

Behavioral Responses of the Chronically Instrumented Sheep Fetus to Chemosensory Stimuli Presented in Utero

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Fetal sheep were surgically prepared on Days 113–114 of gestation with an array of chronic instruments for recording electromyographic data (EMG) in oral–facial, axial, and limb muscles and heart rate (FHR). Fetuses also were fitted with an intraoral catheter for infusion of chemosensory fluids (isotonic saline, quinine, colostrum, sucrose) onto the surface of the tongue. Individual subjects received chemosensory infusions on Days 134–137. Fetuses showed consistent oral responses to quinine and milk, but did not respond to isotonic saline or sucrose. Different patterns of motor responses suggested that fetuses discriminated among different concentrations of quinine. The expression of tachycardia to quinine and bradycardia to milk also suggested differential responding to chemosensory fluids that differ in hedonic qualities. Detailed characterization of fetal responses to these stimuli in utero confirm the functionality of the gustatory system in the sheep fetus near term.

Empirical studies of the intrauterine environment and the fetus have suggested that sensory experience may be an important codeterminant of behavioral and neural development during the prenatal period (Lecanuet, Fifer, Krasnegor, & Smotherman, in press). Of the various sensory modalities, chemosensory stimuli (gustation and olfaction) may be the most basic in providing cues that direct maternal–infant interactions and feeding in newborn mammals (Smotherman & Robinson, 1994). The fetus is exposed to a rich chemical environment in utero, providing the potential for experience with a diverse assortment of chemosensory stimuli. The amniotic fluid that surrounds the fetus is continually replenished and undergoes systematic changes in composition during gestation. The constituents of amniotic fluid derive partly from maternal nutrition (dietary cues) and physiology (e.g., hormones) and partly from fetal and placental processes (e.g., respiration, micturition, hormone production; Lev & Orlic,

1972; Marsh, King, & Becker, 1963; Tam & Chan 1977). The fluid bathes the oral, nasal, and pharyngeal cavities and is ingested and respired by the fetus, permitting direct access of chemical cues to the receptors of many chemosensory systems including the lingual taste buds, pharyngeal and epiglottal taste buds, main olfactory epithelia, vomeronasal system, and trigeminal system (Schaal & Orgeur, 1992). Moreover, experiments have suggested that blood-borne chemical cues may gain direct access to the capillary beds of chemosensory receptors to evoke sensory potentials (Maruniak, Mason, & Kostelc, 1983; Maruniak, Silver, & Moulton, 1983). The environment of the fetus clearly is capable of supporting prenatal chemosensory experience during normal gestation.

Direct evidence that fetuses can detect and organize behavioral responses to chemosensory stimuli has been provided in experiments with fetal rats. Responsiveness to complex chemosensory solutions is manifested in increases in gross motor activity, autonomic responses such as changes in heart rate, and expression of species-typical behavior patterns (Smotherman & Robinson, 1987, 1988; Smotherman, Robinson, Ronca, Alberts, & Hepper, 1991). For example, intraoral infusion of a solution prepared from lemon extract evokes an increase in motor activity that rises 4- to 5-fold above baseline levels and persists for 30–60 s after infusion. At the peak of behavioral activity, rat fetuses exhibit a facial wiping response, involving placement of the forepaws against the side of the face, that is similar in form to the displays expressed by postnatal rats on exposure to aversive chemical stimuli. In contrast, intraoral infusion of milk results in little change in overall activity, but elicits a stereotypic response including an increase in mouthing movements that is followed by general redistribution of movements to caudal areas of the body. These changes in motor behavior culminate 2–4 min after milk infusion in a stretch response involving caudal extension of the body and rearlimbs, which closely resembles the stereotypic behavioral response of

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neonatal rats on milk letdown at the lactating nipple (Robinson & Smotherman, 1992). The wiping response to lemon and stretch response to milk illustrate how the fetal rat, which is an altricial species that is born in a relatively immature state, can distinguish different chemosensory stimuli. Moreover, these findings demonstrate that the fetus's first exposure to milk can promote the expression of elements of postnatal suckling behavior that will be crucial in processing this biologically important stimulus after birth (Smotherman & Robinson, 1994).

Apart from studies of fetal rodents, few data are available regarding the responsiveness of fetuses to chemosensory stimuli in utero (Schaal & Orgeur, 1992). Studies of the developing gustatory system have indicated that taste buds are found in fungiform papillae on the tongue as early as E50 (Embryonic Day 50) in the fetal sheep, with changes in the fine structure of taste buds continuing to occur through term (gestation is about 150 days; Mistretta, 1990; Mistretta & Bradley, 1986). Recording of electrical activity in chorda tympani and glossopharyngeal nerves has confirmed that taste receptors on the tongue of the fetal sheep can detect a variety of chemicals as early as E110. However, development of central systems that process taste information, such as the nucleus of the tractus solitarius (NTS) and parabrachial nucleus of the pons (PBN), is delayed relative to the appearance of functional taste receptors on the tongue (Mistretta & Bradley, 1983). Salt solutions applied to the tongue can evoke potentials in peripheral nerves but do not elicit central responses on E114, but neurons that constitute the first gustatory relay within the NTS respond to a variety of chemical solutions by E125 (Bradley & Mistretta, 1980). These anatomical and neurophysiological findings suggest that the fetal sheep can perceive gustatory stimuli by approximately the middle of the third trimester of gestation. Because the sheep fetus develops during a long gestation and is widely used as a model of human fetal physiology, these data may be especially relevant for understanding the development of sensory capacities in the human fetus and preterm infant.

The chemical senses offer advantages for investigation of prenatal sensory function for pragmatic reasons. Because chemical cues originating in the external world are transmitted to the fetus by way of maternal circulation, it is unlikely that the fetus ever experiences a brief, temporally delimited exposure to chemosensory stimuli before birth. In contrast, specific chemical stimuli can be presented directly to the fetal tongue in a precisely controlled pulse delivered on a programmed schedule (Smotherman & Robinson, 1991). This combination of a maternally modulated chemical environment in utero and precise experimental control over parameters of stimulus presentation ensures that (a) the fetus lacks specific experience with pulsatile cues of the same intensity, temporal pattern, and chemical quality as the test stimulus and (b) the test stimulus will be presented against a relatively stable, nonfluctuating background of chemical noise in utero. These experimental considerations are particularly important in accurately measuring changes in fetal responsiveness to stimulation. In the present study, an array of complex chemosensory fluids were presented to fetal sheep on E134–137 to begin to describe the prenatal development of behavioral responsiveness to gustatory stimuli. These fluids, including isotonic

saline, quinine, sucrose, and milk, were infused onto the surface of the tongue of fetuses prepared with chronic instrumentation that permitted measurement of oral–facial, gross motor, autonomic, and electrocortical responses to stimulation.

