancement of this form of analgesia from a single infusion of amniotic fluid was measurable within 5 minutes after infusion of amniotic fluid, and persisted for about 30 minutes (15). The normal interval between the delivery of successive rat pups is 20 to 40 minutes.

Therefore, both the dose characteristics and the time course of the effect of POEF on analgesia in nonpregnant rats fit nicely with the characteristics of rat parturition.

Nonopioid Analgesics

Although the analgesia-enhancing effect of ingestion of placenta and amniotic fluid had been demonstrated on a number of different opioid-mediated, or partly opioid-mediated, forms of analgesia (morphine injection, vaginal/cervical stimulation, footshock), and the ability of opioid antagonists to block the effect had also been demonstrated, we had not yet tested for enhancement of nonopioid analgesia. We used aspirin-mediated analgesia for this test, because this form of analgesia, particularly in subjects pretreated with the long-term opioid blocker naltrexone, is considered to be the result of purely nonopioid processes (20, 36, 81). The experiment was conducted using the formalin test rather than the radiant-heat tail-flick latency test. The former, involving inflammation pain produced by a subcutaneous injection of formalin, is a more sensitive assay for pain threshold in tests of nonopioid analgesics (20, 36, 37, 81). Since we used morphine-mediated

analgesia as a control, this paradigm also provided us with the opportunity of testing the effect of ingestion of amniotic fluid on opioid-mediated analgesia in an assay other than the radiant-heat tail-flick latency test, one that involves non-reflexive lifting and licking of an inflamed paw rather than the reflexive movement of the tail.

The results showed that although morphine-mediated analgesia was enhanced in the formalin test by ingestion of amniotic fluid, aspirin-mediated analgesia (in naltrexone-pretreated rats) was not (see Fig. 4). Therefore (a) POEF is ineffective in modifying analgesia produced by at least one major nonopioid analgesic; and (b) the enhancing effect of POEF on morphine analgesia is not specific to either thermal pain mechanisms or to neuroanatomical pain systems related to the tail (48).

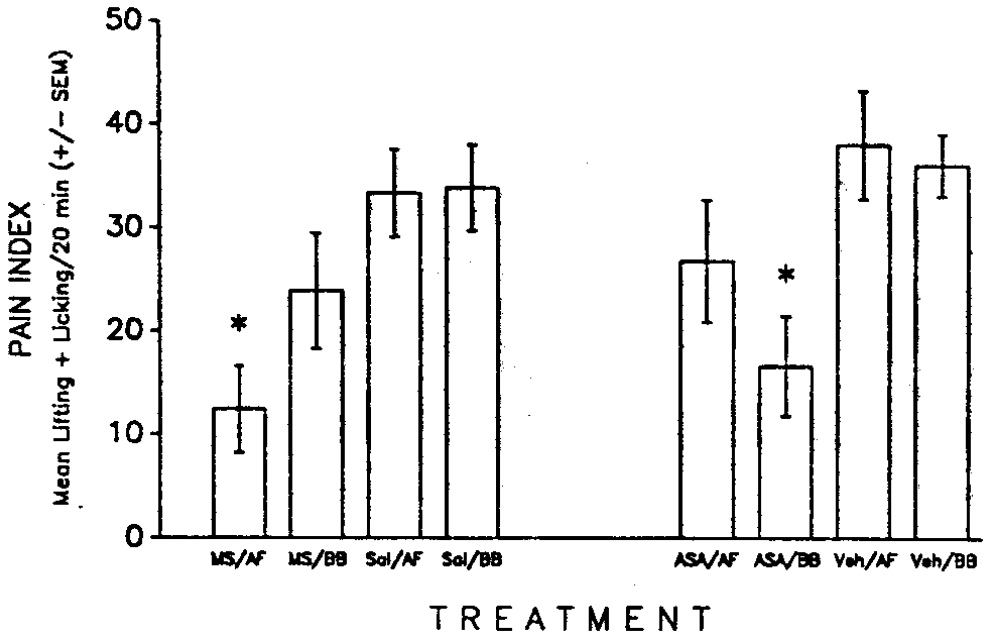

FIG. 4. Pain index (mean s ± S.E.M. spent paw licking and paw lifting over 20 min) for rats in the morphine condition (morphine sulfate = MS; saline control = Sal) or the aspirin condition (aspirin = ASA; vehicle control = Veh) that were infused with 0.25 ml of either amniotic fluid (AF) or beef bouillon (BB). Rats in the aspirin condition were pretreated with naltrexone. (n = 12/group; *significantly different from Sal or Veh, $p < 0.05$). [from (48) with permission].

POEF: ENHANCEMENT OF PERIPARTURITIONAL ANALGESIA

The initial series of studies on the analgesia-enhancing effects of placenta and amniotic-fluid ingestion was conducted on nonpregnant rats. The use of nonpregnant rats enabled us to study the basic parameters of the phenomenon without the confound of ongoing physiological changes occurring during the rat's 22.5-day pregnancy. However, the use of nonpregnant subjects prevented us from testing what we considered to be the major benefit of placentophagia, the enhancement by POEF of pregnancy-mediated analgesia.

