Gastric Vagotomy Blocks Opioid Analgesia Enhancement Produced by Placenta Ingestion

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TARAPACKI, J. A., A. C. THOMPSON AND M. B. KRISTAL. *Gastric vagotomy blocks opioid analgesia enhancement produced by placenta ingestion.* PHYSIOL BEHAV 52(1) 179–182, 1992.—Ingestion of amniotic fluid or placenta by rats has been shown to enhance opioid-mediated analgesia induced by morphine injection, footshock, vaginal/cervical stimulation, or late pregnancy. This enhancement by ingestion appears to be specific to the central actions of opioids. The present study was designed to examine the possibility that information traveling via the vagus nerve might be involved in mediating this effect. Rats that had undergone either selective gastric vagotomy or sham vagotomy were injected with either morphine sulfate or vehicle and fed either placenta or a meat control. Enhancement was observed in rats that had undergone sham vagotomy but not in those that had undergone gastric vagotomy. These results support an interpretation of vagal involvement in the enhancement of opioid-mediated analgesia by placenta.

Placenta   Opioids   Vagotomy   Placentophagia   Morphine   Pain   Analgesia   Tail-flick latency
Vagus   POEF   Rats

PLACENTOPHAGIA, the ingestion of placenta and amniotic fluid, is common among placental mammals during parturition (12). A significant consequence of this behavior is the specific enhancement of opioid-mediated analgesia (13). Placenta or amniotic fluid ingestion does not, by itself, produce analgesia (15,18), nor does it appear to enhance nonopioid-mediated analgesia (14), but it does enhance analgesia produced by morphine injection (14,15,17,18), footshock (17), vaginal/cervical stimulation (18), and late pregnancy (16). Furthermore, this potentiation can be reversed by opioid antagonists (6,15,17). We refer to the active substance(s) in placenta and amniotic fluid as POEF, for placental opioid-enhancing factor (13).

The evidence indicates that gastrointestinal involvement is essential to the effects of placenta and amniotic fluid. All of the studies demonstrating these effects have involved ingestion, and amniotic fluid was not effective when it was injected subcutaneously or intraperitoneally (1). Meanwhile, although opioids have both central and peripheral effects, enhancement by POEF ingestion appears to be specific to the central actions (6). An obvious question that presents itself, then, is how is this potentiation conveyed from the gastrointestinal system to the CNS? The present experiment was designed to begin to address this question. Specifically, we sought to determine whether the enhancement might be conducted by way of the vagus nerve.

There are several lines of evidence that suggest the possibility of vagal involvement in the transmission of the amplification of opioid analgesia by POEF. First, the majority of fibers composing the abdominal vagus are afferent (2,23), making it the major neural relay for visceral information. These afferents relay information from a variety of receptors in the gastrointestinal system (2). Second, the vagus is known to be involved in the regulation of nociception. It has been demonstrated, for example, that activation of vagal afferents produces behavioral antinociception (24,26). Furthermore, this effect is antagonized by naloxone, thereby implicating the vagus in the mediation of analgesia from peripheral opioids. Additional support for this kind of role for the vagus comes from reports that vagotomy reduces the antinociceptive effects of footshock, of an enkephalin analog (24), and of both IV and IP morphine injections (25,30).

The approach taken in the present experiment involved testing the opioid-potentiating efficacy of placenta ingestion in rats that had been subjected to selective gastric vagotomy. Although this approach would not, if enhancement were blocked by gastric vagotomy, preclude the possibility that the critical contribution might be descending activation by vagal efferents (e.g., vagal activation of digestion), the demonstration that enhancement of opioid analgesia by placenta ingestion persisted after gastric vagotomy would eliminate the vagus from further consideration.

METHOD

Subjects

Eighty female Long–Evans rats averaging 250.0 ± 2.0 g were used as subjects. The rats were distributed equally among eight groups. Rats that were removed for failing to meet various criteria were replaced until an *n* of 10 rats/group was achieved. They were maintained on a 14 h on/10 h off light/dark cycle with the light phase beginning at 0600 (EST). Testing was conducted be-

tween 0830 and 1100. Upon entering the experiment, the rats were housed individually in clear plastic cages (46 × 25 × 21 cm) fitted with wire tops and containing approximately one liter of wood shavings. Solid food (Agway Prolab Rat/Mouse/Hamster Formula 3000) and tap water were available ad lib except as described below.