Method

Subjects

Subjects were 7 sheep fetuses produced in timed-matings of Rambouillet-Columbia ewes (*Ovis aries*). Ewes were bred and maintained for the first 100 days of gestation in the breeding facilities at Cornell University. Pregnant ewes were transferred to the Laboratory for Pregnancy and Newborn Research (New York State College of Veterinary Medicine), housed in individual metabolism stalls (1.25 × 0.50 × 0.85 m), and provided with ad libitum alfalfa cubes and water. Ewes and fetuses were scheduled for surgical preparation approximately 1 week after transfer to the laboratory. At all times, animals received care and treatment in accordance with guidelines established by the National Institutes of Health under a protocol approved by the Cornell Institutional Animal Care and Use Committee.

Surgery

All subjects were singleton fetuses surgically prepared on 112–114 days gestational age (DGA). Ewes receive a ketamine (5–10 mg/kg) and glycopyrrolate preanesthetic; general halothane anesthetic is administered following maternal tracheal administration. This procedure also results in anesthesia of the fetus, as halothane readily crosses the ovine placenta (Towell, Figueroa, Markowitz, Elias, & Nathanielsz, 1987). A prophylactic antibiotic treatment of 1 g ampicillin sodium (Polycillin-N; Bristol Laboratories, Syracuse, NY) and 1 g chloramphenicol sodium succinate (LyphoMed, Melrose Park, IL) was administered intravenously to the ewe at surgery. Surgical access to the fetus was provided through a midline laparotomy and incision through the uterine wall.

Instrumentation involves surgical placement of chronic catheters and electronic leads in both the ewe and fetus. Chronic polyvinyl catheters placed in the maternal and fetal carotid arteries were used to periodically obtain blood samples to monitor blood gases and the general condition of the ewe and subject fetus. Transducers connected to the fetal carotid catheter also provided measurements of fetal blood pressure, with changes in blood pressure used to calculate heart rate. An open ended catheter was placed in the fetal trachea to measure intratracheal pressure. An additional catheter was sutured to the dorsal midline of the tongue to permit delivery of chemosensory solutions to the fetus. This catheter was prepared with several holes along the side to distribute the infused solution over a broad area of the tongue. Electrical leads consisted of multistrand bipolar stainless steel wires (Cooner AS632). Paired leads were sewn 5 mm apart into the horn and body of the uterus to record myometrial activity and to determine occurrence of spontaneous nonlabor contractions (Sadowsky et al., 1992). Electrocortical activity (ECoG) was measured with stainless steel leads inserted through the frontal bone into the dura to the left and right of the midline. Wires placed above and below each eye recorded eye movements (EOG). ECoG and EOG are part of standard instrumentation implemented in studies of fetal sheep, and are used for monitoring fetal well-being and behavioral state (Towell et al., 1987). Electromyographic (EMG) recordings were collected from stitch electrodes placed into selected muscles in the oral area, neck, forelimbs, and rearlimbs to record voluntary motor responses of the fetus. Oral muscles included the masseter (jaw adductor) and geniohyoid (tongue levator, swallowing). EMG leads also were used to

measure esophageal activity. This approach to measuring oral activity is similar to that used by Travers and Norgren (1986) to characterize responses of rats to intraoral infusions of sapid fluids. Muscles instrumented in the neck included the nuchal (head levator) and brachiocephalicus (forelimb protractor and head levator). Antagonistic muscle pairs in the right foreleg, brachialis (flexor) and triceps (extensor), and the right hindleg, tibialis (flexor) and gastrocnemius (extensor), also were instrumented with EMG leads.

Following surgical preparation of the ewe and fetus, all fetal catheters and EMG leads were grouped to exit the lateral abdominal wall of the ewe at a single point. Surgical closure was accomplished in layers, consisting of the embryonic membranes and uterus, abdominal wall, and external skin of the abdomen. The ewe was transferred on the day of surgery to the metabolism stall in the laboratory. During the 4 days following surgery, the ewe received an antibiotic regimen consisting of continuous intravenous infusion of chloramphenicol at a rate of 2 g/day. Blood samples (0.5 ml) were collected daily from both maternal and fetal carotid catheters and analyzed on a ABL2 (Radiometer, Copenhagen) blood gas analyzer for PO₂ and PCO₂ (mm Hg) and pH. A heparin solution (10 units per milliliter of physiological saline) was continuously infused at a rate of 0.5 ml/h to ensure that the vascular catheters remained open, but no infusions were delivered through the oral catheter until the days of behavioral testing. At the conclusion of the experiment, the ewe and the subject fetus were administered an overdose of sodium pentobarbital. A necropsy was performed to verify placement of the various EMG leads and positioning of the intraoral catheter.

Experimental Design and Protocol

Behavioral responses of fetal subjects to various chemosensory fluids were measured in a series of tests conducted over a 3-day period commencing 21 days after surgical preparation (range: 134–137 DGA on the 1st day of chemosensory testing). Three classes of chemosensory stimuli were presented, one on each day of testing. On the 1st day, fetuses were tested with three concentrations (0.03-, 0.003-, and 0.0003-molar solutions) of quinine hydrochloride (QHCl). On the 2nd day, fetuses were tested with three concentrations (0.3-, 0.03-, and 0.003-molar solutions) of sucrose (SUC). On the 3rd day, fetuses were tested with three different kinds of milk. Sheep colostrum was collected from newly parturient ewes; postpartum sheep milk was collected from lactating ewes 1–2 weeks after birth. The third milk consisted of commercially available bovine light cream (half-and-half), which has been used extensively in experiments documenting prenatal development of behavioral responses to suckling stimuli in fetal rats (Robinson & Smotherman, 1992; Smotherman & Robinson, 1994). All three milks were sterilized by irradiation and frozen in aliquots until the day of testing. The decision to present QHCl first was based on pilot studies which suggested that it would evoke pronounced but short-lasting responses, whereas milk has been shown to produce persistent responses. On each day of testing, fetal subjects also received two control infusions of isotonic saline. Presentation of both control infusions and all three experimental infusions occurred between 12 and 5 pm.

All test infusions consisted of 1 ml of the experimental solution (or saline control) delivered into the mouth of the fetus through a tongue catheter in a 2–3 s pulse. The same catheter was used for all infusions. Because the volume of the catheter (2.8 ml) exceeded the volume of experimental solution to be delivered, it was necessary to load each experimental solution into the catheter and to advance it to the distal end of the catheter prior to infusion. Loading the experimental solution was accomplished at the same time as infusion of the preceding solution; the next solution to be delivered was introduced into the proximal end of the catheter, resulting in intraoral delivery of the solution already in the distal end of the catheter. A small bubble of

air was introduced into the tubing immediately in front and behind the solution to be delivered to prevent diffusion of the fluid into the oral cavity before the intended time of stimulus presentation. To advance the next solution to the distal end, an additional volume of saline (1.8 ml) was injected into the catheter behind the test solution. These “push” injections resulted in 1.8 ml of saline introduced slowly (5–10 s pulse) into the fetus's mouth. On each day of testing, fetuses thus received a series of control and experimental infusions and push injections. Testing commenced with a control infusion of saline, followed by the three experimental infusions (separated by push injections), and concluding with a second control infusion of saline. The order of presentation of the three experimental solutions was counterbalanced across fetal subjects.