A critical test of the effect of parturitional placentophagia on pain threshold, maternal behavior, lactation, postpartum reproduction, or physiology, involves an examination of those processes in mothers prevented from ingesting placenta and amniotic fluid. Methodologically, this group would serve as the "deprived" group with which normal mothers could be compared. As in a removal-replacement model, the experimenter could then attempt to eliminate differences found in the deprived group by returning birth materials to them for ingestion (the replacement group). However, parturition places certain limitations on this model. It is extremely difficult (we believe it actually impossible) to prevent delivering rats from ingesting placenta, not to mention amniotic fluid, without (a) disrupting parturition, (b) distressing the mother sufficiently to alter her

endogenous opioid levels and therefore confound the pain-threshold characteristics as well as other aspects of reproductive physiology (as in [6]), or (c) creating a situation in which it is technically difficult or impossible to measure pain threshold. Attempts to remove placentas as they are delivered cause mother rats to become agitated and to delay or stop delivery. Furthermore, ingestion of amniotic fluid was found to be at least as effective in the enhancement of analgesia as was ingestion of placenta. Therefore, since amniotic fluid is excreted gradually over a relatively long period of time, whereas a placenta is delivered as a single mass, preventing amniotic-fluid ingestion presented an even more difficult problem.

The short-term solution to the problem was to examine the effects of administration of amniotic fluid shortly before the delivering rat's own amniotic fluid became available for her to ingest (51). Therefore, the parturient control rats, orogastrically infused with beef bouillon rather than amniotic fluid, became the "deprived" group, even though they too were about to give birth and ingest their own amniotic fluid. Some of the rats were pretreated with naloxone to effect a temporary blockade of opioid receptors, and thereby reduce pregnancy-induced analgesia. Pain thresholds were determined using a hot-water tail-withdrawal assay while hand-cradling the parturient rats. The results showed quite clearly that amniotic-fluid infusion does enhance pregnancy-induced analgesia. The prepartum pain thresholds of parturient rats receiving an infusion of donor amniotic fluid 2-6 hours prepartum were elevated to the level observed during delivery in control rats, which also ingested their own amniotic fluid during delivery (see Fig. 5). Additionally, we found that (a) 0.75 ml amniotic fluid produced more enhancement than did 0.25 ml; and (b) prepartum naloxone treatment not only eliminated pregnancy-mediated analgesia prior to delivery, but also rendered the amniotic fluid ineffective in modifying the pain threshold.

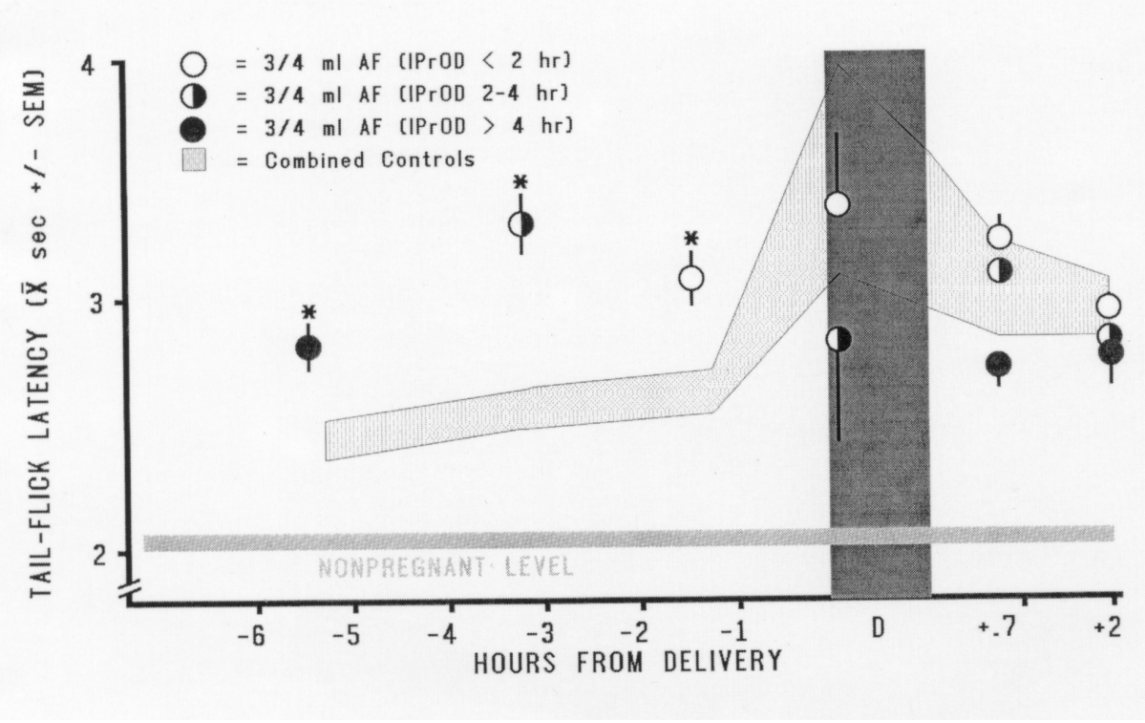

FIG. 5. Effect on pain threshold of infusion of 0.75 ml amniotic fluid in the hours immediately prior to the onset of delivery. IPrOD = *I*nterval between the *Pr*epartum TFL test and the *O*nset of *D*elivery. The Combined Controls group consists of controls for the intubation procedure, for both doses of amniotic-fluid infusion, and for prepartum testing; these groups did not differ from each other. This combined group (depicted by the gray band) represents the course of pregnancy-mediated analgesia. The 0.75-ml amniotic-fluid infusion significantly elevated pregnancy-mediated analgesia at each of the three IPrODs (*$p < 0.01$). [from (51) with permission].