Surgical Procedure

Food deprivation began 12 h before surgery. The rats were anesthetized with an injection (0.65 ml/kg, IP) of a 4:1 mixture of ketamine (Ketaset, 100 mg/ml) and xylazine (Rompun, 20 mg/kg), supplemented (0.05 ml) as necessary. The details of the surgery were similar to the procedures developed in Smith's laboratory (10,27). Briefly, a 4–5-cm ventral midline incision was made just caudal to the xiphoid cartilage. The stomach and lower esophagus were retracted from the abdominal cavity, and a 3-0 silk suture was placed along the greater curvature of the stomach. Gentle traction was maintained by clamping a hemostat on the free ends of the suture and hanging it over the edge of the operating table. An operating microscope was used to identify the two major vagal trunks which were then separated from the esophageal wall. Two 3-0 silk sutures were tied around each trunk 5–10 mm apart at the gastro-esophageal border. The nerve between the sutures was sectioned, and the ends were painted with 70% alcohol. It was frequently necessary to section the celiac branch of the vagus along with the left gastric branch, but the accessory celiac was left intact in all vagotomized subjects. The stomach and esophagus were irrigated frequently during the surgery with physiological saline to reduce adhesion. Finally, the abdominal incision was closed with two layers of interrupted 3-0 silk sutures. Urea-sulfa powder was applied to the incision, and an injection (0.05 ml, IM) of Combiotic was administered to counter the possibility of infection. Sham vagotomy consisted of the same procedure except that the vagal trunks were manipulated but not ligated or cut. Because of the weight loss rats are known to sustain following vagotomy (4,11,19,27), heavier rats were assigned to the gastric vagotomy condition.

One potential confound that was necessary for us to address in this experiment involved the stomach distention and retention of food that is a consequence of vagotomy (4,9,20). It was important for us to be certain that the enhancer, which would be ingested, would not be absorbed or otherwise differentially affected by excessive stomach contents in the vagotomized subjects. There is evidence in the literature of decreased retention of liquid diets by vagotomized rats (8,29); one study found no differences between the stomachs of vagotomized and control rats maintained on a restricted feeding schedule consisting of canned milk (7). However, a few of our pilot rats exhibited distended stomachs after a 14-day recovery period even though they were restricted to a nonfat liquid diet (skim milk and Poly-Vi-Sol multiple vitamins). Rather than risk the ambiguity that would result if the enhancement did not appear in our vagotomized subjects, we decided to test our rats without allowing their stomachs to become distended. Following surgery, therefore, the rats were allowed access to only tap water and a 10% sucrose solution until they were tested on the second postsurgery day. A comparison was made of separate groups of rats maintained on this regimen and euthanized at the time testing would have been conducted. The gastric vagotomy rats showed no abnormal stomach distention, and their mean stomach weight/body weight ratio (0.20 ± 0.03) did not differ significantly from that of the sham-vagotomy rats (0.21 ± 0.03).

Apparatus

During testing, the rats were restrained in opaque, polyvinyl tubes (5 × 2 cm) to which they had been habituated. Pain thresholds were measured with a tail-flick algesiometer that has been described previously (17) and is similar to the apparatus that is standard in other laboratories (3,5,31). The number of s it took for a rat to move its tail from the heated stimulus field was the dependent variable (tail-flick latency, or TFL). Four consecutive trials were conducted at 30-s intervals; the test score was the mean of the last three trials. To prevent tissue damage to subjects experiencing analgesia, trials were terminated at 8 s if no response occurred.

Habituation

In addition to being habituated to the restraining tubes, on the 3 days prior to undergoing surgery the rats were exposed to the 10% sucrose solution and to the substance they would be expected to ingest during testing: placenta or ground beef. Placenta was obtained surgically from day 21 pregnant donors euthanized with CO_2. Both the placenta and the ground beef were frozen at $-20°C$ and stored until needed. For presentation, the substances were warmed to $37°C$.

Procedure

Each rat was weighed 2 h before being tested. Vagotomized rats averaged 247.1 ± 3.0 g, and sham-vagotomized rats averaged 253.0 ± 2.6 g. For the 2 h prior to the test, and during the test, the rats were deprived of food and water. Within each surgery condition, two groups of rats received 1 mg/kg morphine sulfate (IP) followed by the presentation of either one placenta (group M/P) or an equal weight (about 500 mg) of ground beef (group M/B). The other two groups were given saline (1 ml/kg, IP) before receiving placenta (group S/P) or ground beef (group S/B).

