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BRIEF COMMUNICATION

Amniotic-Fluid Ingestion Enhances Morphine Analgesia During Morphine Tolerance and Withdrawal in Rats

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DOERR, J. C. AND M. B. KRISTAL. *Amniotic-fluid ingestion enhances morphine analgesia during morphine tolerance and withdrawal in rats*. PHYSIOL BEHAV **50**(3) 633–635, 1991.—Ingestion of placenta and amniotic fluid has been shown to enhance opioid-mediated analgesia in rats produced by morphine injection, footshock, vaginal/cervical stimulation, and during late pregnancy. The present study was designed to investigate the effects of amniotic fluid ingestion on the characteristics of morphine dependency and withdrawal. Tail-flick latencies in Long-Evans rats were determined before and after repeated daily injections of morphine sulfate. It was found that ingestion of amniotic fluid after establishment of the morphine dependency, coupled with an injection of an otherwise ineffective dose of morphine, enhanced analgesia in morphine-dependent rats, and reversed hyperalgesia seen during withdrawal from morphine dependency.

Amniotic fluid POEF Morphine Withdrawal Opiate Tolerance Analgesia Addiction Pain

PLACENTOPHAGIA, ingestion of placenta and amniotic fluid, is observed during parturition in most placental mammals (6). A major consequence of this behavior is enhancement of opioid-mediated analgesia. Enhancement by ingestion of amniotic fluid or placenta of opioid-mediated analgesia has been demonstrated by using an injection of morphine sulfate (7, 8, 10), by application of footshock (10) or vaginal/cervical stimulation (11), and during the analgesia characteristic of late pregnancy (4,9). This enhancing effect has also been shown to be dose dependent, but not in a linear fashion: ingestion of a low dose of placenta or amniotic fluid enhances opioid-mediated analgesia, whereas ingestion of a high dose of these substances attenuates analgesia (7). Finally, the fact that amniotic-fluid ingestion or placenta ingestion does not produce analgesia in the absence of concurrent opioid-induced analgesia (9,11), and the fact that administration of opioid antagonists reverses the enhancement phenomenon (3, 8, 10), demonstrate that the enhancement is opioid mediated, but is not due to the release of endogenous opioids. Therefore, placenta and amniotic fluid appear to contain an opioid-enhancing substance, which we have named placental opioid-enhancing factor (POEF) (7).

The development of tolerance to chronic morphine administration and the withdrawal symptons that occur following drug abstinence are well documented. There are many effects of morphine injection including, but not limited to, analgesia, dose-dependent changes in body temperature, depressed respiration and locomotion, and changes in body weight (14). Tolerance can be assessed for most individual morphine effects and is indicated by an eventual lack of response, or a diminishing response, to the administration of a constant dose of morphine (14). Dependency can be assessed by determining whether abrupt cessation of morphine results in the withdrawal syndrome, which includes such characteristics as hyperalgesia, deranged thermoregulation, decreased body weight, wet dog shakes and irritability (14). The present study was conducted to determine whether ingestion of amniotic fluid by orogastric infusion enhances analgesia in morphine-dependent rats, and if it reverses withdrawal symptoms, specifically hyperalgesia, produced by abstinence. Previous research has led us to hypothesize that POEF ingestion should render an otherwise ineffective dose of morphine potent enough to ameliorate the withdrawal syndrome and to relieve symptomatic hyperalgesia.

METHOD

Forty-two Long-Evans virgin female rats, 75–120 days old, were used. All rats were maintained in a colony with a 14 h on/10 h off light/dark cycle, with the onset of the light phase at 0500 h (EST). Rats were housed individually in 45 × 24 × 20-cm plastic cages. Food (Agway Prolab Rat/Mouse/Hamster Formula 3000) and water were available ad lib. Prior to testing, all rats were habituated to the test procedures. Each rat was intubated

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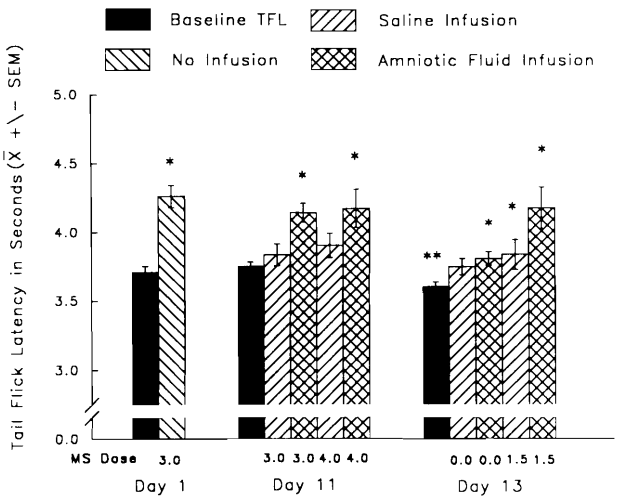

FIG. 1. Mean TFL (±SEM) of morphine-naive rats (Day 1), morphine-tolerant rats (Day 11), and rats in morphine withdrawal (Day 13), both before (Baseline) and after morphine injection and orogastric infusion of either amniotic fluid or saline. *Significantly greater than Baseline on that day ($p<0.05$). **Significantly lower than Day 1 Baseline ($p<0.01$).

without infusion and restrained for 1 h/day for five consecutive days.

Pain thresholds were measured using a radiant-heat tail-flick latency (TFL) test. The tail-flick algesiometer used was similar to those used in other laboratories (1, 2, 12). The apparatus and procedures have been described elsewhere (7). Rats were restrained in opaque polyvinyl chloride tubes (5×21 cm) during the TFL test. TFL, measured in seconds, was the dependent variable. Each TFL test comprised four TFL trials conducted at 30-s intervals; the mean of the last three trials was the score for that test. If no response occurred within 8 s, the stimulus was terminated to avoid tissue damage.