The results were not entirely straightforward. Rats that received 0.25 ml amniotic fluid within two hours

before the start of delivery showed hyperalgesia during the periparturitional period, and many of those showed heightened aggressiveness for as long as two days afterward. A larger dose at an interval of two hours or less, or the 0.25-ml dose at longer intervals, did not produce the same effect.

The effects on other periparturitional phenomena, such as placentophagia, maternal behavior, and parameters of delivery, were also examined. The parameters of delivery (onset of delivery after treatment, duration of delivery, inter-pup interval, pain threshold during and after delivery) were relatively unaffected either by the amniotic-fluid infusion, which was administered 1-7 hours prior to the onset of delivery, or by the naloxone injection, which was administered at least one hour prior to the onset of delivery. The effects on behavior were more difficult to assess; the group sizes were large enough for analysis of the pain-threshold data, but were not sufficient for in-depth analysis of all the behaviors observed. We did not observe any effects on placentophagia during delivery of either prepartum naloxone or amniotic-fluid treatment, despite the fact that these treatments affected pain threshold. However, the dose and time of administration of naloxone were chosen so as to block opioid receptors prior to the onset of delivery, but to have little or no effect during delivery, and to be too low to produce gastrointestinal discomfort (e.g., too low to cause hypophagia or to be effective in establishing a conditioned flavor aversion).

The main behavioral effects observed in our study on prepartum administration of amniotic fluid, in addition to the aggression mentioned above, were the following (50): (a) Rats receiving a low dose (0.25 ml) of amniotic fluid prior to delivery showed increases in general contractions during delivery and decreases in general and anogenital self-grooming postpartum. (b) Rats that received a higher dose (0.75 ml) of amniotic fluid prepartum showed increases during delivery of the lordotic type of contractions, in anogenital grooming, and in nest building, all apparently at the expense of crouching over pups and of resting, which were decreased. (c) The 0.75-ml prepartum dose of amniotic fluid was associated with postpartum increases in grouping of pups and decreases in general self-grooming. (d) Some of the rats receiving 0.75 ml prepartum showed an unusual type of prepartum contraction involving a hunched body posture, which we called "turtle" contractions; these were almost never observed in untreated rats or in rats infused with control fluid. Finally, all these behavioral effects, observed after prepartum administration of amniotic fluid, were absent in the amniotic-fluid-treated rats that had been pretreated with naloxone. More research will need to be done before we can make more general statements about the role of amniotic-fluid ingestion on periparturitional behaviors.

POEF: GENERALIZABILITY OF EFFECT

How far can the effect of POEF be generalized? As mentioned above, (a) the effect has been demonstrated to result from ingestion of either rat placenta or rat amniotic fluid; (b) it is effective in enhancing several types of opioid-mediated or partially opioid-mediated analgesia but without effect on nonopioid-mediated analgesia; (c) it has been documented using several entirely different pain-threshold assays; and (d) enhancement has been found in both nonpregnant and parturient rats. A recent series of experiments has been conducted in an attempt to probe further the general extent of the opioid-enhancement phenomenon.

Males vs. Females

All of the previous studies had been conducted on females, since parturitional placentophagia is sex-specific. However, male rats, too, are able to experience the enhancement of morphine-induced analgesia brought about by ingestion of amniotic fluid (1). Therefore, the mechanism for enhancement is not sexually dichotomous in rats; it is present in both sexes (see Fig. 6) and its activation does not require physiological/endocrinological events associated with female reproductive physiology or with parturition.

