Development of ingestive behavior

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Ross, Michael G., and Mark J. M. Nijland. Development of ingestive behavior. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R879–R893, 1998.—Swallowing represents a primary physiological function that provides for the ingestion of food and fluid. In precocial species, swallowing activity likely develops in utero to provide for a functional system during the neonatal period. The chronically instrumented ovine fetal preparation has provided the opportunity for recent advances in understanding the regulation of in utero swallowing activity. The near-term ovine fetus swallows fluid volumes (100–300 ml/kg) that are markedly greater, per body weight, than that of the adult (40–60 ml/kg). Spontaneous in utero swallowing and ingestive behavior contribute importantly to the regulation of amniotic fluid volume and composition, the acquisition and potential recirculation of solutes from the fetal environment, and the maturation of the fetal gastrointestinal tract. Fetal swallowing activity is influenced by fetal maturation, neurobehavioral state alterations, and the volume of amniotic fluid. Furthermore, intact dipsogenic mechanisms (osmolality, angiotensin II) have been demonstrated in the near-term ovine fetus. It remains unknown to what degree, if any, fetal swallowing may be influenced by nutrient appetite, salt appetite, or taste. Nevertheless, the development of dipsogenic and additional regulatory mechanisms for ingestive behavior occurs during fetal life and may be susceptible to changes in the pregnancy environment. This review describes what is currently known regarding the in utero development of ingestive behavior and the importance of this activity for fetal and perhaps ultimately adult fluid homeostasis.

Fetal swallowing and amniotic fluid

Amniotic fluid volume and composition are maintained during intrauterine life by a balance of fetal fluid acquisition and loss. The primary physiological function of all multicellular animal life forms, representing the only significant means by which nongaseous material is extracted from the environment. Ingestive behavior acts to facilitate entry of both fluid and food to the gastrointestinal tract. The physical act of swallowing requires extensive motor coordination of pharyngeal, laryngeal, esophageal, and diaphragmatic muscles. Thus it is not surprising that central (brain stem) nuclei and motoneuron interconnections are required to “program” a swallow. Beyond the motor coordination, neural regulatory influences modulate swallowing behavior. In the newborn or adult, reflex mechanisms, including laryngeal chemo- or mechanoreceptors, serve to protect the airway. In addition, subconscious or conscious drives, including thirst, hunger, and sodium appetite, stimulate complex behaviors consummating in swallowing.

Swallowing and ingestive behaviors are likely developed in utero in all mammalian species in which there is significant fetal fluid production (urine and lung liquid) into an amniotic cavity. One may suggest that fetal development of this physiological behavior serves only to provide a functional system during the neonatal period. However, as will be described, in utero swallowing contributes importantly to several critical developmental processes, including the regulation of amniotic fluid volume and composition, the acquisition and potential recirculation of solutes from the fetal environment, and the maturation of the fetal gastrointestinal tract. Furthermore, swallowing responses to putative dipsogens (e.g., hypertonicity, angiotensin II (ANG II)) have been demonstrated in the near-term ovine fetus. Thus the development of dipsogenic regulatory mechanisms may occur during fetal life, particularly in precocial species, and may be susceptible to changes in the pregnancy environment. This review describes what is currently known regarding the in utero development of ingestive behavior and the importance of this activity for fetal and perhaps ultimately adult fluid homeostasis.
production and resorption. After embryogenesis, the fetus excretes significant volumes of urine into the amniotic cavity and, in species such as sheep, to the allantoic cavity (a fetal membrane-enclosed diverticulum developed from the hindgut and attached to the fetal bladder via the urachus). Fetal urine flow represents the single most important factor regulating amniotic fluid volume, as marked increases or decreases in urine flow rates are correlated with polyhydramnios (65, 143) and oligohydramnios (56), respectively. Relative to urine flow, proportionately smaller volumes of lung fluid are secreted into the amniotic cavity. Although there are gestational changes in amniotic fluid volume, a near balance of fetal fluid production and resorption results only in small daily fluid volume changes. Fetal swallowing serves as a major route for amniotic fluid resorption, recirculating water and solutes to the fetus. In addition, sheep, subhuman primates (43), and perhaps humans also absorb amniotic fluid via an intramembranous pathway. The intramembranous pathway has been defined in the ovine model as water flux across the amnion membrane into the fetal vasculature lining the chorionion (41), or, in humans, across the amnion into the fetal vasculature of the placenta.