In other experiments conducted in our laboratory dealing with the enhancing effects of placenta ingestion, 3 mg/kg (IP) had been the appropriate threshold dose of morphine sulfate (14,15,17,18). The 1 mg/kg dose used in this investigation was chosen after pilot rats that had undergone sham vagotomy exhibited antinociceptive responses to the 3 mg/kg dose that were beyond the limits of our TFL procedure, presumably due to the presence of elevated levels of endogenous opioids. Results obtained from additional pilot rats suggested that 1 mg/kg (IP) morphine sulfate might be an optimum effective threshold dose under these circumstances. Before testing vagotomized rats, groups of sham-vagotomized rats were tested to be sure that a measurable amount of analgesia was obtained. In addition, in order to determine whether the presumed contribution of endogenous opioids was due to the surgery itself or to another of our manipulations (e.g., anesthesia), separate groups of rats were prepared without incisions. These rats were habituated, anesthetized, and maintained on the same diet and schedule as our test rats.

In all cases, testing was conducted by an experimenter who was blind to the condition of the rats. It began with an assessment of the subject's baseline TFL (T_b), followed immediately by the morphine or saline injection. Fifteen min after the injection, the rat was presented with placenta or ground beef in an untippable glass dish. Only those rats that ate all the proferred meat were tested further. Ten min later, the subject was again tested for TFL (T_{10}), then once more after another 25-min interval (T_{35}). For purposes of analysis, each latency was converted into percent change from baseline.
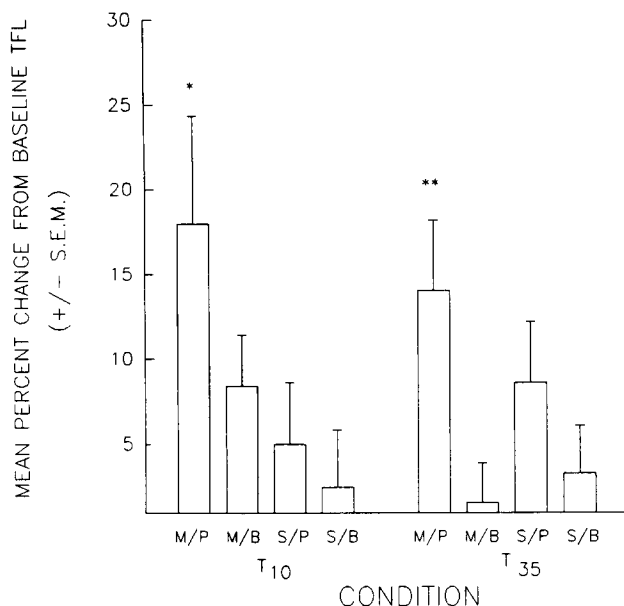
FIG. 1. Mean percent change from baseline tail-flick latency ($\pm$SEM), at the 10-min (T_{10}) and 35-min (T_{35}) tests, of rats receiving sham gastric vagotomy. M/P = morphine + placenta; M/B = morphine + beef; S/P = saline + placenta; S/B = saline + beef. *M/P > S/P; **M/P > S/B; $p < 0.05$.

Verification of Vagotomy

After being tested, the rats were allowed ad lib access to solid food and tap water for 3 days before being euthanized to verify the completeness of the vagal transections. Because the assessment of vagotomy can be difficult (19,22), two criteria were established, both of which had to be met. First, a microscopic examination was conducted for the presence of any fiber connections between the cut ends (10,27). The discovery of any connection was considered grounds for rejection. And, second, a stomach weight/body weight ratio (4,20) of 0.03 was selected as the minimum acceptable, as it was a ratio that none of our pilot sham-vagotomized rats achieved. Ten rats were removed from the study for failing to achieve a ratio of 0.03; two of these also were found to have connecting fibers during postmortem examination.

RESULTS

Since sham-vagotomized and vagotomized rats were tested separately, the sham-vagotomy and vagotomy conditions were also analyzed separately as $2 \times 2 \times 2$ ANOVAs: drug (morphine, saline) $\times$ enhancer (placenta, beef) $\times$ time, with repeated measures on the time variable.

The results obtained from the sham-vagotomized rats are presented in Fig. 1.

The ANOVA revealed a significant drug $\times$ time interaction, $F(1, 36) = 4.58$, $p < 0.05$, and a significant main effect of enhancer, $F(1, 36) = 4.83$, $p < 0.05$. Probes were then conducted of the interaction. They revealed, first of all, that the effect of morphine (mean percent change from baseline = +10.49 $\pm$ 2.83%) on the sham-vagotomized rats was significant, $F(1, 36)$ = 4.61, $p < 0.05$, but the effect of saline (+4.84 $\pm$ 2.15%) was not ($p > 0.05$). The analysis of the data acquired from the rats prepared without surgery (nonincision control) uncovered no significant effect of 1 mg/kg morphine, either alone or in any

interaction. In addition, when the effect of morphine on the two groups was compared, the mean percent change from baseline of the sham-vagotomy group (+10.49 $\pm$ 2.83%) was significantly higher than that of the nonsurgery group (+4.37 $\pm$ 1.66%), $t(29)$ = 1.93, $p < 0.05$. These findings suggest that it was the surgery itself, rather than another aspect of our procedure (e.g., anesthesia), that was responsible for the discernable effect of the low dose of morphine in the sham-vagotomy group. This interpretation is consistent with other studies that have found increases in endogenous opioids in response to the stress of surgery (21,28).