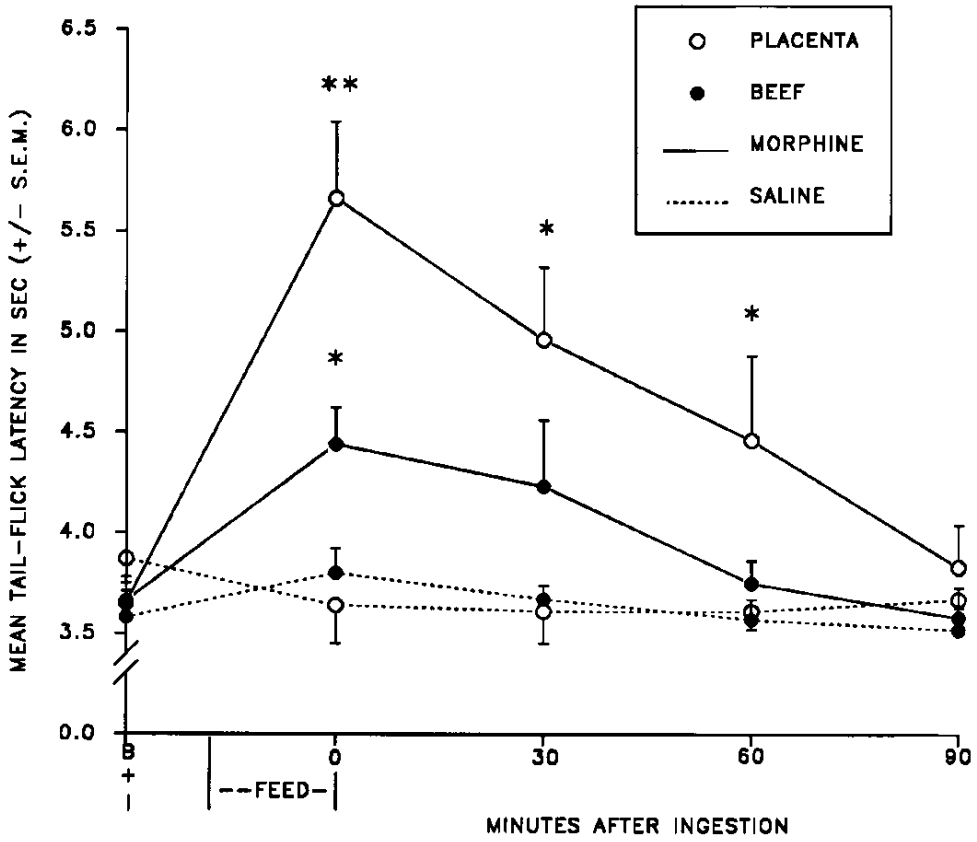

FIG. 6. Mean (± S.E.M.) tail-flick latency of male rats that ate either placenta or beef after receiving either a morphine (3 mg/kg, IP) or saline (1 ml/kg, IP) injection (n = 8/group). I = injection; *significantly different from saline groups ($p < 0.01$); **significantly different from all other groups ($p < 0.01$). [from (1) with permission].

Species of Donor

Using tail-flick latency in the rat as an assay for POEF activity, we have documented such activity in homogenates of both human and bottlenose dolphin (Tursiops truncatus) placenta (1). Both placentas came to us serendipitously: the human placenta was a healthy, intact, placenta donated by a local mother and her obstetrician; the dolphin placenta was donated by the Niagara Falls Aquarium (Niagara Falls, NY). Both placentas were obtained within an hour or two after delivery and were immediately frozen. Eventually, pieces of each were thawed and processed. Orogastric infusion of human-placenta cytosol and voluntary ingestion of small pieces of dolphin placenta were both found to enhance vaginal/cervical stimulation-induced analgesia in rats. Interestingly, both humans and dolphins are among the rare exceptions to the mammalian tendency to engage in parturitional placentophagia regularly (43). These findings strongly suggest that POEF is a common mammalian substance, which in turn, suggests the possibility that all mammalian species, including humans, possess the capacity for responding to POEF.

Route of Administration

Since periparturitional placentophagia is an ingestive behavior, all our initial studies demonstrating an effect of internalized amniotic fluid and placenta involved ingestion: substances were either eaten or were infused orogastrically. To investigate the specific involvement of gastrointestinal processes, we tested the effect on opioid-mediated analgesia of amniotic fluid that had been injected either subcutaneously or intraperitoneally (1). Neither route of injection produced enhancement at the dose of morphine used, suggesting that gastrointestinal involvement is necessary. This involvement may take one of a number of forms. One

possibility is that the POEF molecule is activated by the environment of the stomach (involvement of all but the very beginning of the small intestine is less likely, in light of the finding that POEF activity can be detected within five minutes after infusion [15]). Perhaps acidity or enzyme activity is required to form or unbind the POEF molecule, which is then absorbed into the circulation. An alternative explanation is that POEF activity depends on contact with receptors found only in the lining of the stomach (2). Whatever the case, a specific relationship between an ingestive behavioral process and mediation by specific gastrointestinal events is not without precedent: the phenomenon of flavor-aversion learning largely conforms to this model (24), in that flavor is uniquely linkEd to gastrointestinal discomfort, and not to other forms of discomfort.

POEF in Other Tissue

The animal-derived control substances used in our laboratory (ground beef, commercial beef bouillon, chicken-egg albumin) have been found not to contain POEF activity. However, this does not mean that POEF activity is not to be found in any animal tissues other than afterbirth materials. To date, the only other substance we have tested that we felt was likely to possess POEF activity is liver from pregnant rats from which we also harvested placenta and amniotic fluid. Pregnant-rat liver, when eaten by nonpregnant rats in amounts equivalent to the optimum effective amount of placenta (500 mg), did not produce enhancement of morphine-induced analgesia (1).

Effect on Other Opioid-Mediated Processes -- Thermoregulation

Opioids are involved in phenomena other than analgesia, e.g., feeding, reproductive behavior, social behavior, and thermoregulation. Differences may exist between analgesia and these other processes in regard to the particular opioid receptors involved, whether the process is being mediated centrally or peripherally, and if centrally, the location within the CNS of mediation of the processes. To test the generality of the effect of POEF on opioid-mediated processes, we examined the effect of amniotic-fluid ingestion on morphine-mediated changes in body temperature (1, 45). Injections of low doses of morphine (< 5.0 mg/kg) reliably produce hyperthermia in rats (9, 38, 60). We examined effects of ingestion of amniotic fluid on both hyperthermia and analgesia in the same rats, in groups receiving injections of morphine of 0, 2, 3.5 or 5 mg/kg. We found that, as expected, morphine produced both hyperthermia and analgesia in a dose-dependent fashion, but only the analgesia was modified by amniotic fluid ingestion (see Fig. 7).

Therefore, POEF probably does not affect all opioid-mediated phenomena. At this time, we do not know whether differential receptor effects (e.g., no enhancement of processes mediated by æ opioid receptors), or different neuroanatomical loci of action, are primarily responsible for the results.

