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Milk-Induced Analgesia and Comforting in 10-Day-Old Rats: Opioid Mediation

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BLASS, E. M. AND E. FITZGERALD. *Milk-induced analgesia and comforting in 10-day-old rats: Opioid mediation.* PHARMACOL BIOCHEM BEHAV **29**(1) 9–13, 1988.—The effects of slow intraoral milk infusions on the opioid-mediated behaviors of ultrasonic vocalizations and paw removal from a hot plate (48–49°C) were evaluated in 10-day-old rats. Milk reduced distress vocalization by circa 30% while increasing paw lift latencies by about 60%. Alterations in both behaviors were fully reversed by naltrexone (0.5 mg/kg) pretreatments. These data demonstrate the calming and analgesic effects of milk. Implications for a possible role of opioid peptides in mother-young relationships are discussed.

Opioids Milk Analgesia Stress reduction

OPIOID systems' development provides neuroscientists with an excellent opportunity to relate the ontogeny of structure to that of function [2, 7, 14–16, 29]. Recently this relationship has been expanded to behaviors during ontogeny that may have an opioid basis [4–6, 15, 23]. For example, opioid based pain and stress sensitivity have been demonstrated in infant rats [16,27], chicks [24,26] and puppies [25] among others [10,11]. Specifically morphine injections elevate infant pain thresholds [17], appear to palliate stress caused by maternal separation [18] and can serve as a reward for infant classical conditioning [16,28]. These behavioral alterations are blocked by naltrexone.

Blass, Fitzgerald and Kehoe [5], in studying 10-day-old rats, demonstrated behavioral parallels between oral sucrose infusions and morphine injections. Specifically, 3.5, 7.5, or 11.5% sucrose infusions caused markedly elevated paw lift latencies and diminished level of ultrasonic distress vocalization. Both of these alterations were fully normalized by pretreatment with the opioid antagonist naltrexone. The current study exploits these paradigms to assess the effects of milk on distress and pain reactions.

Identifying the animals' responses to milk goes beyond cataloguing substances to which there is opioid sensitivity. Milk is especially significant because it is the substance that the mother deposits with her infants at the time that she leaves the nest. Therefore potential changes in stress or pain responsivity are ecologically significant. Alterations in coping caused by milk may potentially influence the spontaneous rate of distress vocalization emitted by infant rats when they are left by the mother [3, 12, 13]. Modulation of distress vocalization may influence maternal return to the nest or may have deleterious consequences to the infants by advertising their location to predators [1,3].

Accordingly, the current experiments assess infant rats' responses to milk, infused intraorally, and evaluate whether stress amelioration caused by milk infusion is naltrexone reversible. This study, therefore, specifically determines, against cannulated control and unoperated rats, the effects of intraoral infusions of milk on ultrasonic distress vocalization and paw lift latencies. It also evaluates whether differences resulting from intraoral milk infusions are opioid based by injecting the animals with naltrexone (0.5 mg/kg b.wt.) or isotonic saline prior to milk infusions.

METHOD

Subjects

Subjects were derived from Sprague-Dawley female rats, raised in our colony, and outbred with Charles River males obtained from Camm Research Laboratories (Wayne, NJ). Pregnant females were individually housed during the last trimester in plastic cages (38×30×77 cm) that were covered with stainless steel wire lids. The lids always held Purina laboratory pellets. Water was available either through a bottle, accommodated by the lid, or through an automatic watering system that protruded through the front of the cage. The floor of each cage was covered with a layer of wood chips. Temperature was maintained at a constant of 25°C, with humidity uncontrolled. Lights in the colony room were on from 0700 to 2100 hours daily.

As term progressed, females were checked for birth daily, late in the afternoon. Pups then discovered were considered to have been born on that day and accordingly designated as 0 days of age; on the day following birth, they were considered to be 1 day of age, etc. Litters were culled to ten pups on the day after birth. Pups 10 days of age weighing between 19–28 grams served in these experiments. Each pup was studied once only. Each litter contributed only one pup/experimental condition.

