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AMNIOTIC-FLUID INGESTION BY PARTURIENT RATS ENHANCES PREGNANCY-MEDIATED ANALGESIA

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Summary

Amniotic fluid and placenta contain a substance (POEF, for Placental Opioid-Enhancing Factor) that, when ingested, enhances opioid-mediated analgesia in nonpregnant rats; ingestion of the substance by rats not experiencing opioid-mediated analgesia, however, does not produce analgesia. It is highly likely that periparturitional analgesia-enhancement is a significant benefit of ingestion of the afterbirth (placentophagia) during delivery. Here we report that prepartum ingestion of amniotic fluid (via orogastric infusion) does indeed enhance the endogenous-opioid-mediated analgesia evident at the end of pregnancy and during delivery; that the degree of enhancement is greater with 0.75 ml than with 0.25 ml; and that the prepartum enhancement of analgesia can be blocked with the opioid antagonist naloxone.

Ingestion of either placenta or amniotic fluid has been shown to enhance various types of opioid-mediated analgesia in nonpregnant rats. These include analgesia induced by morphine injection, footshock, and vaginal/cervical stimulation (1-4), but not analgesia produced by aspirin injection (5). In the absence of opioid-mediated analgesia, however, ingestion of placenta and amniotic fluid do not produce analgesia (1-4). Opioid antagonists such as naloxone and naltrexone not only block opioid-mediated analgesia, but also render placenta ingestion and amniotic-fluid ingestion ineffective (1,3,5). We have named the active substance in placenta and amniotic fluid POEF, for *P*lacental *O*pioid-*E*nhancing *F*actor (4).

Most nonaquatic, nonhuman, mammalian mothers engage in placentophagia (6), or at least lick amniotic fluid from themselves or the infant. Since a measurable increase in pain threshold, produced by elevated endogenous opioids, is present just prior to delivery (7), we have been exploring the possibility in rats that a significant benefit of parturitional placentophagia is an enhancement of this pregnancy-mediated analgesia during the periparturitional period. The evidence we have gathered so far supports this hypothesis: amniotic fluid and placenta are effective when ingested (1-5); one placenta or 0.25 ml amniotic fluid (the amounts available during the delivery of one rat pup) are the optimum doses for enhancement of a 3-mg/kg, IP injection of morphine sulfate (4); and enhancement produced by administration of 0.25 ml amniotic fluid by orogastric tube is detectable within 5

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minutes and lasts for about 30 minutes (8), the approximate duration of the interpup interval during delivery. In the present study we sought to answer the direct question, Does ingestion of amniotic fluid enhance pregnancy-mediated analgesia?

Since amniotic fluid is available to the mother before emergence of the first neonate, and placenta is not available until well after delivery of the pup, amniotic fluid is probably the more important substance in enhancement of pregnancy-mediated analgesia. The ideal design in which to test the opioid-enhancing effects of amniotic-fluid ingestion at delivery would require prevention of ingestion. However, the ingestion-prevention techniques we have tried or contemplated so far either have interfered with delivery, would stress the mother inordinately and compromise the endogenous opioid-release pattern, or interfere with the measuring of pain thresholds. To avoid these problems, we administered donor amniotic fluid, by orogastric tube, a few hours before the mother's own amniotic fluid became available to her; mothers not receiving the donor amniotic fluid served as controls.

Methods

Subjects. Two hundred thirty-three pregnant Long-Evans rats, 3-4 months old, served as subjects. Prior to the last two days of pregnancy, they were housed individually in hanging wire-mesh cages (24.5 x 18 x 18 cm), in a colony maintained on a 14 hr on/10 hr off light cycle (lights on at 0500 hrs, EST). On about Day 20 of pregnancy, each rat was switched to a 10-gal glass aquarium containing 3 cm of coarse sawdust, 10 pellets of food, and a water bottle. The aquaria were covered with a weighted wire-mesh lid and were separated from each other by cardboard partitions. Mirrors were placed behind the aquaria to facilitate observation of the onset of delivery. Food (Agway Prolab Rat/Mouse/Hamster Formula 3000) and water were available *ad lib*. until the day of expected delivery.