Ultimately, fetal fluid acquisition for body growth and amniotic fluid volume results from maternal to fetal transplacental water and electrolyte transfer, although this averages only 20–30 ml/day throughout the course of human gestation. Simultaneous with the excretion of ~1,000 ml/day of urine and lung fluid, the near-term ovine or human fetus swallows 500–1,000 ml/day, volumes that far exceed that normally acquired across the placenta. Fetal swallowing represents a major route of amniotic fluid resorption, serving to recirculate urine and lung fluid volumes to the fetus. Nevertheless, the fetus is not dependent on ingested fluid to maintain body water homeostasis. Ovine fetal esophageal ligation does not affect fetal urine flow rate (37, 121), and external drainage of amniotic fluid does not impact fetal urine flow or lung fluid production rates (40). Thus net maternal to fetal placental water flow (providing for fetal fluid production) is able to markedly increase under these conditions (74, 138) to compensate for absent or reduced swallowing (66). The composition of swallowed fluid may impact on fetal fluid dynamics, however, as a result of gastrointestinal fluid absorption and fetal responses to altered plasma composition. Thus intraruminal infusions of isotonic saline do not significantly alter fetal plasma osmolality or fetal urine flow, although infusions of hypertonic saline or water induce significant decreases and increases in urine flow rate, respectively (111, 112).

One may further examine the dynamics of amniotic fluid electrolytes, resulting from both urine and lung fluid production. Whereas intramembranous flow has been accepted as a route of water exchange, the transfer of electrolytes across the amniotic membrane is less clear (42, 59). Notably, should intramembranous transfer of electrolytes occur, the gradient of sodium and chloride between the fetal blood and amniotic fluid would favor transfer into the amniotic cavity. Thus swallowing likely represents the only route for fetal resorption of the principal amniotic fluid electrolytes. These concepts suggest that spontaneous fetal swallowing must occur in all species in which the fetus is producing significant quantities of fluid (i.e., urine or lung liquid).

Despite the considerable evidence of the role of fetal swallowing in amniotic fluid volume regulation, the association of reduced human swallowing and excess amniotic fluid (polyhydramnios) remains controversial. Human studies have primarily used the absorption of tracers injected into the amniotic cavity for determination of swallowed volume. In anomalous human anencephalic infants, swallowing was determined by absorption of intra-amniotic radioactive colloidal gold (2). Eight of nine fetuses demonstrated polyhydramnios, and minimal swallowing (~25 ml/day) was measured in five of eight cases. However, the one fetus with normal amniotic fluid also demonstrated reduced swallowing (10 ml/day). Although the author concluded that anencephaly is associated with polyhydramnios independently of fetal swallowed volume, it is more likely that a net balance of alterations in other sites of fluid exchange obscured the effect of absent swallowing in several cases. Conversely, increased amniotic fluid volume is not found invariably in human fetuses with anencephaly, as some of these fetuses may be capable of normal swallowing activity (2). Another controversy surrounding the role of fetal swallowing in amniotic fluid volume regulation has been the finding that many, although not all, human fetuses with esophageal atresia develop polyhydramnios. In an early publication, Lloyd and Clatworthy (73) described 220 cases of alimentary tract obstruction, including 53 infants with esophageal atresia. The observation that only 7 of these 53 pregnancies (14%) developed polyhydramnios has continued to influence the question as to whether impaired swallowing contributes to disturbances of amniotic fluid volume regulation. Notably, there was no report of the type of esophageal malformations in these infants, a factor that may have significant impact on the amniotic fluid consequences. As >90% of all esophageal atresia cases are associated with a fistula connecting the lower esophageal segment with the trachea, fetal lung liquid or inspired amniotic fluid may be directed into and absorbed by the gastrointestinal tract. Thus the presence of this fistula suggests that polyhydramnios should be expected in only a proportion of esophageal atresia cases. Similarly, additional case reports of fetuses with esophageal atresia and normal amniotic fluid volume have not differentiated those fetuses with tracheal-esophageal fistula. Swallowed amniotic fluid is likely absorbed in the proximal small intestine, as evidenced by the report that 23 of 35 (66%) infants with atresia or high-grade stenosis of the proximal jejunum or duodenum developed polyhydramnios (73). Animal studies similarly suggest an important role of fetal swallowing in amniotic fluid volume regulation. Esophageal ligation of fetal monkeys results in acute
polyhydramnios (87), and esophageal occlusion of fetal sheep produces a marked increase in ovine amniotic fluid volume (37). However, amniotic fluid volume normalized after 2–3 wk in monkey fetuses with esophageal ligation, and chronic (3 wk) esophageal ligation of ovine fetuses did not change amniotic fluid volume from normal controls (135). Notably, neither fetal urine flow nor lung fluid productions were monitored in studies demonstrating normalization of amniotic fluid volume (87, 135). Although fetal urine flow rate may not change in response to esophageal ligation (135), it is possible that fetal urine or lung fluid secretion decreased in esophageal-ligated fetuses [as a result of fetal stress responses to the surgical preparation (37)], accounting for the normalization of amniotic fluid volume. Alternatively, the return to normal amniotic fluid volume in animal fetuses with esophageal ligation may represent adaptive fetal responses, with absorption of additional fluid via alternative sites, such as intramembranous water resorption (41, 59).

