Milk-induced Hypoalgesia in Human Newborns

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ABSTRACT. ***Objective.*** **To determine whether milk and its components reduce crying in newborns during and after blood collection for phenylketonuria evaluation.**

Methodology. **Seventy-two normal newborns ingested 2 mL of milk (Similac), Ross Special Formula, fat, protein, lactose, sucrose, or water for the 2 minutes preceding blood collection via heel lance. Crying duration during and for the 3 minutes after the procedure was determined by scorers who were blind to the ingestive substance.**

Results. **Sucrose and Similac each reduced crying during the blood collection procedure. Sucrose, fat, protein, and Ross Special Formula were effective during the 3-minute recovery period. Neither water nor lactose were effective during or after blood collection.**

Conclusion. **Milk and some of its components are antinociceptive in human newborns. Based on previous studies, reduced crying during and after painful stimulation may be mediated through endogenous opioids. These findings are of potential clinical significance: natural protective mechanisms, normally engaged during suckling, may safely and noninvasively be activated to reduce newborn crying to painful stimulation.** ***Pediatrics*** **1997;99:825–829;** ***fat, hypoalgesia, infants, milk, opioids, protein.***

ABBREVIATION. RSF, Ross Special Formula.

Suckling, the defining mammalian behavior, is the vehicle for infant nutrition.[1] Suckling also reduces energy expenditure[2] through reduced crying,[3–8] heart rate,[9] gross motor activity,[9] and decreased reactivity to noxious stimulation.[8,10,11] The last attribute is the focus of this report, which determines whether milk and its components are also antinociceptive in human newborns.

Parallel studies in rat and human infants have revealed features shared by both species concerning pain reduction and its underlying mechanisms. In both species nonnutritive suckling is extremely antinociceptive.[8,10,12] Furthermore, sugars tasted in volumes of 250 to 400 μL reduce spontaneous crying[4–7] and reduce crying during circumcision or blood collection via heel lance.[8,11] Chemosensory-induced antinociception in rats is naltrexone reversible, implying mediation through opioid systems, probably through μ receptors.[5,14,15] In this regard, crying was not reduced by sucrose[9] in infants born to women who were maintained on methadone during pregnancy, who might be expected to have lower levels of circulating endorphins.[16,17] A ramp-like time course characterizes sweet-induced changes that can last for up to 7 minutes in human[7] and rat[15] infants. Extended change cannot be attributed to lingering aftertaste because sucrose most effectively reduced crying when delays were imposed between ingestion and testing.[11,14]

Intraoral infusions of milk also elevate pain threshold in rats,[5] although not as much as the sugars. Milk's effectiveness in rats is probably through its fat moiety because infusions of corn oil reduce distress (ultrasonic) vocalization in 10-day-old rats and lengthen escape latencies from a 48°C surface.[15] These reactions to milk and corn oil are also naltrexone reversible. Interestingly, lactose, the milk sugar, does not reduce crying in human infants in a concentration range of .17 to .51 M[18] or influence heat-escape latencies in rats, even at concentrations threefold that found in milk.[13]

The current studies determine whether milk and its components, alone or in combination, reduce newborn crying during and after the heel lance procedure for withdrawing blood for phenylketonuria evaluation. Accordingly, human newborns were allowed to taste 2 mL of sucrose, commercially available milk (Similac), a special formula prepared by Ross Laboratories, or its constituents of fat, protein, or lactose for 2 minutes preceding blood collection. Changes in crying caused by these substances were evaluated against water infusions, which have previously been demonstrated not to have a hypoalgesic effect.[8,11]

Based on the findings cited above, we predicted that sucrose would be the most effective substance; milk and Ross Special Formula (RSF) would also attenuate crying, as would fat. Neither water (relative to nontreated infants from previous studies) nor lactose should reduce crying either during blood collection or during the 3-minute period after blood collection. No predictions could be made concerning the efficacy of protein on the basis of previous studies.

METHODS

Subjects

A total of 72 infants born in the Tompkins Community Hospital, Ithaca, New York, were randomly assigned to nine experimen-

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tal groups. All infants were delivered vaginally. Sixty-one were white, 8 Asian, and 3 black. Forty-two of the infants were female. Birth weights ranged between 2976 and 3697 kg, with a mean of 3321 kg. All infants had Apgar scores of 8 or above 1 minute after delivery, and 9 or above at 5 minutes. Body weights were equivalent among the experimental groups as were gender and racial distributions. Infants were between 22 and 40 hours old when their heels were lanced for the standard blood collection for phenylketonuria evaluation. Data were collected between 7 AM and 8 AM with the assistance of a single nurse to minimize variability. Infants had not eaten for 2.5 to 3.0 hours and were almost always asleep at the time of the experiment.