Groups. The experimental groups consisted of rats receiving either 0.25 ml (n = 57) or 0.75 ml (n = 37) of rat amniotic fluid (AF). Some of the experimental rats (n = 27) were pretreated with the opiate antagonist naloxone (naloxone HCl, 0.1 mg/kg in a vehicle of 1.0 ml 0.9% saline, s.c.); some (n = 18) were pretreated with naloxone vehicle only; and some were not pretreated (n = 49). Infused control groups received either 0.25 ml (n = 56) or 0.75 ml (n = 37) of dilute beef bouillon (BB). Some of these rats (n = 27) were pretreated with naloxone, some (n = 17) were pretreated with vehicle, and some (n = 49) were not pretreated. Uninfused control rats received either pretreatment with naloxone (n = 5), pretreatment with vehicle (n = 6), or no pretreatment (n = 10). Naloxone was chosen as the opiate blocker because it is short-lasting; this decreased the possibility that an acute administration of the blocker would affect delivery if administered more than an hour beforehand (chronic treatment with naltrexone has been reported not to affect the parameters of delivery [7]). Two additional control groups were run, one consisting of unmanipulated (observed only) pregnant rats (n = 10), and a second of pregnant rats unmanipulated prepartum, but receiving pain-threshold tests postpartum (n = 15). Forty-one cycling adult female rats, matched for intervals between TFL tests, served as nonpregnant controls.

Procedures. Four tests for pain threshold were conducted: one test prepartum, one after the birth of the first pup, and one each at 40 min (0.7 hr) and 2 hr after the last pup was delivered (postpartum tests). The dependent variable was the latency for a hand-cradled rat to withdraw its tail from 55.5° C ($\pm$ 0.5°) water; the longer the latency, the more analgesia. This assay is widely used, and is considered to be reliable and innocuous (9-11). We decided that hand-cradling and the tail-dip procedure were preferable to placing delivering rats in

restraining tubes for the more common assay using a spot of hot light beamed onto the tail (1-4). The tail-flick latency score (TFL) was calculated as the mean of the last two of three trials separated by 30-sec intervals. Tail-withdrawal occurs in normal nonpregnant rats in about 2.2 sec. Each trial was terminated at 30 sec if no tail-withdrawal response occurred; with water at 55° C, rats do not sustain tissue damage to the tail. (In this study, no latency was greater than 10 sec.) The tests were conducted blind to the treatment of the rats. All rats received handling and cradling experience before they were mated.

Since the exact time for the onset of delivery cannot be predicted in rats with much precision, a set of criteria, which included a 7-hr window of opportunity, was decided upon in advance. Four hundred eleven rats were bred for the study. To increase the probability of deliveries occurring on the morning of Day 22 of pregnancy (day of impregnation is Day 1) to about 75%, the light cycle was reversed (on at 1500 hr, off at 0500 hr, EST) for pregnant rats on about Day 8 (12). On Day 22, food and water were removed 3 hr after lights-on; 3 hr later, those rats that appeared to be nearing delivery received their drug injection (naloxone, vehicle, or none), followed 20 min later by an orogastric infusion (amniotic fluid, beef bouillon, or none), followed 20 min later by a prepartum TFL test. The interval between that TFL test and the start of delivery is referred to as the *IPrOD* (for *I*nterval between the *Pr*epartum TFL test and the *O*nset of *D*elivery). The actual IPrOD was different for each rat, but rats could easily be grouped into three ranges, *post hoc*, for purposes of analysis: < 2 hr; 2-4 hr; > 4 hr. Subjects were removed from the study if they were observed to gain access to vaginally discharged fluid during the prepartum manipulations, or if pups were delivered after the 40-min postpartum TFL test (16%). Two hundred eight rats were tested once prepartum (IPrOD < 2 hr, $n = 68$; IPrOd 2-4 hr, $n = 85$; IPrOD >4 hr, $n = 55$) and at least at 2 hr postpartum. One hundred sixty-three rats were also tested at 40 min postpartum, and some ($n = 50$) also after the first pup was delivered; first-pup tests were added when disruption was found to be minimal, but since accuracy of prediction of impending delivery increased with practice, none of the first-pup tests were conducted on rats with IPrOD >4 hr.