To provide control over the volume and timing of each experimental infusion, the solution to be delivered was advanced to the distal end of the catheter (push injection) at least 15 min before the next experimental infusion. An interval of at least 30 min separated consecutive infusions of different experimental solutions (range: 30 to 68 min). In addition to the delay, two criteria were used to restrict the moment when the infusion was delivered: all test infusions occurred (a) during a stable epoch of low voltage ECoG activity and (b) at least 5 min after the most recent nonlabor contraction of the myometrium, as determined by uterine EMG.

Data Acquisition and Analysis

Each channel from EMG leads and transducers for blood pressure and heart rate was connected to a data acquisition system (DAS) 24 to 48 h after surgery. The DAS consisted of preamplifiers and other conditioning circuitry, where signals were raised to standard levels used throughout, and a system for averaging signals from the 15 independent channels in 1-s intervals, converting analog signals to 8-bit digital integers, and providing a synchronized time reference. EMG and ECoG signals were amplified (100–200 μ V full scale deflection for uterine EMG; 50–200 μ V for ECoG and fetal EMG channels), high and low pass filtered at 3 and 30 Hz, full wave rectified, and then low pass filtered at 10 Hz before digitizing. Digital data were passed to a DOS-based microcomputer, where they were saved to disk. This DAS also permitted simultaneous display of different data channels as printed output. The output of this system provided digital summaries of data in the 15 channels in 1-s intervals, which were continuously recorded from the time of connection to the DAS through the completion of the experiment.

Preliminary examination of the data was conducted to determine the most appropriate time scale for characterizing fetal responses to chemosensory stimulation. In this article, all behavioral variables are reported in 15-s intervals comprising a 1-min baseline period terminating 15-s before infusion and a 3-min period after infusion. A marker channel activated at the time of chemosensory testing was used to identify the onset of each infusion to the nearest second; to ensure that the baseline and postinfusion periods did not include artifacts resulting from procedures for delivering intraoral infusions, the 15-s period containing the 2–3 s experimental infusion was excluded from the analysis.

The range of digitized activity data from the various EMG channels often did not extend to zero, indicating the presence of background noise. To distinguish muscle activity from background noise within each channel, the baseline period for each experimental infusion was independently examined to identify the actual minimum score recorded. Muscle activity was defined as any score in a 1-s interval that exceeded a criterion threshold established at 2% of the full-scale range above the observed minimum. With this criterion, activity in each of the muscle EMG variables was summarized for successive 15-s intervals as a percentage of time active (number of 1-s intervals active/15 s). Background noise and a true zero was less problematical

for FHR, ECoG, and EOG, which exhibited activity closer to the middle of the digital range. Recorded scores in these variables were averaged in 15-s intervals before statistical analysis. Response measures in all variables were compared across 16 15-s intervals (4 baseline + 12 postinfusion) in a series of one-way repeated-measures analyses of variance (ANOVAs). Following significant overall repeated measures effects, post hoc comparisons by the method of Fisher PLSD ($p < .05$) were used to determine whether any post infusion interval differed from 15-s intervals in the baseline period.

Results

Fetal Response to Isotonic Saline

Fetuses were presented with isotonic saline in a control infusion at the outset of each of the 3 days of chemosensory testing. Changes in the nine EMG variables, heart rate (FHR), electrocortical activity (ECoG), and electroocular activity (EOG) were examined for evidence of behavioral response to control infusions. No significant changes in any variable were indicated by the repeated measures ANOVAs between the baseline period before infusion and the 3-min period after saline infusion ($ps > .05$). Mean activity exhibited in the EMG variables before and after infusion is summarized in Figure 1. Some of the muscles were relatively quiet during the baseline period, showing activity in less than 20% of recorded 1-s

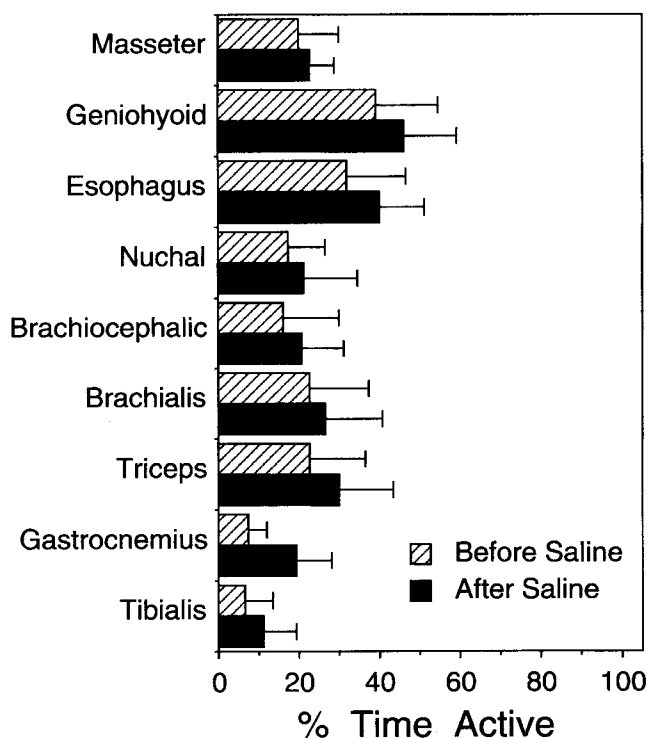


Figure 1. Percentage of 15-s intervals that various fetal muscles exhibited activity before and after intraoral infusion of isotonic saline. Bars show the mean activity of seven fetal subjects; lines depict SEM. Saline infusion did not result in a significant change in electromyographic activity in any of the nine muscles, suggesting that the physical delivery of a fluid through the intraoral catheter is not sufficient to evoke behavioral responses in the fetal sheep.

intervals. However, certain muscles, notably including the oropharyngeal muscles geniohyoid and esophagus, exhibited spontaneous activity of 30–40% during the baseline period. Fetal heart rate changed little after infusion of saline with resting FHR of 152.0 ± 5.7 bpm and mean FHR after infusion of 151.1 ± 4.0 bpm. The absence of change in any behavioral variable after infusion indicated that sheep fetuses did not respond to isotonic saline delivered into the mouth.

