Early Human Development 115 (2017) 32–37

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Myristic acid in amniotic fluid produces appetitive responses in human newborns

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ARTICLE INFO

Keywords:
Appetitive behavior
Fatty acids
Myristic acid
Newborn recognition
Prenatal learning
Olfactory stimulation

ABSTRACT

Background: A mixture of eight fatty acids (lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, elaidic acid, and linoleic acid) that are contained in human amniotic fluid, colostrum, and milk produces appetitive responses in newborns, suggesting the existence of a transition of sensorial cues that guide newborns to the maternal breast.
Objective: To explore the ability of each of these eight fatty acids individually to produce appetitive responses in newborns.
Methods: The study included 12 healthy human newborns < 24 h after birth. Using a longitudinal design, cotton swabs that were impregnated with each of the eight fatty acids and control substances (i.e., vehicle, saline, and vanilla) were placed approximately 1 cm from the newborns' nostrils for 30 s. Positive responses that were suggestive of acceptance included appetitive movements (i.e., suckling) and sniffing that were directed toward the cotton swab. Lateral movements of the head away from the swab were considered negative responses. Remaining stationary with no changes in facial expressions was considered indifference.
Results: Compared with controls (i.e., vehicle, saline, and vanilla) and the other fatty acids tested, myristic acid produced the longest duration of positive facial responses (suckling and sniffing). No significant differences in negative facial responses were observed in response to the odoriferous stimuli. No reactions that were suggestive of disgust were observed.
Conclusion: A complex combination of stimuli, including the odor of myristic acid, may integrate sensory cues that guide newborns to the maternal breast.

1. Introduction

Among the signaling systems, chemical cues that consist of pheromones [1] can cause notable behavioral responses, including anxiety [2], when perceived by other individuals in the group. The opposite is also true. Some pheromones can act as cues that indicate the existence of a safe environment [1] by informing other individuals of the same species about the absence of danger or presence of food through sensorial systems. Some chemical cues begin to achieve salience in the intrauterine milieu before birth under two conditions: a functional sensorial system and the presence of such cues in the intrauterine milieu (i.e., amniotic fluid). Such prenatal training may guide newborns to similar substances that are contained in colostrum and milk, acting as a guide to the maternal breast and nursing.

In humans as early as the 24th gestational week, the olfactory mucosa is well developed [3] and contains ciliated olfactory receptors that have a mature appearance [4]. Olfactory marker proteins (an indicator of neuroreceptor functionality) [5] and connectivity with mitral cells in the main olfactory bulb [6] are present in epithelia by the 28th gestational week and in the main olfactory bulb between the 32nd and 35th gestational weeks [7]. Similarly, at approximately the 12th gestational week, the human vomeronasal organ is well developed [8] and visible in the developing fetus [3,9,10] and newborns [11]. From an anatomical perspective, human fetuses have a reasonably well developed olfactory system before birth.

Some specific components of the interphase between mothers and newborns (e.g., amniotic fluid, colostrum, and milk) may serve as sensorial cues. Shortly after birth, human newborns and other mammals display movements of the head toward the maternal mammary gland [12,13], its own amniotic fluid odor [14–16], and maternal

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http://dx.doi.org/10.1016/j.earlhumdev.2017.08.009
Received 22 June 2017; Received in revised form 18 August 2017; Accepted 25 August 2017

axillary odor [17]. These observations suggest that prior exposure to sensorial cues, likely during intrauterine life, facilitates orientation toward the natural source of feeding (i.e., the mammary gland). Human amniotic fluid is a complex mixture of many substances. Eight fatty acids from amniotic fluid, colostrum, and maternal milk have consistently been identified, and an artificial fatty acid mixture that is based on the content and concentration of fatty acids in human amniotic fluid (i.e., lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, elaidic acid, and linoleic acid) produces appetitive responses in human newborns [18]. Therefore, at least in humans, the initial steps toward infant-mother interactions appear to occur after birth when the newborn seeks substances that are similar to those that it experienced during intrauterine life, such as those that emanate from the maternal breast [15,19], including fatty acids [19,20].

However, unknown is whether the entire fatty acid mixture produces these seeking behaviors or whether only some of the fatty acids do. Therefore, the present study evaluated human newborns a few hours after birth. We recorded appetitive responses to each of these eight fatty acids and compared the results with scents from vanilla, the vehicle, and neutral saline.

2. Materials and methods

2.1. Ethics

The present study strictly followed the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments that involve humans. The local research ethics committee (Biomedical Research Institute of the National Autonomous University of Mexico and Hospital Civil de Perote, Veracruz, Mexico) approved the study. During the invitation sessions, all of the mothers received a detailed explanation of the purpose and risks of the study. Two physicians who did not participate in the experimental protocol gave the explanations to the mothers. We did not touch their newborns at any time during the study, with the exception of placing the newborn in the bed. The mothers were present near the bed throughout the behavioral tests.