Surgical Procedure

Intraoral jaw cannulae were constructed from a 10 cm length of PE 10 intra-medic polyethylene tubing by flanging one end of the tube with heat as described by Hall [8]. Implantation was achieved by using a curved length of piano

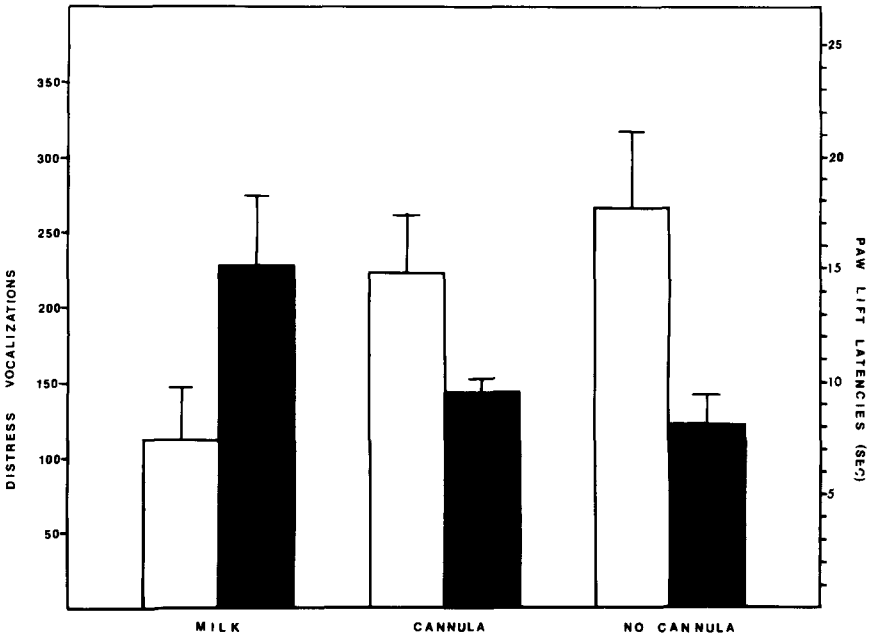

FIG. 1. Mean number of distress vocalizations (open columns) and mean paw lift latencies of 10-day-old rats receiving milk infusions, cannula implanted rats or unopened control rats.

wire. One end of the piano wire was friction-fit to the cannula, the other inserted beneath the tongue and maneuvered out of the animal's ventral jaw surface. This surgical procedure was completed in about 20–25 seconds and was conducted without pup anesthetization.

Testing Environment

All testing was conducted in an environmentally controlled chamber (Forma Scientific Inc.) that was adjusted to maintain a constant temperature of 32.5°C at circa 90% humidity. For recording paw lift latencies we followed the procedures of Kehoe and Blass [17] in which a stainless steel plate (maintained at 48–49°C) was connected in series to a variable DC power supply and clock/counter that was accurate to 0.01 sec (Lafayette Instrument Co.). The pup's right forelimb, two hind limbs and trunk were firmly but gently supported in the experimenter's right hand. This allowed the animals left paw to contact the hotplate surface thereby activating the timer. The timing circuit was broken by paw lift and this score constituted the paw lift latency (PLL), one dependent variable.

Ultrasonic distress vocalizations (DV) were recorded with the aid of a Holtage bat detector (set at 42 Hz). A pup was separated from its littermates six hours after surgery. The PE 10 cannula was linked by PE 50 tubing to an infusion pump and the rat was placed in an isolated container (6.5″ diameter, 4″ high) lined with clean chips. The infusion pump was activated as appropriate and distress vocalizations were recorded for 8 min. Infusions were presented as a continuous flow for 3 min 20 sec during which time a volume of 0.2 cc's of commercially available milk, warmed to body temperature, was delivered. This volume represented approximately 1% of the animal's body weight. All of the infused milk was swallowed by the pups.

Procedure

Forty-eight pups 10 days of age were studied. Each pup was randomly assigned to one of six conditions that were derived from two drug treatments (naltrexone vs. saline) and three experimental manipulations: milk infusion, cannulae implantation and no surgical intervention. No two pups in any given litter were ever assigned to the same condition. The pups received their cannula or were handled 2–6 hours prior to testing and returned to their mother. All pups were separated from the mother 15 minutes before the start of the session. They were group housed in a Plexiglas bin, lined with soiled nest chips, and were allowed to acclimatize to the test chamber. Each pup was injected at that time with either naltrexone or isotonic saline. Fifteen minutes after injection the pup was connected to the infusion pump and the pump was turned on for animals receiving milk. The pup was placed in the isolation container for 8 minutes and its distress vocalizations recorded on a minute by minute basis. Each pup was then tested for withdrawal from the hot plate. The infant's snout and ventral temperatures were determined and that of the hot plate verified. To control for potential experimenter bias one experimenter controlled the substance that was utilized and another tested the animal.