Fetal Response to Quinine

Fetal subjects received intraoral infusions of QHCl in three concentrations on the 1st day of chemosensory testing. Fetal responses to each of these concentrations were assessed in separate repeated measures ANOVAs comprising four 15-s intervals before and twelve 15-s intervals after infusion. The ANOVA examining fetal responses to the high concentration of QHCl (0.03 M) indicated significant changes in activity of masseter, $F(15, 90) = 2.10, p < .02$; geniohyoid, $F(15, 90) = 2.20, p < .02$; and esophagus, $F(15, 90) = 2.20, p < .02$ (Figure 2). QHCl elicited an increase in masseter activity that persisted for 45 s, with a second peak in activity occurring about 2 min after infusion. Activity in the other EMG variables did not differ between the baseline and postinfusion periods ($ps > .05$). Post hoc comparisons indicated that activity in the geniohyoid was elevated for 75 s after QHCl infusion. Infusion resulted in an initial epoch of esophageal activity that continued for 60 s, followed by less pronounced increases that were most evident at 90 s and again 135–180 s after infusion. The high concentration of QHCl did not evoke significant EMG responses in the brachiocephalic, nuchal, or limb muscles. Significant changes in FHR also were indicated by the overall ANOVA, $F(15, 90) = 2.28, p < .01$ (Figure 3). During the baseline period, mean FHR was 152.7 ± 5.4 bpm. Infusion of QHCl evoked a modest tachycardia that was most evident 45–60 s after infusion.

Examination of fetal responses to the medium concentration of QHCl (0.003 M) also indicated significant changes in several EMG variables. Responses were evident in masseter, $F(15, 90) = 2.80, p < .001$; geniohyoid, $F(15, 90) = 1.81, p < .05$; esophagus, $F(15, 90) = 2.05, p < .02$; and nuchal, $F(15, 90) = 2.33, p < .01$ (Figure 2). Other EMG variables did not change after QHCl infusion. Post hoc comparisons indicated that the medium concentration of QHCl resulted in a pronounced increase in masseter activity, increasing nearly 5-fold over baseline levels, that persisted for the entire 3-min period after infusion. Geniohyoid activity also increased markedly after infusion with nearly all postinfusion intervals elevated relative to the baseline period. Esophageal activity after QHCl infusion was elevated at all points after infusion. Finally, nuchal activity was more variable during the baseline period, but increased significantly at all points during the 3-min period after infusion. Infusion of the medium concentration of QHCl did not result in significant changes in EMG activity in brachiocephalic or any of the four limb muscles, or in FHR (Figure 3).

Infusion of the lowest concentration of QHCl (0.0003 M) also evoked fetal responses, which were evident in significant increases in activity of the esophagus, $F(15, 90) = 1.78, p < .05$; brachialis, $F(15, 90) = 1.88, p < .05$; and triceps, $F(15,$

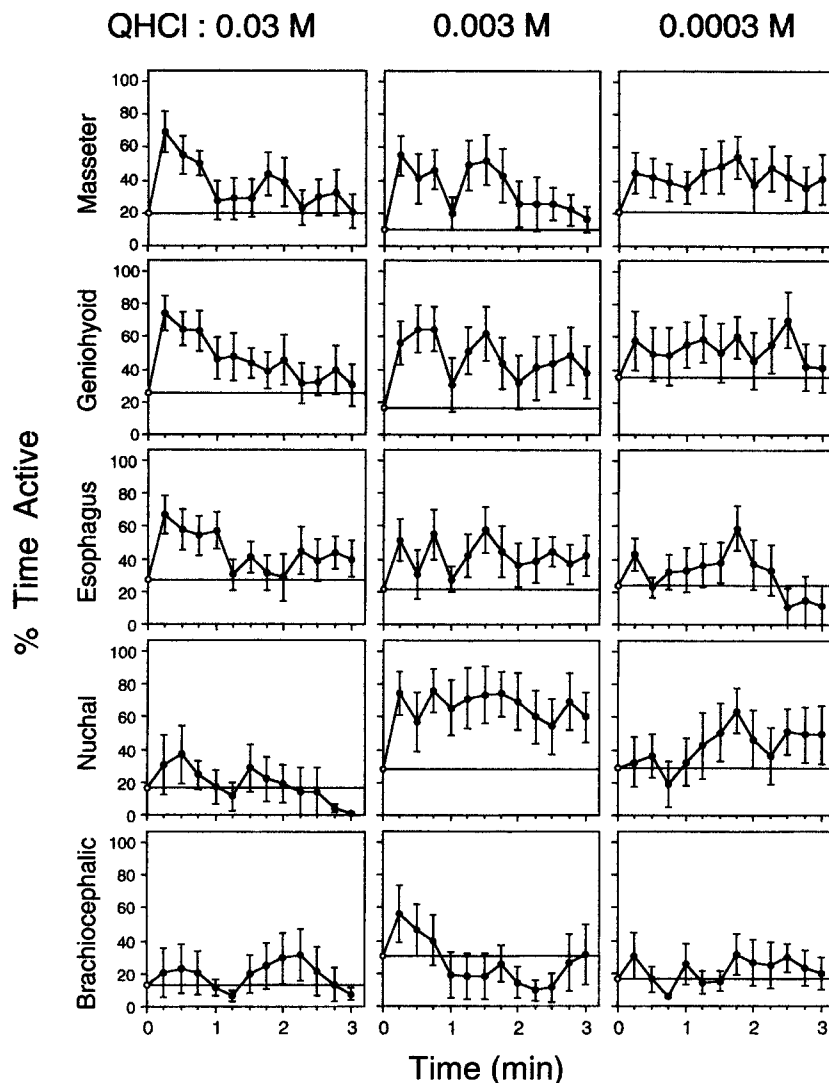


Figure 2. Changes in masseter, geniohyoid, esophagus, nuchal, and brachiocephalic electromyographic (EMG) activity after intraoral presentation of QHCl in one of three concentrations. Each graph presents the mean \pm SE_M during twelve 15-s intervals after intraoral infusion (solid circles), with the mean baseline activity depicted as an open circle and a horizontal line. Analyses of these data indicated that the fetal sheep showed elevated oral activity (masseter, geniohyoid, and esophageal EMG) after infusion of the high and intermediate concentrations of QHCl, and elevated esophageal activity at the low concentration.

90) = 2.52, $p < .005$ (Figure 2 and Figure 3). Esophageal activity was elevated with increases most evident 105 and 180 s after infusion. Brachialis showed a modest increase after infusion with the most pronounced elevations at 60 s and again 105–180 s after infusion. Triceps activity also was modestly elevated above baseline during 90–150 s and again at 180 s after infusion. The oral muscles masseter and geniohyoid did not exhibit significant changes in activity after infusion of the low concentration of QHCl, nor were responses in nuchal, brachiocephalic, gastrocnemius, or tibialis evident. No significant changes in FHR were indicated.