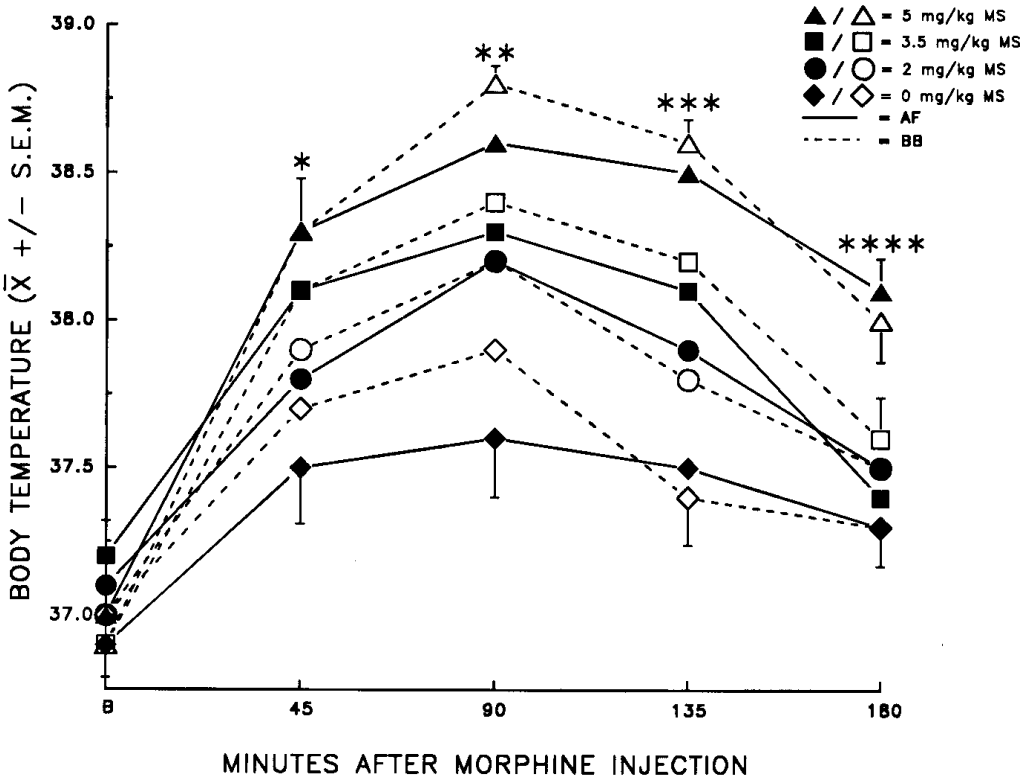

FIG. 7. Mean (± S.E.M.) body temperature (in ° C) of virgin female rats before (B) and after injection of morphine (MS) and an orogastric infusion of amniotic fluid (AF) or beef bouillon control (BB). No significant differences between AF and BB infusions were found ($p > 0.05$). Differences indicated are for morphine dose only ($p < 0.05$). Pain thresholds measured at 45 min were significantly higher in the 2 mg/kg MS + AF Group than in the 2 mg/kg MS + BB Group ($p < 0.05$). *5 mg/kg = 3.5 mg/kg > 2 mg/kg = 0 mg/kg; **5 mg/kg > 3.5 mg/kg = 2 mg/kg > 0 mg/kg; ***5 mg/kg > 3.5 mg/kg > 2 mg/kg > 0 mg/kg; ****5 mg/kg > 3.5 mg/kg = 2 mg/kg = 0 mg/kg. [from (1) with permission].

Effect on Other Opioid-Mediated Processes -- Opiate Tolerance and Withdrawal

Individuals receiving daily injections of a given dose of an opiate show a diminution of the analgesic response, and many other effects of opiates, to that dose after several days. Eventually, a stronger dose is required to elicit the magnitude of response seen initially with the repeated dose (tolerance). If administration of the opiate is halted abruptly, after tolerance has been formed, the individual soon manifests a series of transitory symptoms including hyperalgesia, weight loss, thermoregulatory disturbances, shakes, and irritability (opiate-withdrawal syndrome) (80). We reasoned that since POEF enhances morphine-induced analgesia, i.e., reduces the amount of morphine necessary to produce a given level of analgesia, it might have a similar effect on the phenomena of morphine tolerance and withdrawal (16). Rats were made tolerant to a daily injection of 3 mg/kg morphine. After ten days of treatment, the 3-mg/kg dose no longer produced analgesia. On Day 11, that dose, coupled with an orogastric infusion of amniotic fluid, again produced measurable analgesia. A control infusion of saline on Day 11, to test for dishabituation, did not produce analgesia in conjunction with 3 mg/kg morphine. The next day (Day 12), the tolerant rats were allowed to go into withdrawal, during which they exhibited hyperalgesia. On Day 13, during withdrawal, the rats were treated with 1.5 mg/kg morphine, or with a vehicle control, and were infused either with amniotic fluid or with a fluid control. The morphine injection coupled with amniotic-fluid infusion produced significantly more relief from withdrawal symptoms, particularly hyperalgesia, than did any other combination of treatments. In addition, amniotic-fluid infusion in rats receiving no morphine produced a measurable reduction in withdrawal hyperalgesia (see Fig. 8).

This was the first time we detected an effect of amniotic fluid in the absence of the induction of opioid-mediated analgesia. It is possible that POEF enhanced an otherwise undetected endogenous-opioid release occurring during *withdrawal* (34).