FETAL SWALLOWING: SOMATIC AND GASTROINTESTINAL DEVELOPMENT

Fetal swallowing contributes importantly to somatic growth and gastrointestinal development as a result of the large volume of ingested fluid. Pitkin and Reynolds (100) estimated that 10–15% of fetal nitrogen requirements result from swallowing of amniotic fluid protein. Amino acids and glucose are absorbed and used by the fetus if they are administered into the fetal gastrointestinal tract (22, 23). Furthermore, intragastric ovine fetal nutrient administration partially ameliorates fetal growth retardation induced by maternal malnutrition (21). Intra-amniotic nutrient administration does not reverse fetal growth retardation resulting from oxygen deprivation, however (36). Further evidence for the role of swallowing in fetal growth results from studies demonstrating that impairment of fetal rabbit swallowing at 24 days gestation (term = 31 days) induces an 8%-weight decrease (compared with controls) by 28 days (133). The fetal gastrointestinal tract is directly impacted, as esophageal ligation of fetal rabbit pups results in marked reductions in gastric and intestinal tissue weight and gastric acidity (92). Reductions in gastrointestinal and somatic growth were reversed by fetal intragastric infusion of amniotic fluid (92). Similarly, esophageal ligation of 90-day ovine fetuses (term = 145–150 days) induces a 30% decrease of small intestine villus height (131) and a reduction in liver, pancreas, and intestinal weight (5). Although ingestion of amniotic fluid nutrients may be necessary for optimal fetal growth, trophic growth factors within the amniotic fluid also importantly contribute. Thus the reduction in fetal rabbit weight induced by esophageal ligation is reversed by gastric infusion of epidermal growth factor (91). Similarly, oral epidermal growth factor accelerates stomach growth of neonatal rats, compared with control littermates (34). Studies in human infants support the association of fetal swallowing and gastrointestinal growth, as upper gastrointestinal tract obstructions are associated with a significantly greater rate of human fetal growth retardation compared with fetuses with lower gastrointestinal obstructions (25, 99).

HUMAN FETAL SWALLOWING

Despite its importance, fetal swallowing has been relatively inaccessible to study in human fetuses. Early human studies used techniques no longer acceptable. In 1963, McLain (84) performed X-ray amniographs of human fetuses and demonstrated increased gastrointestinal motility and increased propagation of gastrointestinal contents with advancing gestational age. In 1965, Pritchard (102) injected radioactive chromium-labeled red blood cells into the amniotic fluid and measured chromium content in amniotic fluid at cesarean section and chromium recovered from infant diapers during the first five days of life. Results demonstrated that the term fetus swallows 155 ml · day⁻¹ · kg⁻¹, with a range of 72–262 ml · day⁻¹ · kg⁻¹ (102). In 1970, Abramovich (2) injected colloidal gold into the amniotic cavity of pregnant women throughout gestation. The 18-wk fetus swallowed only 4–11 ml/day (18–50 ml · day⁻¹ · kg⁻¹), although there was a marked increase in volume swallowed with advancing gestation. In a later report (3), near-term fetuses were demonstrated to swallow 68 ml · day⁻¹ · kg⁻¹. However, only 7.7–47.1% of gold was recovered from the neonate. Thus this volume of fetal swallowing may be an underestimate. Similarly, radio-labeled proteins have been injected into the amniotic cavity of women at term, with evidence that at least two-thirds of the amniotic fluid volume was cleared of protein per day and the volume of amniotic fluid swallowed by the fetus correlated directly with the volume of fluid within the amniotic cavity (45). Our laboratory recently developed a mathematical model of human amniotic fluid dynamics (77). The model results are consistent with the aforementioned human studies in demonstrating a marked increase in fetal swallowed volume with increasing gestational age. Furthermore, the model indicates that an increase in swallowing relative to urine and lung liquid production accounts for the reduction in amniotic fluid volume near term and post term. More recent studies using prenatal ultrasound have confirmed that the human fetus chews, swallows, and even regurgitates during intrauterine life (11).