Across the three concentrations, it was evident that QHCl consistently evoked responses in muscles associated with the oral processing of chemosensory fluids. Masseter and geniohy-

oid activity suggest repeated mouthing and tongue responses to this fluid, whereas esophageal activity may reflect either swallowing or pharyngeal rejection of this bitter-tasting solution. The increase in nuchal activity, observed at the medium concentration, and the antagonist foreleg muscles brachialis and triceps, observed at the low concentration, also suggest that moderate QHCl stimulation can promote gross motor responses involving the head and limbs.

Fetal Response to Sucrose

Fetal subjects received intraoral infusions of sucrose (SUC) in three concentrations on the 2nd day of chemosensory testing. The ANOVAs comparing activity in the nine EMG

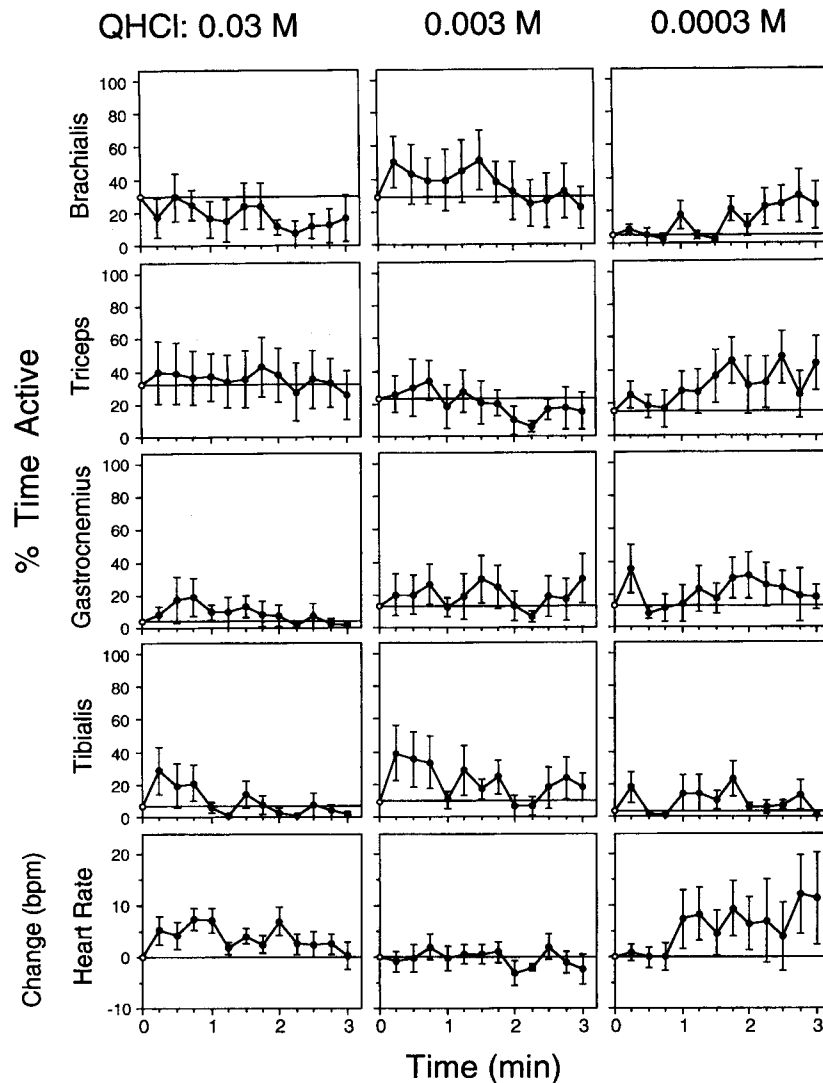


Figure 3. Changes in brachialis, triceps, gastrocnemius, and tibialis electromyographic (EMG) activity, and fetal heart rate after intraoral presentation of quinine hydrochloride (QHCl). Fetal heart rate is expressed as a change, in beats per minute, from resting heart rate before infusion. Analyses of these data indicated that the high concentrations of QHCL elicited brief tachycardia, whereas the low concentration resulted in elevated EMG activity in brachialis and triceps muscles.

variables and FHR indicated no significant changes in fetal activity after infusion at any of the three concentrations (0.3, 0.03, and 0.003 M) (Figures 4 and 5). These behavioral measures provided no evidence that fetal sheep detected or responded to any concentration of sucrose.

Fetal Response to Milk

Fetuses received intraoral infusions of different types of milk on the 3rd day of chemosensory testing. Infusion of sheep colostrum evoked significant changes in activity of both masseter, $F(15, 90) = 2.02, p < .05$, and geniohyoid muscles, $F(15, 90) = 2.47, p < .005$. In contrast to fetal responses evoked by QHCl, post hoc comparisons revealed that colostrum resulted in significant decreases in masseter and geniohyoid activity

that occurred several minutes after infusion (Figure 6). The decrease in masseter activity was most evident 165 s after infusion, and reduced geniohyoid activity was observed from 120–180 s after infusion. It should be noted that fetuses showed somewhat elevated activity of the geniohyoid during the baseline period before infusion, which probably contributed to the ability of the analysis to detect a decrease in activity. No significant responses were found in other behavioral variables.

Infusion of postpartum sheep milk evoked behavioral responses that appeared to differ qualitatively from colostrum. The series of ANOVAs indicated significant changes in the activity of masseter, $F(15, 90) = 1.93, p < .05$; geniohyoid, $F(15, 90) = 3.63, p < .0001$; and esophagus, $F(15, 90) = 2.58,$