The probes also revealed that the effect of drug was significant at T_{10} (+13.17 $\pm$ 3.61%), $F(1,36)$ = 4.80, $p < 0.05$, but not at T_{35} (+7.80 $\pm$ 2.70%, $p > 0.05$). This appears to have been due to a dissipation in the effectiveness of morphine over time and was particularly apparent in group M/B (T_{10} = +8.39 $\pm$ 3.03%; T_{35} = +1.60 $\pm$ 2.29%).

As to the main effect of enhancer, rats fed placenta clearly showed greater increases from baseline (+11.38 $\pm$ 2.93%), and therefore higher pain thresholds, than did rats fed beef (+3.95 $\pm$ 1.86%). The interaction of placenta ingestion with morphine injection did not reach statistical significance, as it had in other investigations conducted in our laboratory (15,17,18), probably due to the variability contributed by the apparently elevated endogenous opioids. Nevertheless, the effect of enhancer in the sham-vagotomized rats provided a criterion against which we could compare the results obtained from the vagotomized rats (see Fig. 2).

The ANOVA performed on the data from the vagotomized rats revealed only a significant main effect of time, $F(1, 36)$ = 4.95, $p < 0.05$. In contrast to the sham-vagotomy condition, no effect of enhancer was uncovered, either as a main effect or in an interaction with morphine. Therefore, placenta ingestion did not enhance TFL in morphine-treated rats that had undergone gastric vagotomy.
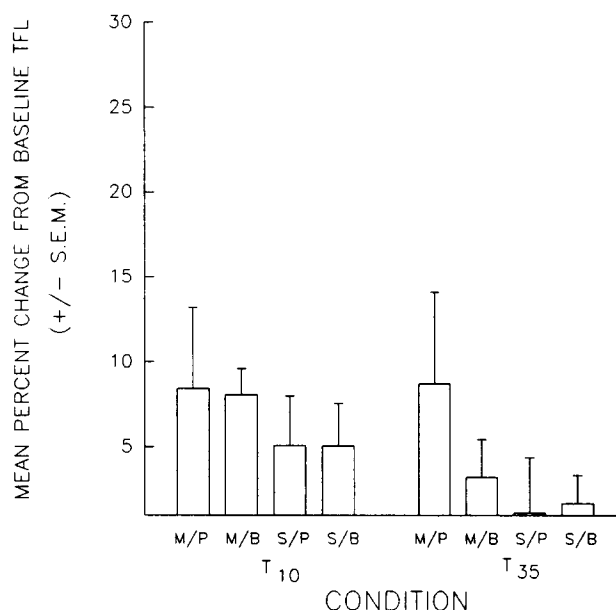

FIG. 2. Mean percent change from baseline tail-flick latency ($\pm$SEM), at the 10-min (T_{10}) and 35-min (T_{35}) tests, of rats receiving gastric vagotomy. M/P = morphine + placenta; M/B = morphine + beef; S/P = saline + placenta; S/B = saline + beef.

DISCUSSION

The data clearly show that enhancement of opioid-mediated analgesia by placenta ingestion, and therefore presumably POEF ingestion, requires an intact gastric vagus nerve. We have hypothesized that afferent vagal information, acting on the brain, provides the critical link between the gastrointestinal tract and the central nervous system by which ingested POEF influences the central analgesic actions of opioids. However, an alternative explanation, that the effect of vagal efferent influences on digestion might play a role in the creation or activation of the POEF molecule, has not been ruled out. We are currently conducting a study designed to examine this alternative.

There did appear to be one suggestion, in the present study, that the enhancer (POEF) might have had some effect in the absence of vagal participation. Rather than decreasing, the pain threshold (the mean percent change from baseline TFL) for vagotomized rats in group M/P remained constant from T_{10} to T_{35} ($T_{10} = +8.41 \pm 4.78\%$; $T_{35} = +8.68 \pm 5.40\%$). The amount of variability in the data precludes any firm conclusion, but this might be an indication that although placenta did not enhance analgesia in these rats, perhaps it helped to sustain it. This could have occurred through a nonvagal, possibly humoral mechanism.

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