Testing occurred between 0900 and 1200 h. Rats were given a daily injection of 3.0 mg/kg morphine sulfate (MS, 1 ml/kg, IP) for ten days. On Day 11, half the subjects were injected with 3.0 mg/kg MS and half were injected with 4.0 mg/kg MS. (We expected the lower dose to be ineffective, but felt that the higher dose MS might produce a detectable, and therefore enhanceable, level of analgesia.) Fifteen minutes after the MS injection, half the rats in each group received an orogastric infusion of 0.25 ml amniotic fluid; the remaining rats received 0.25 ml saline. Infusions were administered through a length of PE 160 tubing, fitted to a blunted 16-ga hypodermic needle mounted on a 1-cc glass syringe. Techniques for the collection, storage, and infusion of amniotic fluid have been described previously (7,8). On Day 12, all rats were injected with 0.9% saline vehicle (1 ml/kg, IP), instead of MS, to precipitate withdrawal. On Day 13, half the rats were injected with 1.5 mg/kg MS (1 ml/kg, IP) and half were injected with saline vehicle. This particularly low dose of MS was used so that we were confident that the MS alone would not reverse the hyperalgesia that is characteristic of withdrawal. Secondly, amniotic fluid has a biphasic effect and pilot work using higher doses of MS found amniotic fluid to be inhibitory. Again, half the rats in each group were infused with 0.25 ml amniotic fluid and half with 0.25 ml saline. Subject treatments were balanced in that two rats from each group on Day 11 were assigned to each group on Day 13. TFL was determined for each rat on Days 1, 11, and 13. On each of the three days of testing, the baseline TFL was first determined, followed immediately by drug injection, and then by infusion of fluid (if any). A second TFL test was administered 50 min after injection.

A separate group of 9 rats received daily injections of saline (0.9%, 1 ml/kg, IP), but were otherwise maintained and tested identically to rats treated with MS.

RESULTS

There was no change in the baseline TFL of those rats receiving daily injections of saline. The baseline TFLs were 3.62±0.05, 3.54±0.06 and 3.57±0.06 on Days 1, 11, and 13, respectively, $F(2,16)<1.0$.

In morphine-treated rats on the first day of testing (Day 1), morphine injection produced a significant increase in pain threshold (see Fig. 1). TFL was elevated from 3.71±0.04 s, at baseline, to 4.26±0.08 s following morphine injection, which represents about a 15% increase, $F(1,32)=81.23$, $p<0.001$. On Day 11 this same dose of MS produced no change from baseline TFL. The saline-infused rats (controls for dishabituation and amniotic fluid infusion) showed a latency of 3.75±0.05 s at baseline, and 3.83±0.08 s following 3.0 mg/kg morphine, $F(1,6)=2.04$, $p>0.05$. A 2-way ANOVA performed on the data from Day 11 indicated a significant main effect of infused fluid, $F(1,29)=8.45$, $p<0.01$, in that both doses of morphine were enhanced by amniotic-fluid ingestion. There was no main effect of drug (neither 3.0 nor 4.0 mg/kg morphine produced analgesia) nor was there an interaction between infused fluid and drug. Therefore, amniotic-fluid infusion increased TFLs of rats that received either 3.0 mg/kg or 4.0 mg/kg MS whereas saline infusion did not.

On Day 13, the baseline mean TFL of the morphine-treated rats was slightly but significantly lower (3.60±0.04 s) than it had been on Day 1 (3.71±0.04 s), $F(1,32)=9.86$, $p<0.01$, indicating hyperalgesia due to morphine withdrawal [48 h without morphine (13)]. A 2-way ANOVA performed on the data from Day 13 indicated significant interactions of drug and test time (pre vs. post), $F(1,29)=9.54$, $p<0.05$, and of infused fluid and test time, $F(1.29)=11.89$, $p<0.01$. The simple-effects probes of these interactions revealed that, as expected, MS increased the TFLs of rats infused with either amniotic fluid or saline, $F(1,47)=8.78$, $p<0.05$. Furthermore, amniotic-fluid ingestion significantly increased the TFLs of the rats injected with morphine, $F(1,47)=6.22$, $p<0.05$. The TFLs of those rats receiving 1.5 mg/kg MS injection + amniotic-fluid infusion were higher than those receiving 1.5 mg/kg MS injection + saline infusion which, in turn, were higher than of those rats receiving vehicle injection + saline infusion. An unexpected finding was that amniotic-fluid ingestion increased the TFLs of rats injected with vehicle. This may have been due to enhanced endogenous opioid release in response to withdrawal (5) or to a conditioned response to the injection procedure.

DISCUSSION

Tolerance to a low dose of morphine (3.0 mg/kg) was observed to develop, as indicated by a lack of an analgesic response to morphine injection after 10 days of daily injections. However, after tolerance developed, this otherwise ineffective dose of morphine was capable of evoking analgesia if it was coupled with ingestion of amniotic fluid (but not if it was coupled with ingestion of saline). Withdrawal, precipitated by abstinence, produced hyperalgesia, as indicated by a lowering of baseline TFL. This hyperalgesia could be reversed by a very low dose of MS (1.5 mg/kg) in combination with ingestion of amniotic fluid. Therefore, both tolerance and at least one major withdrawal symptom, hyperalgesia, can be modified by ingestion of amniotic fluid (and therefore ingestion of POEF). This raises the

possibility that POEF, when isolated and perhaps synthesized, might be an effective tool in the management of opioid addiction.

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