No mother had received meperidine or other morphine-like substances during delivery or during the postdelivery period if nursing. Fifty-eight of the 72 infants were breastfed and were essentially equally distributed among the nine groups. No infants were on special diets. An infant was enrolled in the study only after a parent, generally the mother, provided informed written consent; 68% of the parents approached did so. The study and consent forms were approved by the human subjects committees of both Cornell University and the Tompkins Community Hospital.

Eight infants served in each condition, namely, sucrose, water, protein, lactose, dilute fat, concentrated fat, fat/lactose, Similac, and RSF. Each infant was studied as shown by the time line in Fig 1. Seven minutes before blood collection, the infant's foot was placed in a heated matrix, thereby facilitating circulation at the heel, the source of blood collection. Three minutes later, the infant drank exactly 2 mL of its designated solution through a sterile plastic syringe for 2 minutes. An additional 2 minutes elapsed after delivery, during which time the nurse prepared the surgical procedure and removed the matrix from the infant's foot. The procedure itself took place with the infant lying supine in his or her bassinet. For scoring purposes the procedure was considered to start when the nurse squeezed the infants heel immediately before heel-stick, and to end when a Band-Aid was applied to the wound and the foot released. Three to 6 minutes were necessary for blood collection. Mean collection times did not differ significantly among groups, nor did the number of heel-sticks. The entire session from solution administration to 3 minutes after Band-Aid application to the heel was recorded via videotape and later scored for crying. Neither the nurse, who was not present during fluid delivery, nor the person scoring the videotape were informed about experimental condition. Except for Similac, which is opaque, syringe contents were clear and, therefore, unknown to the experimenter. Fluids were delivered at body temperature and prepared as follows:

Sucrose, 12%, was prepared on a weight/volume basis by adding sterile water to 6 g of sucrose until a volume of 50 mL was achieved.

Protein was prepared by adding 35 mL of water to 7.5 mL of protein (Provimin), a gift from Ross Laboratories, and vigorously agitating the mixture.

Lactose was of a 7% concentration, ie, the physiological range, and was prepared by adding water to 3.5 g of lactose until 50 mL was achieved. We did not use a more concentrated lactose solution. In our experience human newborns have found more concentrated solutions aversive, as judged by increased crying and grimacing.[18]

Dilute fat was composed of 1.85 mL of a coconut and soy oil blend mixed with 48.15 mL of water and agitated until homogeneity.

Concentrated fat was composed of 3.7 mL fat and 46.3 mL water. This concentration was apparently slightly aversive to the infants because 5 of the 8 infants in this group cried during the 2-minute interval between consuming the fat and the onset of the procedure. Of the remaining eight groups, only 1 infant cried in each of five groups, 3 each in the protein and Similac groups, and 2 infants cried after receiving the fat/lactose mixture.

Fat/lactose solution was made by combining 3.5 g lactose and 1.85 mL fat, adding water until 50 mL was achieved, and vigorously agitating the solution.

RSF was fabricated according to the percentage of each component that is found in human milk. Accordingly, the recipe of Ross Laboratories was followed, namely, adding water to 7.5 mL protein (Provimin), 3.24 g lactose, and 1.85 mL fat until 50 mL were reached and the entire mixture was agitated vigorously by sonication for 5 minutes.

Similac, a gift from Ross Laboratories, was also warmed to body temperature and presented to the infants as above.

Crying was declared to occur when a cry sound was detected. We did not distinguish among different crying intensities. In actuality crying was intense, almost always commencing when the heel was squeezed by the nurse in preparation for collection. Intensity increased when the heel was lanced.

Statistical Evaluations

Because the primary goal of these studies was to evaluate the effectiveness of milk and its individual components in reducing crying during and after blood collection, the sequence of planned comparisons was sucrose vs water, Similac vs water, and RSF vs water. Individual component comparisons were to be made against water if the milk or special formula evaluation was statistically reliable. Because of the relatively small number of subjects in each group and the lack of normal distribution of scores, the Mann-Whitney *U* statistic was chosen to determine statistical reliability during blood-harvesting. Also, because the collection procedure was of a somewhat variable duration, the percentage of time spent crying during the procedure was used for statistical evaluation. All probability values are presented after Bonferoni corrections for multiple tests.[19]

The effectiveness of the different substances in relieving crying during the recovery period was evaluated statistically via *t* test for difference scores in crying between the first and third minutes after treatment. This is justified by the protracted effects of chemosensory stimulation in both rat[5,13,14] and human infants.[6,7,18] This comparison also acknowledges the possibility that substances could become effective against diminished stress due to the passage of time since blood collection.