Amniotic fluid used for infusion was harvested from CO_2-killed donors on Day 21 of pregnancy. Details of collection and storage have been published previously (3,4). The control substance, for BB, was half-strength, commercially-produced, instant beef bouillon (3,4); the bouillon infusion produces an effect indistinguishable from no infusion (4) or from an infusion of chicken-egg albumin (unpublished observation). Fluids were infused through 11.5 cm of PE 160 tubing fitted to a blunted 16-ga hypodermic needle, which, in turn, was fitted to a 1-cc glass syringe (3-5,8). Each rat received several practice intubations, without infusion of fluid, before mating.

Results

The control groups for the intubation procedure, for prepartum testing, and for AF dose (*i.e.*, the BB groups) did not differ significantly from each other (*intubation and BB controls*: $F[6,39] = 1.36$, $p > 0.05$; *prepartum testing control*: $F[1,76] < 1.00$) on TFL or with respect to parameters of parturition (onset or duration of delivery, interpup intervals). They were pooled for subsequent analyses, and are represented by an irregular gray band on Fig. 1. TFLs for combined controls ($n = 97$) did differ from the nonpregnant TFL level ($F[1,99] = 45.23$, $p < 0.001$); this 22% greater tail-flick latency represents *pregnancy-mediated analgesia* (7,13). The controls showed significantly more endogenous analgesia during (48%) and after delivery (27%) than they had shown immediately before ($F[3,39] = 32.26$, $p < 0.01$; *Newman-Keuls*: 1st pup $>$ 2 hr postpartum = 40 min postpartum $>$ prepartum).

A 2 x 2 x 3 x 2 ANOVA was performed (Fluid [AF, BB] x Dose [0.25 ml, 0.75 ml] x IPrOD [<2 hr, 2-4 hr, >4 hr] x Time [prepartum, 2 hr postpartum]). This was followed by a 2 x 3 x 3 ANOVA comparing Fluid x IPrOD x Time [prepartum, 40 min postpartum, 2 hr postpartum] only for those rats receiving the 0.75-ml dose, because few rats in the 0.25-ml dose groups were tested at 40 min postpartum.

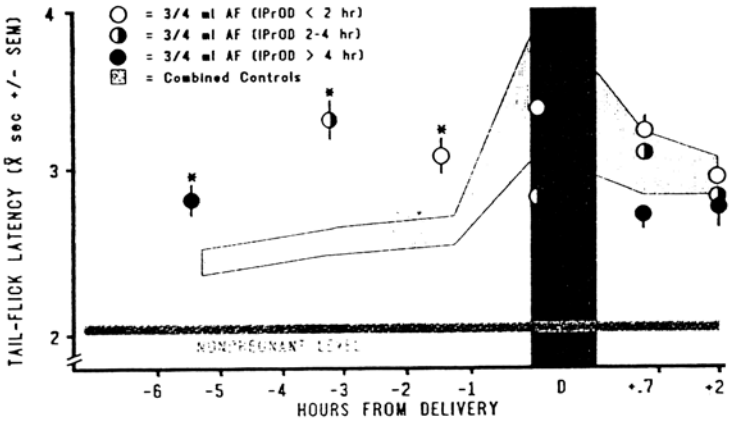

FIG. 1

Effect on pain threshold of infusion of 0.75 ml amniotic fluid in the hours immediately prior to the onset of delivery (the 0.25-ml doses are omitted from the graph for the sake of simplicity). The Combined Controls group (IPrOD <2 hr, $n = 22$; IPrOD 2-4 hr, $n = 37$; IPrOD >4 hr, $n = 23$; postpartum tests only, $n = 15$) 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 (* = $p < 0.01$).