2.2. Mother volunteers and newborns

We obtained written consent from 12 mothers to include their babies in the study. Mothers who were previously diagnosed with any psychiatric or neurologic pathology were excluded from the study. All of the mothers were right-handed, and none were smokers. They were all in optimal health, confirmed by a complete clinical physical examination.

The inclusion criteria included a minimum gestational age of 39 ± 0.23 weeks (range, 37–40 weeks), birth weight appropriate for gestational age (range, 2.7–3.7 kg), and minimal Apgar score of 9.0 immediately and 5 min after delivery. Well-being and the absence of hunger were ensured by feeding the newborns with colostrum from the maternal breast 15 min before the behavioral test. None of the newborns received any artificial milk formulation or bottle-feeding before the tests.

In addition to the newborn and its mother, two researchers were in the examination room. One researcher applied the stimuli, and the other researcher videorecorded the session and coordinated the activities (i.e., the sequence of stimulus presentation) while explaining each step of the experimental procedure to the mother. Under these conditions, only one researcher was near the newborn, at a distance that was necessary to apply the stimuli while not touching the newborn. The other researcher and mother were at least 2 m away from the newborn, remaining as silent and stationary as possible.

2.3. Olfactory stimuli

Each of the eight fatty acids that were previously identified in human amniotic fluid was prepared individually, strictly following the same concentration that was found in amniotic fluid in previous studies [18]. Pure fatty acids were individually dissolved in a volume of 100 mL of vehicle (96% propyl-*n*-glycol and 4% ethanol) at < 40 °C. All of the fatty acids were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). The final preparation of each of the fatty acids was based on previous reports [18,21] and the physiological concentrations of fatty acids that are detected in amniotic fluid: linoleic acid (4.4 mg/L), palmitoleic acid (7.1 mg/L), stearic acid (3.7 mg/L), myristic acid (3.0 mg/L), elaidic acid (1.5 mg/L), lauric acid (0.4 mg/L), oleic acid (8.0 mg/L), and palmitic acid (15. 3 mg/L). We included three control odors: control-neutral (0.9% saline), control-aromatic (4% vanilla dissolved in distilled water), and control-vehicle (96% propyl-*n*-glycol and 4% ethanol). The presentation of each sensorial stimulus lasted 30 s. Thus, a total of 11 olfactory stimuli were applied, with a 60 s interval between each stimulus presentation. The total duration of testing was < 17 min for each newborn. The sequence of presentation of the various stimuli varied between newborns according to a Latin-square design. Afterward, each mother took care of her newborn.

2.4. Behavioral test and data analysis

Detailed procedures have been published elsewhere [18]. Briefly, the tests were conducted in a nursery room at a temperature of 25 °C. The newborns were individually placed in a warming bed (Infant Warner IW 703, Fisher Paikel Heath Care) with controlled temperature (37 °C) and humidity (< 60%). Different disposable swabs were introduced in Vacutainer tubes, impregnated with the corresponding olfactory stimulus, and vertically placed 1 cm above the newborn's nose. Particular attention was paid to preventing the cotton swabs from touching the newborn's skin. All of the sessions were recorded with a digital videocamera (Sony, DCR-SR85, 25 × optical zoom, Carl Zeiss lens) for further analysis.

Two other independent observers who were blind to the sequence and nature of the stimuli separately scored the presence or absence of responses. The correlation between the observers' scores reached 0.886 ($p < 0.001$). The videorecordings were analyzed several times. The presence of responses that were suggestive of mucosal irritation (e.g., grimacing, crying, hiccupping, and sneezing) were first explored. For the evaluation of these behaviors, the presence and absence of each sensory stimulus presentation over 30 s was considered and marked on a binary scale (1 or 0).

Subsequently, to classify the newborns' reactions to the stimuli, the data were analyzed based on categories of positive and negative facial responses according to previous reports [22], with slight modifications. Movements of the mouth and suckling behaviors were considered appetitive responses because they are directed toward the maternal breast or milk bottles [23]. Positive responses were scored when movements of the mouth (i.e., suckling) and nose (i.e., sniffing) were directed toward the swabs. Negative responses included lateral movements of the head away from the swab. Remaining stationary without any perceptible changes in facial expression was considered an indicator of indifference.

2.5. Statistical analysis

Possible gender differences were analyzed using the Mann-Whitney *U* test. Behaviors that were suggestive of mucosal irritation (e.g., grimacing, crying, hiccupping, and sneezing) were scored in binary form (1 or 0). The Cochran *Q* test was used to analyze the presence or absence of these behaviors. Because the data did not follow a normal distribution, we used nonparametric tests and Statistica 7.0 software. The duration of positive and negative responses to the stimuli was