RESULTS

Overall, the findings support the predictions made at the outset of the study. Sucrose most effectively reduced crying both during the treatment and recovery phases. Neither lactose nor water were effective in either phase. Similac significantly reduced crying during treatment, but, unexpectedly, not during the posttreatment interval. Fat solutions, protein, and RSF reduced crying during recovery but not during blood collection itself. Thus, milk and some of its components can reduce crying, but not during intense acute stimulation.

Mean amount of crying for infants in each group during blood collection is presented in Fig 2 as a percentage of treatment duration. Infants who received water before heel lance cried during 92% of the procedure. This is comparable to our previous

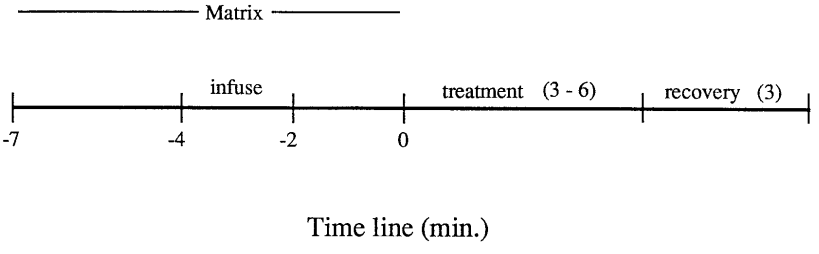

Fig 1. Time line depicting the experimental protocol followed in this experiment, starting 7 minutes before blood collection, when a heated matrix was placed on the infant's foot, and terminating 3 minutes after the collection procedure.

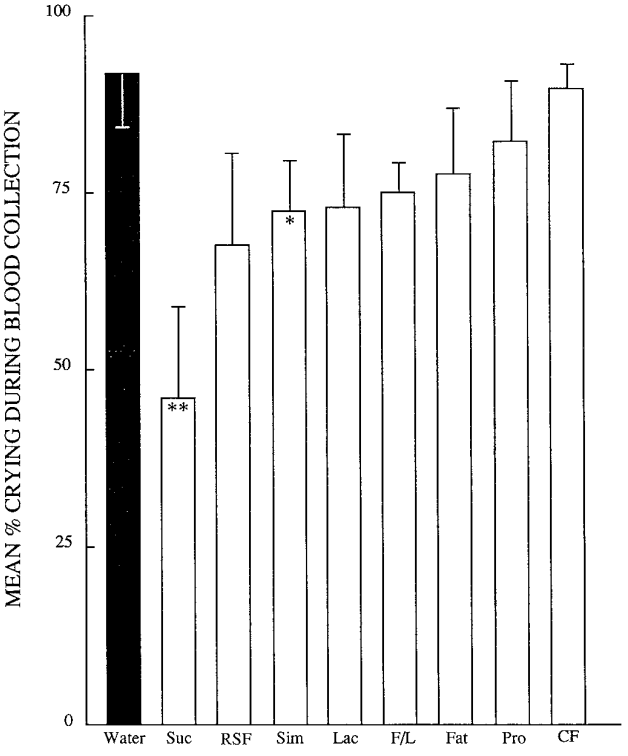

Fig 2. Mean percentage (± standard error) of crying during blood collection procedure of newborns who had received 2 mL of either water, sucrose (Suc), Ross Special Formula (RSF), Similac (Sim), lactose (Lac), fat/lactose (F/L), fat, protein (Pro), or concentrated fat (CF) 2 minutes before blood collection (*P = .038; **P = .015).