The 4-way ANOVA revealed a significant Fluid x Dose x Time interaction ($F[1,121] = 5.72$, $p < 0.02$). Probes of the interactions for simple effects indicated that the prepartum administration of AF (regardless of dose) produced a significant elevation of pregnancy-mediated analgesia (about a 13% increase in TFL for 0.25 ml AF, and about a 25% increase for 0.75 ml AF), whereas administration of BB did not (*0.75 ml AF*: $F[1,189] = 28.16$, $p < 0.001$; *0.25 ml AF*: $F[1,189] = 6.32$, $p < 0.02$). In fact, the prepartum level of analgesia for the 0.75-ml AF rats was significantly *greater* than the postpartum levels ($F[1,121] = 9.06$, $p < 0.01$), whereas for the 0.25-ml AF rats, prepartum and postpartum levels were the same ($F[1,121] < 1.00$). For BB rats, on the other hand, prepartum levels were significantly lower than postpartum levels (*0.25 ml BB*: $F[1,121] = 36.14$, $p < 0.001$; *0.75 ml BB*: $F[1,121] = 49.25$, $p < 0.001$).

The 4-way ANOVA also produced a significant Fluid x IPrOD x Time interaction ($F[1,121] = 3.48$, $p < 0.04$). When the data were collapsed across doses, the greatest effect of AF administration was observed with the 2-4 hr IPrOD (administration occurring 2-4 hr before the onset of delivery; TFL latencies were 24% above those of pregnant controls). With this IPrOD, rats receiving AF showed significantly higher levels of analgesia prepartum than they did postpartum ($F[1,121] = 9.38$, $p < 0.01$). At IPrODs of <2 and >4 hrs, prepartum and postpartum levels of analgesia were equivalent (*IPrOD <2 hr*: $F[1,121] = 2.81$, $p > 0.05$; *IPrOD >4 hr*: $F[1,121] < 1.00$). For all three IPrODs, rats receiving BB showed significantly lower levels of analgesia prepartum than they did postpartum (*IPrOD <2 hr*: $F[1,121] = 29.75$, $p < 0.01$; *IPrOD 2-4 hr*: $F[1,121] = 60.83$, $p < 0.001$; *IPrOD >4 hr*: $F[1,121] = 12.94$, $p < 0.01$). Regardless of IPrOD, AF-treated rats showed higher levels of analgesia than did BB-treated rats on every test (*IPrOD <2 hr*: $F[1,121] = 5.05$, $p < 0.03$; *IPrOD 2-4 hr*: $F[1,121] = 17.41$, $p < 0.01$; *IPrOD >4 hr*: $F[1,121] = 9.22$, $p < 0.01$).

The 3-way ANOVA that included the 40-min postpartum test (0.75-ml doses only) revealed that for both control rats and rats receiving AF, the level of analgesia shown at 40 min postpartum was significantly higher than that shown at 2 hr postpartum (*AF rats*: $F[2,116] = 5.31$, $p < 0.01$; *Newman-Keuls*: prepartum = 40 min postpartum > 2 hr postpartum; *BB rats*: $F[2,116] = 50.35$, $p < 0.001$; *Newman-Keuls*: 40 min postpartum > 2 hr postpartum > prepartum).

A paradoxical result suggested a possible long-lasting effect of the low dose of AF. The postpartum pain thresholds of the rats receiving 0.25 ml AF at the shortest IPrODs were significantly *lower* than those of any of the other groups (*0.25 ml AF at <2-hr IPrOD*: mean = 2.41 $\pm$ 0.11 sec; *0.25 ml BB at <2-hr IPrOD*: mean = 2.90 $\pm$ 0.12 sec; $F[1,189] = 8.53$, $p < 0.01$)(the postpartum thresholds of the rats receiving 0.75 ml AF at the shortest IPrODs were among the highest of all the groups at each postpartum test; see Fig. 1). In addition, the hyperalgesia in those few low-dose/short-IPrOD rats was associated with heightened aggressiveness that lasted for as long as two days (21% incidence *vs.* 3% for BB). The low dose of AF administered within 2 hr before the onset of delivery may have rendered the rats refractory to their own endorphins or POEF during and after delivery.