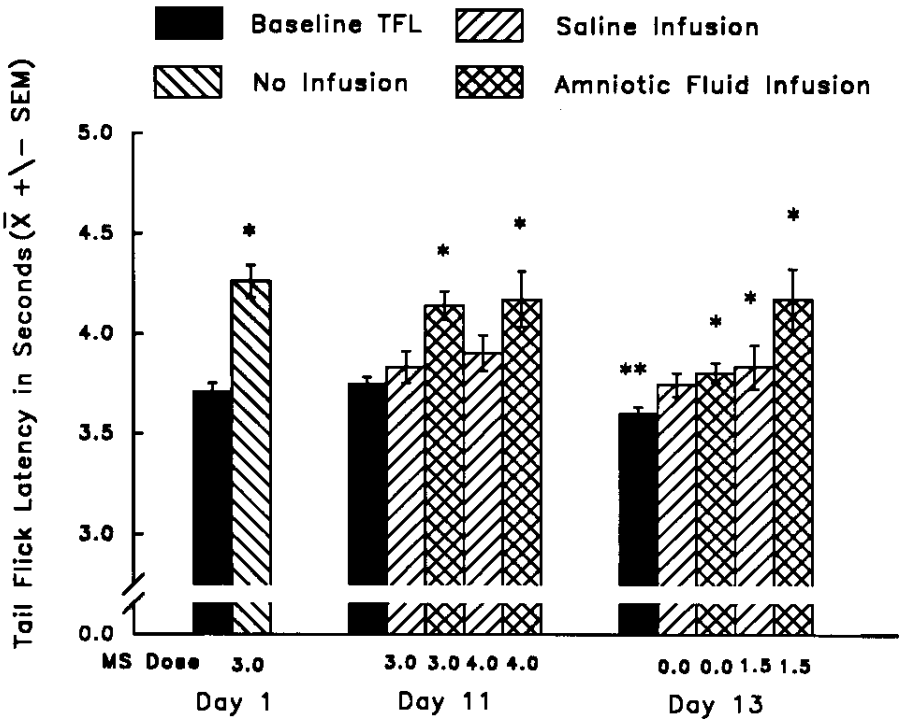

Fig. 8. Mean TFL (± S.E.M.) of morphine-naive rats (Day 1), morphine-tolerant rats (Day 11), and rats in morphine withdrawal (Day 13) both before (Baseline) and after morphine injection and orogastric infusion of either amniotic fluid or saline. *significantly greater than Baseline on that day ($p < 0.05$). **significantly lower than Day 1 Baseline ($p < 0.01$). [from (16) with permission].

Effect on Other Processes -- Pseudopregnancy

The study we conducted on the enhancing effect of ingestion of 0.25 ml amniotic fluid on analgesia produced by various levels of vaginal/cervical stimulation produced an unexpected result that we have not yet explored systematically: amniotic-fluid ingestion tended to block pseudopregnancy induced by vaginal/cervical stimulation (75). Nonpregnant rats in diestrus received either 75, 125, 175, or 225 g vaginal/cervical pressure after receiving an orogastric infusion of 0.25 ml of either amniotic fluid or saline. Among the rats receiving 225 g vaginal/cervical stimulation, a significantly greater proportion of those infused with saline entered pseudopregnancy afterward than did those infused with amniotic fluid. It is not clear yet what mechanism underlies this effect, or whether POEF or another constituent of amniotic fluid is responsible. In reducing the likelihood of pseudopregnancy resulting from vaginal/cervical stimulation, ingestion of amniotic fluid during delivery may serve also to decrease the probability of pseudopregnancy resulting from delivery, thereby increasing the chances for the occurrence of postpartum estrus and consequently a pregnancy eventuating from postpartum copulation. Although placenta deprivation during parturition was found to have no effect on the characteristics of postpartum estrus (70), the effects of deprivation of both placenta and amniotic fluid on the characteristics of postpartum estrus have not been examined.

MODE AND LOCUS OF POEF ACTION

Perhaps the most burning question is one that if answered first, would more readily provide answers to the issues of how and where POEF acts: that question is what is the nature of the POEF molecule? However, until this question is answered, we have to satisfy ourselves with less direct approaches to many of the important questions that remain.

Mode of Action of POEF

Virtually all the studies we have conducted on POEF suggest that it acts by enhancing the action of opioids or opiates that are present in the system, rather than by producing an increase in endogenous opioids or because it is, itself, an ingestible opioid. Although placenta and amniotic fluid are known to contain opioids (22, 35, 39), in our experiments on non-withdrawing rats administration of amniotic fluid or placenta has never produced analgesia in rats not treated with an opioid-mediated, or partly opioid-mediated, analgesic (e.g., 1, 44, 45, 49, 52). If POEF acts by triggering the release of endogenous opioids, or simply by contributing opioids to the system, we would have expected to see analgesia resulting from high levels of intake of placenta or amniotic fluid, whether or not we applied opioid-mediated analgesia to the subject.