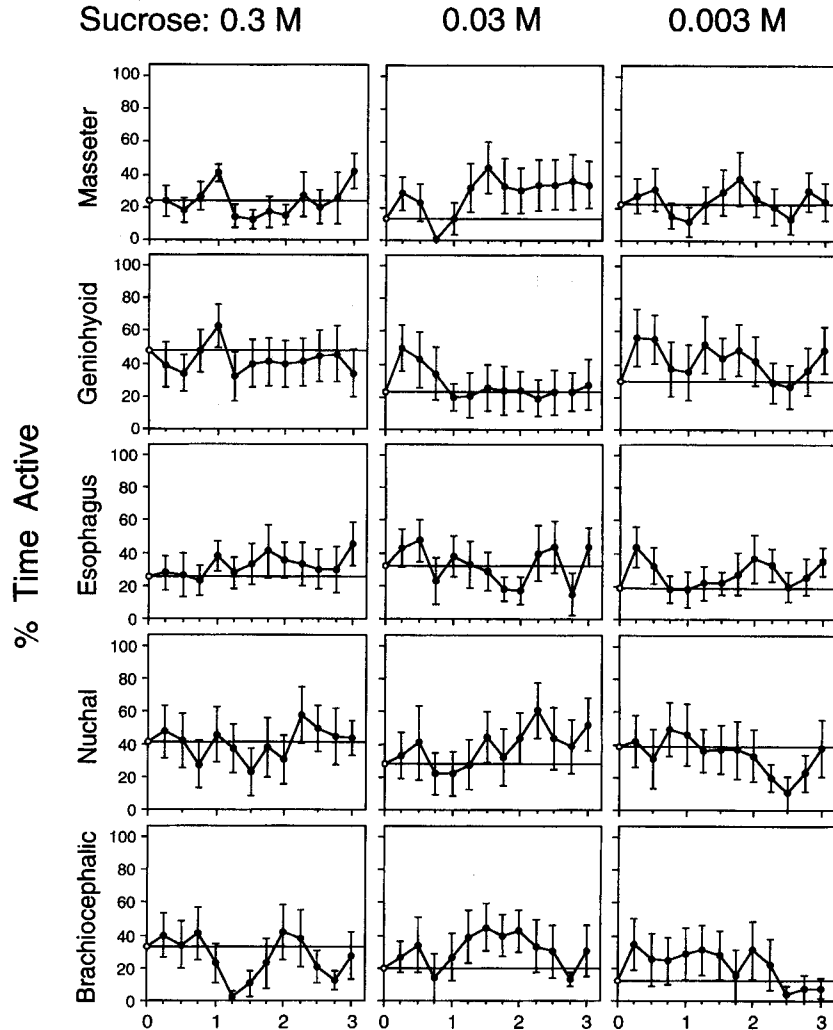


Figure 4. Changes in masseter, geniohyoid, esophagus, nuchal, and brachiocephalic electromyographic activity after intraoral presentation of sucrose. Each graph presents the mean \pm SEM during twelve 15-s intervals after intraoral infusion (solid circles), with the mean baseline activity depicted as an open circle and a horizontal line. Analyses revealed no significant responses to any of the three concentrations of sucrose.

$p < .005$. Milk evoked an increase in masseter activity that was evident 15–45 s after infusion and at several points thereafter (75–135 s, and 180 s, see Figure 6). Geniohyoid activity also increased after milk infusion at all 15-s intervals after infusion. Esophageal activity also was significantly elevated throughout the 3-min period after infusion of milk. In addition to these motor responses, sheep milk evoked a significant change in FHR, $F(15, 90) = 3.21$, $p < .001$ (Figure 7). During the baseline period FHR was 151.7 ± 5.0 bpm. After infusion, the change in FHR consisted of a bradycardia that commenced 60 s after infusion and continued through the end of the 3-min period. Apart from the oral muscles, there was no evidence of gross motor responses associated with infusion of sheep milk.

Infusion of bovine milk to the fetal sheep failed to evoke significant responses in any of the EMG variables, or FHR

(Figures 6 and 7). Like sucrose, these behavioral measures provided no evidence that fetal sheep detected or responded to bovine milk.

Infusion of postpartum sheep milk evokes pronounced and distinctive behavioral responses in the fetal sheep. Like the responses to QHCl, masseter and geniohyoid activity suggest that milk evokes protracted periods of mouthing behavior in addition to esophageal activity. At this level of analysis, it is not possible to characterize further the pattern of the oral and pharyngeal responses. However, it is noteworthy that milk elicits a pronounced bradycardia, in contrast to the modest tachycardia evoked by the high concentration of QHCl. The occurrence of robust responses to mature sheep milk also contrasts with the absence of fetal response to bovine milk and the quieting influence of sheep colostrum on oral muscle activity.

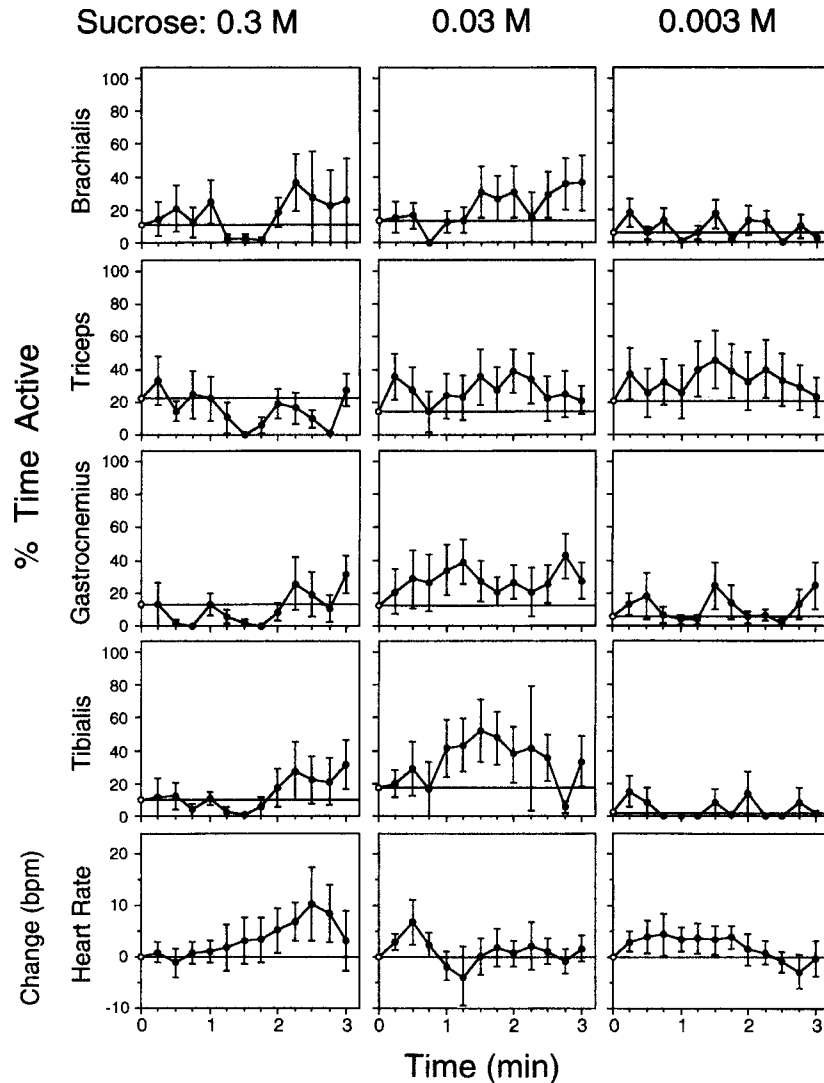


Figure 5. Changes in brachialis, triceps, gastrocnemius, and tibialis electromyographic activity, and fetal heart rate after intraoral presentation of sucrose. Analyses revealed no significant responses to any of the three concentrations of sucrose.