Naloxone, which because of its short half-life did not disturb the timing of parturition (*IPrOD with naloxone*: mean = 176.00 $\pm$ 33.4 min; *IPrOD without naloxone*: mean = 227.50 $\pm$ 24.29 min; $F[5,222] = 1.43$, $p > 0.05$; *duration of delivery with naloxone*: mean = 148.00 $\pm$ 13.56 min; *duration of delivery without naloxone*: mean = 135.00 $\pm$ 11.62 min; $F[5,222) < 1.00$) or the normal level of analgesia observed at delivery of the first pup (*non-naloxone*: mean = 3.59 $\pm$ 0.16 sec; *naloxone*: mean = 3.38 $\pm$ 0.19 sec), completely suppressed pregnancy-mediated analgesia during all three IPrOD ranges, and produced TFLs in all groups of prepartum rats that were indistinguishable from TFLs of nonpregnant rats ($F[1,66] < 1.00$).

Discussion

In sum, administration of amniotic fluid, by orogastric tube, in the few hours before the onset of delivery, elevated pregnancy-mediated analgesia to a level not observed in untreated rats until delivery begins, when the mother's own discharged amniotic fluid becomes available to her. The enhancement was more dramatic for a dose of 0.75 ml amniotic fluid (see Fig. 1) than for a dose of 0.25 ml. It should be remembered that all rats treated with amniotic fluid prepartum still had access to their own delivered amniotic fluid, which became available just prior to the emergence of the first pup, as well as placenta, which was available after delivery of each pup.

We contend that enhancement of pregnancy-mediated analgesia naturally occurs as a consequence of periparturitional ingestion of birth materials (placentophagia), and that the prepartum administration of amniotic fluid produced the enhancement prematurely. That analgesia enhancement is the *most* important benefit of ingestion of amniotic fluid and placenta is, of course, arguable. However, in contrast to various other benefits that have been postulated, such as avoidance of predators, increasing nest cleanliness, satisfaction of specific or general hunger (6), analgesia enhancement would certainly serve as a universal benefit across taxonomic groups.

Why would it make sense to have enhancement of existing analgesia by the action of a substance like POEF, rather than an elevation in analgesia produced by a greater output of endogenous opioids? Too high an opioid level may have a deleterious effect on proper maternal care (14,15). POEF, ingested in amniotic fluid, may therefore provide a mechanism for the reduction of discomfort during and after delivery that does not require more than a minimum of endogenous opioid.

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References

1. M.B. KRISTAL, A.C. THOMPSON and H.L. GRISHKAT, Physiol. Behav. 35 481-486 (1985).
2. M.B. KRISTAL, A.C. THOMPSON, S.B. HELLER and B.R. KOMISARUK, Physiol. Behav. 36 1017-1020 (1986).
3. M.B. KRISTAL, A.C. THOMPSON and P. ABBOTT, Physiol. Behav. 38 809-815 (1986).
4. M.B. KRISTAL, P. ABBOTT and A.C. THOMPSON, Pharmacol. Biochem. Behav. 31 351-356 (1988).
5. M.B. KRISTAL, J.A. TARAPACKI and D. BARTON, Physiol. Behav., 47(1) in press (1990).
6. M.B. KRISTAL, Neurosci. Biobehav. Rev. 4 141-150 (1980).
7. A.R. GINTZLER, Science 210 193-195 (1980).
8. J.C. DOERR and M.B. KRISTAL, Physiol. Behav. 46 913-915 (1989).
9. M.R. FENNESSY and J.R. LEE, *Methods in Narcotic Research*, S. Ehrenpreis and A. Neidle (eds), 73-99, Marcel Dekker, New York (1975).
10. E.F. HAHN, Meth. & Find. exptl. clin. Pharmacol. 7 373-381 (1985).
11. P.A.J. JANSSEN, C.J.E. NIEMEGEERS and J.G.H. DONY, Arzneim-Forsch. 13 502-507 (1963).
12. A.D. MAYER and J.S. ROSENBLATT, J. comp. physiol. Psychol. 94 1040-1059 (1980).
13. S.A. BARON and A.R. GINTZLER, Brain Res. 418 138-145 (1987).
14. R.S. BRIDGES and C.T. GRIMM, Science 218 166-168 (1982).
15. B.S. RUBIN and R.S. BRIDGES, Brain Res. 307 91-97 (1984).