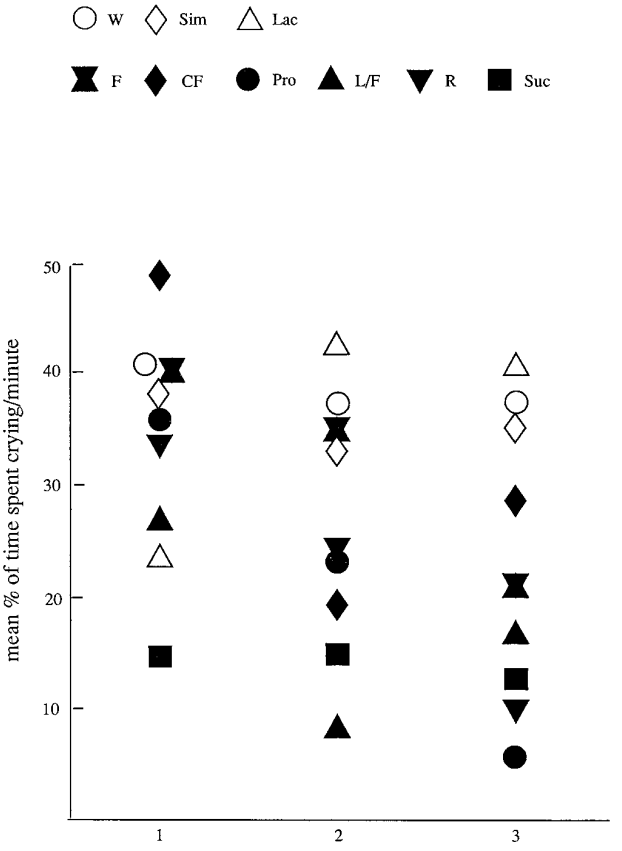

Fig 3. Mean amount of crying each minute for each group of infants during the 3-minute recovery period. Closed symbols represent groups for whom treatment reduced crying (statistical values in text); open symbols represent ineffective treatments. W indicates water; Sim, Similac; Lac, lactose; F, fat; CF, concentrated fat; PRO, protein, F/L, fat/lactose; RSF, Ross Special Formula; and Suc, sucrose.

findings[8,11] and to the amount of crying of infants treated in the standard fashion without any intervention.[8,11] Thus, the ingestive act per se did not reduce crying under the present circumstances of foot restraint, squeezing and heel lance. In contrast, infants who received sucrose cried only 47% of the time during blood collection, a value in keeping with earlier reports.[8,11] This reduction is statistically reliable (Mann-Whitney U = 8, P = .015). Indeed, 3 of the 8 infants who drank sucrose did not cry at all during the procedure. Similac was the only other substance that reliably reduced crying relative to infants who had received water. Infants who received Similac cried a mean of 72.6% of the time. This reduction was statistically reliable as well (U = 12, P = .038). Comparisons of the individual components did not attain conventional levels of statistical reliability. Two comments are noteworthy in this regard. First, the number of subjects in each group was small so some of the differences may have reached statistical significance with larger numbers in each group. Second, as opposed to sucrose, which reduced crying by 50%, relative to water, during the acute pain of blood collection, crying reductions with milk or its components were rather modest. Infants in the latter conditions cried only 5% to 25% less of the time than infants who had received water.

Milk's components were variably effective in reducing crying during recovery (Fig 3). There was essentially no reduction in the mean amount of crying for infants in the Similac and water groups across the 3-minute recovery period. Lactose-treated infants appeared to cry more as the recovery period progressed, but this difference did not reach statistical reliability.

As represented by the filled characters, both fat concentrations and protein all reduced crying during recovery from heel lance (corrected $P < .02$ and .008, respectively) as did RSF ($P < .01$). Of the 40 infants in the fat, protein and RSF conditions, 37 reduced crying between the first and third minutes. This may be contrasted with the 24 infants in the water, Similac, and lactose conditions, of whom 11 actually increased crying during recovery.

Fig 4 presents the number of infants in each condition who cried for 20% or more of the time during each recovery minute. A clear pattern emerges that conforms well with the predicted outcome of water and lactose ineffectiveness and RSF, fat, and maximum sucrose effectiveness (no prediction was made concerning protein). Based on these predictions, the probability (binomial) of the distribution (Fig 4) occurring by chance was $P < .004$. Only infants who received Similac did not behave as predicted.

