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.

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
0.25 ml amniotic fluid and half with 0.25 ml saline. Subject withdrawal. Secondly, amniotic fluid has a biphasic effect and pilot 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 treatments were balanced in that two rats from each group on hibitory. Again, half the rats in each group were infused with treatments were administered through a length of PE 160 tubing, fit­

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 algometer was used similar to those used in other laboratories (1, 2, 12). The apparatus and procedures have been described elsewhere (7). Rats were re­

strained in opaque polyvinyl chloride tubes (5 x 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, dose of MS (1.5 mg/kg) in combination with ingestion of amniotic fluid; the remaining rats received 0.25 ml saline. Infu­

sions 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 injec­

tion. 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.

**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.

REFERENCES