In fact, although our original research suggested that high levels of intake of placenta or amniotic fluid do not enhance opioid-mediated analgesia (44), very high doses of POEF may actually be associated with a reduction of ongoing opioid-mediated analgesia, e.g., hyperalgesia (unpublished observations). A biphasic response such as this might be explained a number of ways. For example, the POEF molecule may possess both agonist and antagonist properties, which emerge differentially at different doses, as is the case with nalorphine, pentazocine, and cyclazocine (see [61] for review). Alternatively, amniotic fluid and placenta may contain both opioid enhancers (e.g., POEF) and inhibitors, but with the inhibitors existing in much smaller concentrations than the enhancers; when the level of intake of amniotic fluid or placenta in a brief period of time is extremely high, the dose of inhibitor may approach optimum whereas the dose of enhancer may be well beyond optimum. Indirect evidence tends to suggest the first explanation. If POEF does not have a mixed agonist/antagonist action, then the antagonist molecule in amniotic fluid must be quite close in size and characteristics to that of POEF, since the concentration of fractions of amniotic fluid that contain POEF produces inhibition rather than enhancement of opioid-mediated analgesia [unpublished observations].

Additional indirect evidence for the mode of action of POEF comes (a) from the finding that amniotic fluid enhances opioid analgesia when the fluid is ingested, but not when it is received by subcutaneous or intraperitoneal injection (1), and (b) from pilot data that show that pretreatment with infused activated charcoal blocks the enhancing effect of infused amniotic fluid on opioid-mediated analgesia [unpublished observations]. As mentioned above, this suggests that either POEF is activated in the gastrointestinal system and then is absorbed, or it requires binding to and activation of available gastrointestinal receptors for mediation of the effect. In the latter case, stimulation of gastric receptors might lead next to either neural or humoral activity (2). Neural activity, conveyed by vagal afferents, might ultimately result in direct neural stimulation of brain areas; involvement of vagal afferent information in the modulation opioid-mediated pain has already been documented (62, 68). Humoral activity might involve the release of a second molecule, e.g., cholecystokinin, which might in turn have an effect on opioid processes.

Locus of Action of POEF

Opioids have both central and peripheral effects. Although the effect of opioids in the central nervous system is more important for analgesia than are processes occurring in the periphery, the existence of peripheral effects makes it necessary to try to separate these from central effects in order to understand the mode of action of POEF on opioid-mediated analgesia. Quaternary naltrexone, or naltrexone methobromide, is an opioid antagonist that, because of its molecular configuration, does not readily cross the blood-brain barrier. It

is therefore quite useful in distinguishing central from peripheral opioid actions (8, 77). Using either systemic or intracerebroventricular application of quaternary naltrexone, in conjunction with either systemic or intracerebroventricular application of morphine sulfate, we have determined (13, 14) that POEF enhances only the central action of morphine in morphine-induced analgesia (see Fig. 9).

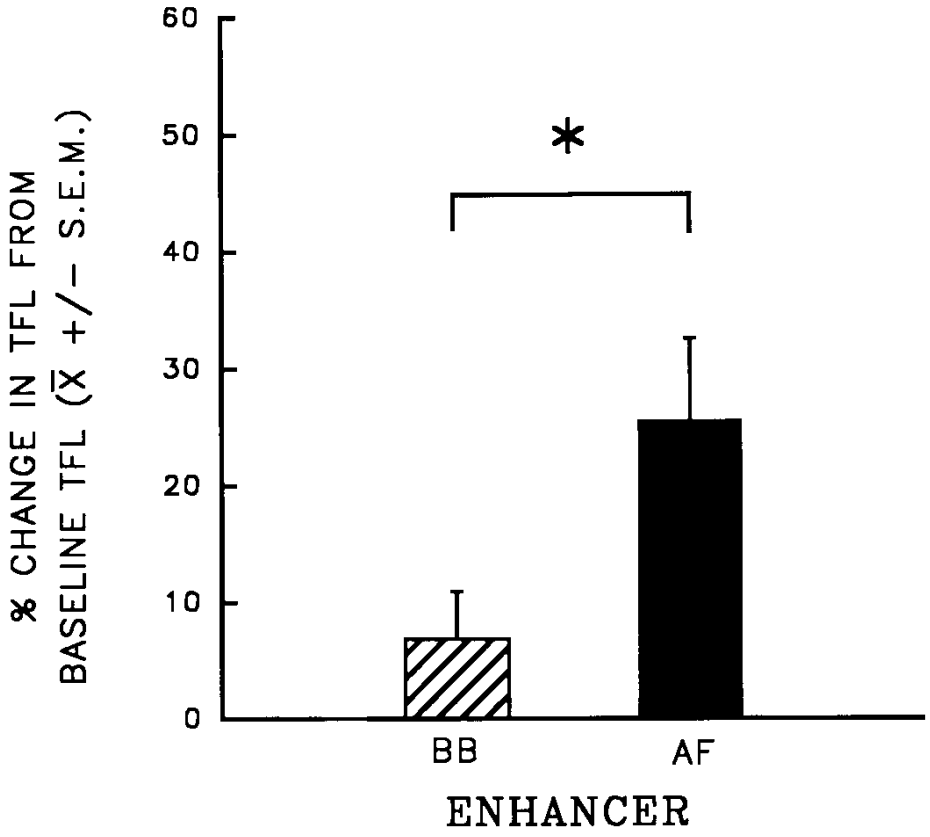

Fig. 9. Mean percent change (± S.E.M.) from baseline tail-flick latency (TFL) of rats pretreated with systemic Antagonist [quaternary naltrexone (QN)], then treated with central Agonist [morphine (MS)] and an orogastric infusion of Enhancer [amniotic fluid (AF) or beef bouillon control (BB)]. n = 7/group; *$p < 0.05$. [from (14) with permission].