Discussion

On the basis of anatomical investigation and electrophysiological measurements of neural activity in the gustatory system, Mistretta and Bradley (1986) predicted that the fetal sheep (and by inference, the human fetus) should exhibit behavioral responses to a wide range of taste stimuli and discrimination among salt, sweet, sour, and bitter stimuli by late in gestation. The present study provides evidence that the E134–137 fetal sheep can indeed express motor and autonomic responses to gustatory stimuli infused onto the surface of the tongue. Consistent oral responses, as measured by increased activity in the masseter and geniohyoid muscles and elevated esophageal activity, were evoked by infusion of quinine. Although oral responses were expressed to both the medium and high concentration of quinine, other behavioral variables indicated that the fetal sheep can distinguish different concen-

trations of QHCl. Changes in heart rate, involving a modest tachycardia, were expressed only to the highest concentration of quinine (0.03 M), whereas gross motor responses, including nuchal, brachialis, and triceps activity, were evoked by lower concentrations. The responsiveness of fetal sheep to varying levels of quinine stimulation accords well with data reported by Steiner on behavioral responses of preterm human infants to gustatory stimuli. Neonates born in the seventh month of gestation expressed different oral–facial reactions to sweet (sucrose), sour (citric acid), and bitter stimuli (quinine and urea) (Steiner, 1979). Further, aversive reactions were more pronounced to higher concentrations of urea, suggesting that preterm infants can discriminate among different intensities of gustatory stimuli (Ganchrow, Steiner, & Daher, 1983). In the present study, autonomic reactions (FHR) were evident only at 0.03 M, whereas oral responses (masseter and geniohyoid)

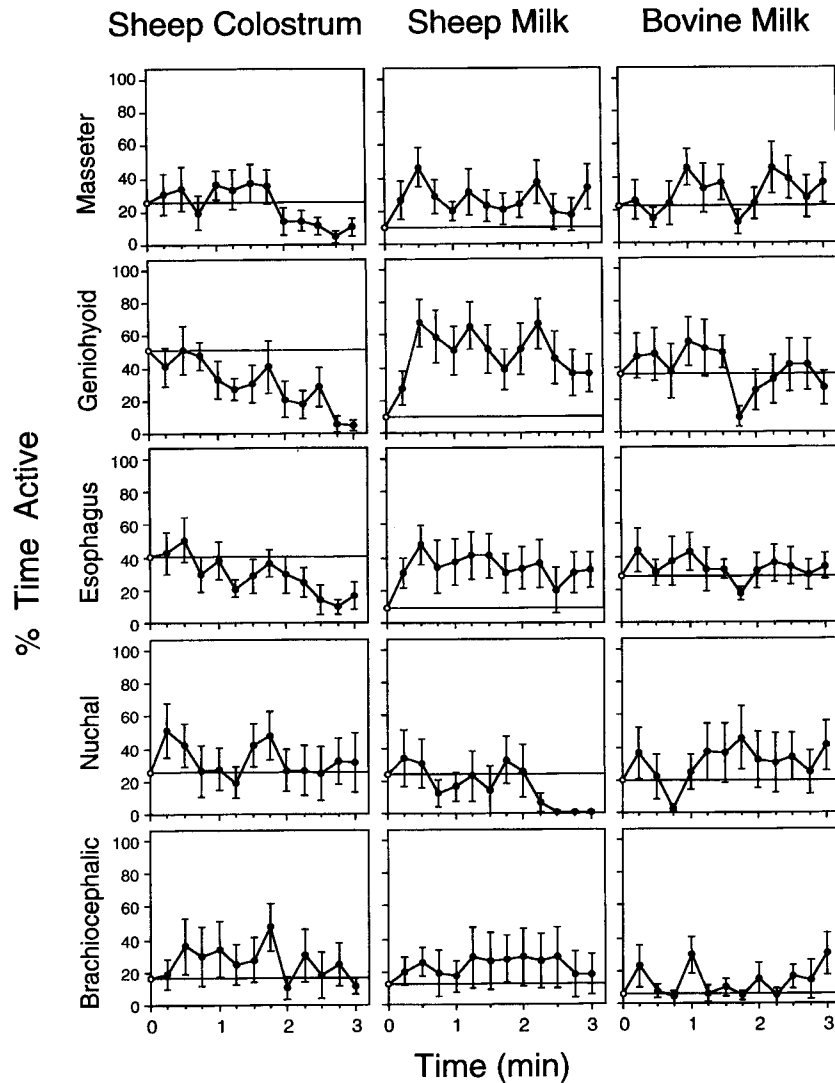


Figure 6. Changes in masseter, geniohyoid, esophagus, nuchal, and brachiocephalic electromyographic (EMG) activity after intraoral presentation of sheep colostrum, sheep milk, or bovine milk. Each graph presents the mean \pm SEM during twelve 15-s intervals after intraoral infusion (solid circles), with the mean baseline activity depicted as an open circle and a horizontal line. Analyses indicated decreased EMG activity in masseter and geniohyoid muscles after infusion of colostrum, but elevated masseter, geniohyoid, and esophageal activity after infusion of sheep milk.

were absent at 0.0003 M, suggesting a similar intensity gradient in responses of the fetal sheep to a bitter gustatory stimulus.

Infusions of fructose (0.5 M) or sucrose (1.0 M) have been reported to evoke electrical activity in the chorda tympani and the nucleus of the solitary tract in the E128 fetal sheep (Mistretta, 1990; Mistretta & Bradley, 1986). Further, there is no indication that sweet sensitivity is developmentally delayed relative to bitter or sour tastes. Anatomical and electrophysiological evidence therefore suggest that the gustatory system of the fetal sheep late in gestation can detect and respond to sweet stimuli such as sucrose. However, the present study found no evidence of behavioral responding across a range of sucrose concentrations (0.003 to 0.3 M). Because the lack of responding probably is not due to immaturity of the gustatory

system, these data imply that the fetal sheep do not indiscriminately respond to gustatory stimuli. Sucrose therefore appears to be discriminated from quinine and milk stimuli that evoke consistent behavioral responses in the fetal sheep.