Statistical Evaluation

Statistical evaluation consisted of two way analysis of variance single measure design and one way analysis of variance [9].

RESULTS

A 1% body weight continuous infusion of commercial milk into the mouth of 10-day-old rats caused two marked alterations in behavior (Fig. 1). First, distress vocalizations were reduced by more than 50% in animals that received milk infusions relative to operated control rats and by almost

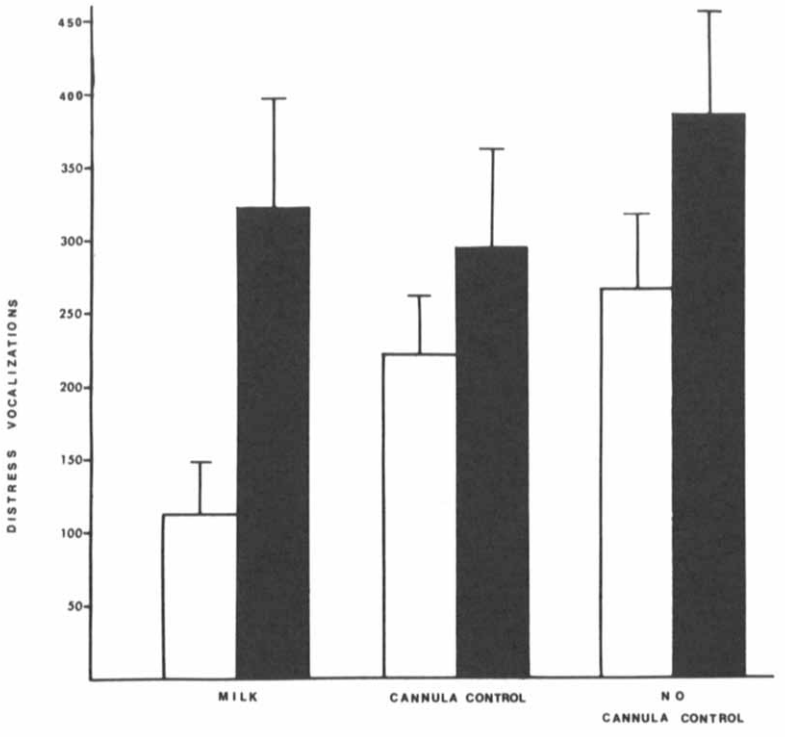

FIG. 2. The effects of naltrexone on levels of distress vocalization in milk-infused, operated control and non-operated rats. Open columns: saline, naltrexone: dark column.

67% relative to unoperated control rats, $F(2,21)=3.48$, $p<0.05$. Second, a complementary profile was obtained for paw lift latencies. Rats receiving milk had a mean paw lift latency of 15.7 sec. This contrasts with the 9.4 and 8.1 sec paw lift latencies for the two control groups. Decreases in DV and increased paw lift latencies by experimental relative to control groups is reminiscent of the profile obtained by Kehoe and Blass [17] in rats injected with morphine.

Figure 2 demonstrates the effects of naltrexone on distress vocalizations in milk-infused rats and in the two control groups. According to analysis of variance there was a main treatment effect, $F(1,42)=8.26$, $p<0.01$. Naltrexone almost trebled the level of distress vocalization in milk-infused rats and increased distress vocalizations in non cannulated rats by about 33%. The effect in the cannula-control animals was considerably more modest. To the point of the current experiment, naltrexone reversibility of distress vocalizations to normal levels in milk-infused rats suggests that the effects of milk infusion may have an opioid basis. This essential phenomenon was also achieved in paw lift latency: naltrexone reduced mean PLL of milk-infused animals from 15.7 (±2.5) to 11.2 (±1.5) seconds and reduced cannula control rat PLL from 9.4 (±1.1) to 6.6 (±0.7) sec. The no-cannula-control rats did not change their latencies: 8.1 (±0.4) without naltrexone to 8.5 (±0.8) sec with naltrexone. This last finding is not fully consistent with our previous findings that baseline paw lift latencies in nonoperated isolated rats were naltrexone-attenuated. PLL increases owing to stress [17], intraoral sugar infusions [4], or morphine injections [17,19] are all naltrexone reversible, thereby implicating opioid mediation of these increases.

Figure 3 demonstrates on a cumulative minute by minute basis the effects of milk on distress vocalization and its reversibility by naltrexone. The delayed DV reduction by milk (left panel) offers insights into its mechanism of action. It was not until the third (i.e., last) minute of infusion that milk-infused rats differed from control rats. The differences increased over time.