SUMMARY AND CONCLUSIONS

The important findings of the last decade of research on the consequences of placentophagia involve two groups of results: those relating to the effect of placenta and amniotic fluid on the skin of neonates on facilitation of maternal responsiveness in adults, and those relating to enhancement of opioid-mediated analgesia.

Studies on the effect on maternal behavior of placenta and amniotic fluid on the surface of the neonate show that they speed up the rate at which maternal behavior develops in adults that are not immediately maternal, and that they probably do so by intensifying the level of adult-infant contact. In this respect, any manipulation that increases adult-infant contact, such as applying other attractive substances to the neonate, or housing the adult and neonate in a very small area, is likely to facilitate the development of maternal responsiveness on the part of the adult. In common with mothers of most other mammalian species, rat mothers are intensely maternal within moments of delivery of the neonate. Therefore, this beneficial effect of the placenta and amniotic fluid stimuli on the skin of the neonate is best observed, or may primarily function, in adults in

which maternal responsiveness develops more slowly, such as in nonparturient rats or in mothers of some nonnesting species.

On the other hand, the effect of ingestion of placenta and amniotic fluid on enhancement of pregnancy-induced analgesia may be a ubiquitous mammalian phenomenon, and may have important general consequences both for mammalian adaptation and, by extrapolation, for the pharmacological management of pain and of opiate addiction.

Ingestion by the mammalian mother of birth fluids and tissues enhances the endogenous-opioid-mediated analgesia existing during late pregnancy and delivery ("pregnancy-induced analgesia"). Amniotic-fluid ingestion provides enhancement of analgesia early in the delivery process, and placenta ingestion provides analgesia enhancement later in delivery and could produce enhancement that lasts into the early postpartum period. The enhancement seems to be accomplished by potentiating the effect of available opioids, rather than by increasing opioids either by triggering the release of endogenous opioids or by adding exogenous opioids to the system. The strategy of producing more opioid effect without adding more opioids is particularly effective because too high a level of opioids has been shown to have a deleterious effect on maternal caretaking behavior (7, 69).

The research on enhancement of analgesia has also shown, among other things, that (a) the substance responsible for the enhancement (POEF) affects several types of opioid-mediated analgesia, but not the nonopioid-mediated analgesia produced by aspirin; (b) POEF ingestion does not, by itself, produce analgesia; (c) the mechanism for responding to POEF is present in male as well as nonpregnant female rats; (d) POEF activity can be measured in a variety of pain assays; (e) although POEF affects tolerance and withdrawal, in addition to analgesia, it does not affect all opiate-mediated phenomena; and (f) POEF activity is present in the placenta of mammals other than rats.

Although we have found that ingestion of amniotic fluid does not modify morphine-mediated hyperthermia, and therefore does not affect to all opioid-mediated phenomena, we do not yet know the extent to which it does participate in opioid-mediated phenomena other than analgesia. We are only now beginning to understand the possible important effects of ingestion of amniotic fluid and placenta, and therefore of POEF, on periparturitional behavior and on postpartum reproductive efficiency.

An interesting question still remains unresolved: why is there so little evidence that humans groups regularly practice or practiced placentophagia? An extensive survey of anthropological files revealed no direct evidence of regular, deliberate, placentophagia among human cultures (43). However, the focus of the survey, and indeed, the focus of the anthropological notes, was on the fate of the placenta, not on the fate of amniotic fluid. The placenta is a large, obtrusive mass of tissue. It easily becomes the focus of attention during delivery, particularly the attention of observers. However, in regard to the significance of ingestion of birth materials and the reduction of periparturitional pain, the placenta may actually be a red herring. For humans and for many other mammals, the POEF content of amniotic fluid may be far more important for purposes of periparturitional pain relief than is that of placenta, although POEF may actually be produced in the placenta. The availability of amniotic fluid long before expulsion of the placenta would make it the more useful vehicle for POEF. Parturient women, in cultures past or present, could have inadvertently ingested amniotic fluid before expulsion of the fetus perhaps by licking their fingers, or during delivery by kissing, or cleaning by licking, the amniotic-fluid-soaked infants; this might be sufficient to produce the effects of ingested amniotic fluid well before the expulsion of the placenta at the conclusion of delivery. This form of ingestion of birth materials, and therefore of POEF, was probably a far more common occurrence than was ingestion of the placenta itself, but may not have been dramatic enough to warrant attention or documentation.

The more information we gather about POEF, the more likely becomes the possibility that POEF may provide a significant new direction in pharmacological management of pain and addiction. For pain management, the

strategy of enhancing the effects of endogenous opioids rather than administering narcotic analgesics has great appeal for both medical and social reasons. Pain control by treatment with synthetic POEF may have additional advantages: (a) if the mechanism of POEF is to potentiate the action of existing opioids rather than to increase circulating opioids, high doses of POEF are unlikely to lead to opioid overdose; and (b) if POEF has mixed agonist/antagonist properties, as some of the research suggests, very high doses -- abusive doses -- may lead to hyperalgesia. Therapy with POEF may therefore be self-limiting.

Tolerance developed to opiates, and withdrawal from opiates, have also been found to be modifiable with POEF, in that treatment with POEF reduces the amount of morphine required to maintain tolerance or to alleviate the hyperalgesia of withdrawal in morphine-dependent rats. This suggests the possibility that POEF, when synthesized, may also prove to be a useful tool in the management and treatment of opiate addiction.

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