In summary, these findings suggest that Similac, RSF, and lactose may have been marginally effective during the acute stress of blood-harvesting (Fig 2). Fat, RSF, and protein all reduced crying after the procedure when, presumably, the pain and stress

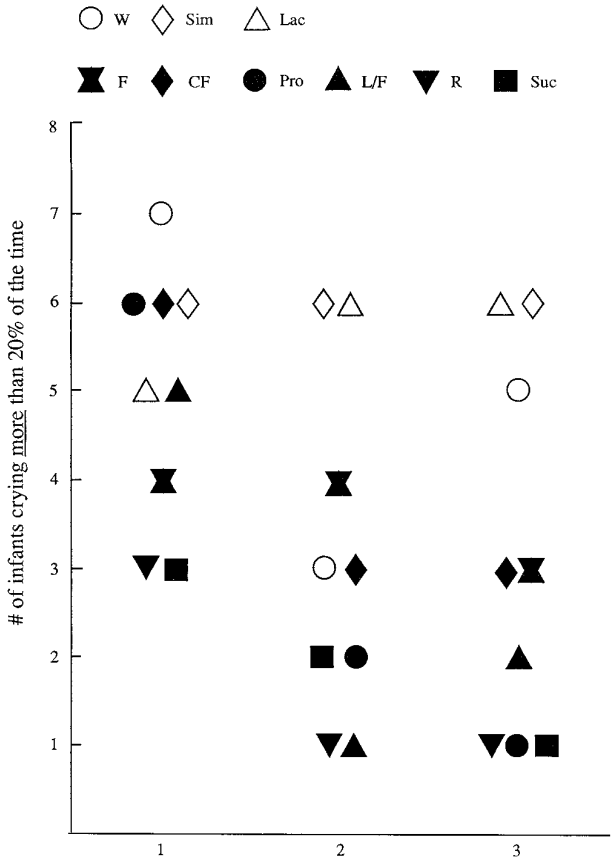

Fig 4. The number of infants in each group who cried for 20% (12 seconds) or more of a given minute during recovery (symbols as in Fig 3).

associated with the blood-harvesting procedure had somewhat lessened. The characteristics of protein as an antinociceptive agent (ie, one combating the general and specific stresses of the procedure) and their underlying mechanisms require further investigation.

DISCUSSION

These findings provide the first evidence for milk-induced antinociception in human newborns. They raise the possibility that the mechanisms underlying sweet-induced antinociception are also mobilized by milk's flavor and that of some of its constituents. Thus, mother's milk itself may be quieting and antinociceptive. Indeed, according to Barr and colleagues,[20] mature breast hindmilk significantly reduced spontaneous crying in 10-day-old infants. Milk flavor, like sweet taste, may be operating through an opioid pathway. Consistent with opioid mediation, sucrose, protein, fats, and RSF all reduced crying during recovery, ie, after 5 to 8 minutes had elapsed since fluid ingestion. Moreover, in rats, milk's and fat's antinociceptive actions were naltrexone reversible.[15]

Lactose ineffectiveness warrants discussion. The current findings are the fourth data set in which lactose was ineffective against either pain or spontaneous crying in isolated rat or human infants.[7,13,16] Because lactose did not potentiate crying reduction by fat during recovery, it is unlikely that lactose, albeit without effect of its own, might enhance fat's action. Moreover, for both isolated rat and human infants, lactose did not reduce vocalization even at concentrations that were twice or three times the physiological range.[7,13,16] This is striking given the ability of other sugars and fats to reduce spontaneous crying, that elicited by noxious stimulation, and, in rats, to increase heat withdrawal latencies. Together these findings suggest that lactose is not a factor in milk's combating stress that infants may experience during the natural course of events.

Protein reduction of crying after blood collection was not predicted. We are unaware of any experimental precedents upon which to base protein's mechanism of action, but given that sucrose, milk, and fat act through opioid mediated pathways, in rats at least, so too may the chemosensory changes initiated by protein. This too awaits empirical validation.

These findings of relatively modest milk effects against more profound sugar actions may appear paradoxical at first because sucrose is not a milk constituent. The paradox is more apparent than real, however, when considering that taste-induced hypoalgesia is not limited to infancy[21] and may continue to serve broad regulatory functions after weaning. Within this context, orogustatory stimulation lowers heart rate,[9] reduces activity,[9] and causes a 13% reduction in energy expenditure.[2] Indeed, one is struck by the linkage between meal termination and lethargy or sleep induction: hypoactivity is considered to be one defining characteristic of satiety.[22]

The effectiveness of milk, some of its components, sweet sugars, and sucking itself against pain in rat and human infants, their ease of administration, and their safety are of clinical interest. These noninvasive approaches seem to engage natural antinociception systems without apparent negative consequences. Alone,[10] or in combination,[8] they may provide relief during mild to moderately painful events of the newborn period.

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