Milk-infused animals essentially stopped calling while DVs in control rats increased linearly. The delayed onset suggests a post ingestive contribution to distress vocalization reduction. The right panel demonstrates that rats receiving intraoral milk infusions exhibited sustained calling when the infusions followed naltrexone injections. Similar effects were obtained in our previous studies with rats receiving sucrose intraoral infusions that were preceded by naltrexone injections [5].

In short, intraoral milk infusions elevate paw lift latencies and decrease ultrasonic distress vocalizations. Both of these effects are reversed by naltrexone administration. The effects are not due to infusion *per se* because water infusions into the oropharynx do not affect either distress vocalizations or paw lift latencies [5]. Furthermore, because inhibition was protracted well after infusion termination, we conclude that the infusion did not interfere with distress vocalizations.

DISCUSSION

These findings extend the range of substances that wh. injected into the mouth of infant rat pups: (a) reduce distress vocalizations, (b) enhance paw lift latencies, (c) have these effects reversed by opioid antagonists. Milk is particu-

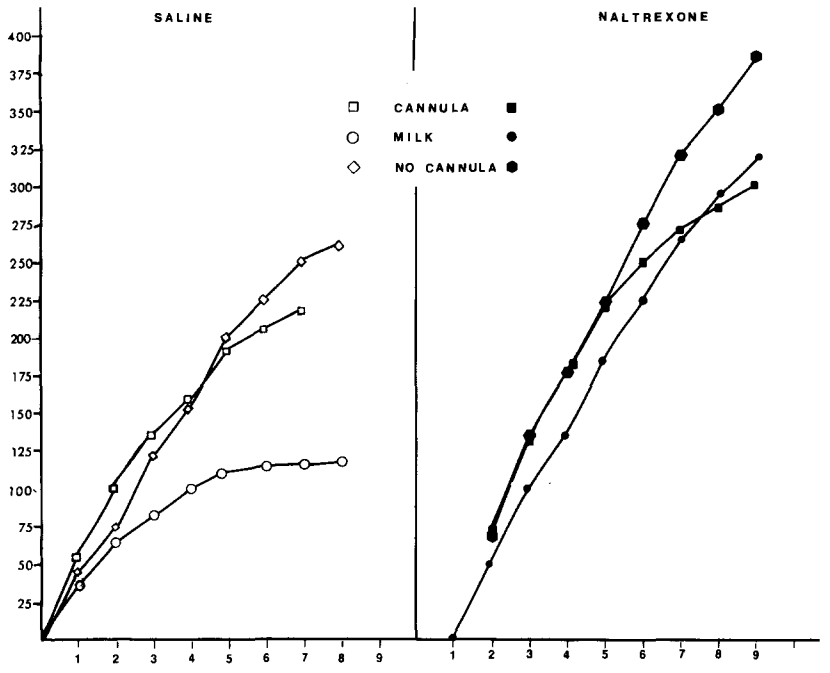

FIG. 3. Cumulative distress vocalizations in rats pretreated with isotonic saline or naltrexone (0.5 mg/kg). Infusion duration was 3 min 20 sec.

larly interesting developmentally because of its centrality as the infant's sole nutritional and hydrational source. The current report of milk's quieting effects during maternal separation provides it with additional immediate significance. We are currently determining whether mother's milk delivered through the natural mode of suckling also has quieting and analgesic properties.

It is informative to contrast the effects of milk and sucrose intraoral infusions. Milk quiets differently than sucrose. This sugar's quieting starts immediately upon delivery and does not require a latency of two to three minutes before it appears. Whether the milk delay reflects a different and slower class of taste receptors (e.g., fats) or whether the mechanism is gastric or beyond, is currently under investigation. Note, however, that quieting for both milk and sucrose endures beyond infusion termination.

Expanding the range of food substances that appears to cause opioid release starts to catalogue the classes of foods that cause changes in the properties of stress and pain systems. The relationship identified here is reciprocal because feeding is enhanced under certain stressful conditions [22]. The two substances selectively eaten, sweets and fats [4, 20, 21], have now been demonstrated behaviorally to cause endogenous opioid release ([5], Shide and Blass, unpublished observations February 1987). Thus, substances differentially selected and consumed also differentially influence distress systems [7]. This reciprocity has obvious significance for our understanding of normal motivational and affective interactions as well as for understanding unusual behaviors presented by clinical populations with affective feeding disorders.

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