Some of the strongest evidence for selective responsiveness to different chemosensory stimuli derives from intraoral infusion of different origins to the fetal sheep. Postpartum sheep milk, obtained from lactating ewes 1–2 weeks after parturition, consistently evoked oral responses (masseter and geniohyoid), and esophageal activity in fetal subjects. Mature sheep milk also elicited a distinctive bradycardia response that differed from the tachycardia evoked by quinine, suggesting that these two fluids were distinguished by the fetus. Tachycardia is typically associated with a defensive autonomic response

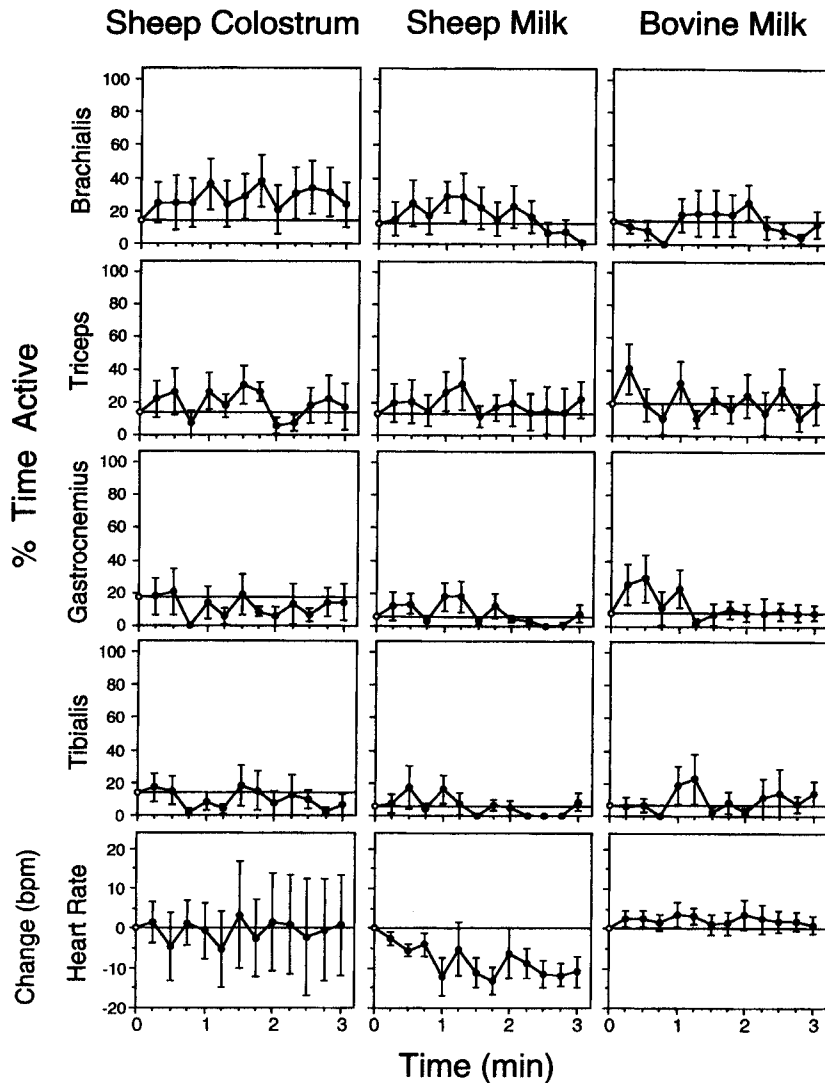


Figure 7. Changes in brachialis, triceps, gastrocnemius, and tibialis electromyographic activity and fetal heart rate after intraoral presentation of three different milks. Analyses indicated significant bradycardia elicited by infusion of sheep milk.

in a broad range of mammals, whereas bradycardia is associated with an orienting response or change in attention (Rohrbaugh, 1984). Both patterns of cardiac response can be elicited by chemosensory stimuli that differ in hedonic quality and have been demonstrated in experiments employing intranasal infusion of olfactory stimuli to the fetal sheep (Schaal et al., 1991). It should be noted that postpartum milk was not collected in the present experiment from the fetus's own mother, but from other ewes that were maintained in the same flock, exposed to the same diet, and housed under similar environmental conditions. Because both milk and amniotic fluid derive much of their composition from environmental sources such as maternal diet (Schaal & Orgeur, 1992), it is likely that the milk infused to subjects in this study shared many constituents with the amniotic fluid to which fetuses were exposed in utero. It is interesting that fetal responses to mature sheep milk differed

qualitatively from responses to colostrum, which diminished oral activity several minutes after infusion, and bovine milk, which failed to elicit responses in any behavioral variable. Colostrum in particular should exhibit similar composition to both postpartum milk and amniotic fluid (Ling, Kon, & Porter, 1961), yet fetuses apparently discriminated this early milk from more mature milk. Newborn lambs can express fine discrimination of chemosensory stimuli, including amniotic fluid produced in their own pregnancy from amniotic fluid derived from a different ewe (Vince & Billing, 1986). The present study implies that the sheep fetus can make an equally subtle distinction among colostrum, mature sheep milk, and bovine milk.

The temporal characteristics of oral-facial muscle and heart rate responses evoked by different concentrations of QHCl and by different milks in the fetal sheep also appear to be

broadly consistent with behavioral responses elicited by milk and novel chemosensory fluids in other species. In the rat fetus and neonate, for example, solutions prepared from complex botanical extracts (e.g., lemon or mint) reliably elicit pronounced but short-lasting responses involving increased motor activity and heart rate change (Smotherman et al. 1991). Intraoral infusion of milk, in contrast, has been shown to produce behavioral effects that are sustained for several minutes or that lead to discrete responses, such as the stretch response that typically occurs during suckling at the nipple, after a 3–5 min delay (Robinson & Smotherman, 1992). In this study, presentation of a novel gustatory stimulus QHCl produced prompt reactions in oral–facial variables (masseter, geniohyoid, and esophagus EMG) that were large in magnitude but brief in duration. On the other hand, presentation of milk tended to evoke responses that were sustained through the 3-min period after infusion (e.g., oral–facial responses to sheep milk), or which were evident only after a delay of several minutes (e.g., oral–facial responses to colostrum). These findings suggest that experimental presentation of milk to the fetal sheep may produce similar behavioral effects as have been reported in the fetal rat (Smotherman & Robinson, 1994).

At the most basic level, the experiment reported in this article provides important confirmatory evidence that the gustatory system of the fetal sheep is functional by E134–137. Data obtained from chronic instrumentation of muscle, cardiac, and cortical variables in fetal subjects extends earlier anatomical and neurophysiological studies and demonstrates that gustatory experiences can be transduced into behavioral responses during the prenatal period. Because the fetal sheep exhibits a long gestation and is an important animal model of human prenatal development, these findings have implications for the sensory capacities of the human fetus and preterm infant. The full-term human infant is clearly able to recognize milk during suckling and to distinguish this biologically important fluid from other chemosensory stimuli, but it is unclear when these chemosensory abilities have their developmental origin (Smotherman & Robinson, 1992, 1994). Preterm infants are, after all, fetuses in an age-atypical environment that possess immature sensory and motor systems and varying degrees of experience with chemosensory factors in the amniotic fluid. Understanding the prenatal development of the chemical senses in general, and responsiveness to milk and other stimuli associated with early feeding, will be increasingly important given the need to manage and care for preterm infants born at progressively younger postconceptional ages.

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