FETAL SWALLOWING IN ANIMAL MODELS

In view of the limited human fetal studies, the majority of our information on fetal swallowing has resulted from animal experiments. As early as 1881, Wiener (134) injected calcium ferrocyanide solutions into the amniotic cavity of rabbit and dog fetuses and detected the chemical in the wall of the fetal stomach and intestines. Similarly, in 1921, Wislocki (137) demonstrated fetal absorption of trypan blue when injected into the amniotic cavity of guinea pigs and cats. Whereas both of these earlier studies employed animals under general anesthesia, Becker et al. (9) demonstrated swallowing of radiographic contrast material in the
nonanesthetized fetal guinea pig. Studies in the ovine fetus have demonstrated marked similarities with human fetal swallowing behavior and rates of fluid ingestion. In 1973, Bradley and Mistretta (15) recorded swallowing of 16–44 ml·day\(^{-1}\)·kg\(^{-1}\) using an electromagnetic flow probe on the ovine fetal esophagus. Greater ovine fetal swallowed volumes (46–278 ml·day\(^{-1}\)·kg\(^{-1}\)) were measured in late-gestation fetuses with a technique of esophageal cannulation and fluid recirculation (53). Tracer dilution studies have reported ovine fetal swallowed volumes of 200–400 ml·day\(^{-1}\)·kg\(^{-1}\) near term (128, 129) and demonstrated an increase in swallowed volume in direct relationship with gestational age or fetal weight. Notably, tracer studies performed in animal models typically estimate swallowed volume by the disappearance of labeled tracer from the amniotic fluid, rather than the appearance in the fetal compartment, and may overestimate the volume swallowed (94). This may result from detachment of the label (and absorption into the fetus via intramembranous flow) or adherence of the label to fetal skin or amniotic membranes. Nevertheless, these studies confirm the similarity of ovine and human fetal swallowed volumes. Tracer dilution studies have further suggested that spontaneous changes in ovine amniotic fluid volume correlate inversely with the rate of fetal swallowing (130), and experimentally increased amniotic fluid volume is associated with marked increases in swallowed volume (12). Similar chemical dilution studies in preterm and near-term fetal bovines confirm swallowed volumes of 195 and 261 ml·day\(^{-1}\)·kg\(^{-1}\) (17). Although volume ingested could not be quantified, injection of iron dextran into the amniotic sac of rabbit fetuses results in brown staining of gastric contents (38). Thus spontaneous fetal swallowing has been demonstrated in all species in which it has been examined.

Our laboratory has been interested in the development and regulation of ovine fetal swallowing for several years. We sought to address several important questions including: What factors regulate spontaneous fetal swallowing activity? Is fetal swallowing activity responsive to intrauterine stimulation or suppression? Are there intrinsic changes in the regulation and rates of fetal swallowing from preterm to near term? As will be discussed, our studies have demonstrated the function of putative dipsogenic mechanisms in the developing ovine fetus and we have recently initiated studies to explore the development of the central neural pathways for dipsogen-mediated swallowing.

**SPONTANEOUS OVINE FETAL SWALLOWING**

We developed a chronic preparation (118) for the quantification of ovine fetal swallowing using electromyogram wires placed on the fetal thyrohyoid muscle and the nuchal and thoracic esophagus and an ultrasonic flow probe placed around the fetal thoracic esophagus (Fig. 1). Additional maternal and fetal vascular and amniotic fluid catheters, as well as fetal electrocortical electrodes, are commonly used. Electromyogram signals are electronically integrated, and signals are digitized. Computer analysis of the integrated electromyogram waveforms detects individual muscle contractions and subsequently defines a “swallow” as a timed sequence of waveform progression from the thyrohyoid to the thoracic esophagus (Fig. 2). The volume swallowed is measured by the integral of the flow velocity waveform from the ultrasound flow meter and expressed as volume per swallow or volume per time.

To determine the rate of spontaneous swallowing activity and volume swallowed in near-term fetuses, swallowing was recorded for 12-h periods in 128-day gestation ovine fetuses (118). Swallowing electromyogram activity averaged 43 swallows per hour with bouts of swallowing of 2-min duration occurring on average every 28 min. The volume swallowed averaged 35 ml/h, extrapolating to 840 ml/day. Thus the near-term ovine fetus swallows ~0.9 ml/swallow. These studies confirmed that daily swallowed volumes of 100–300 ml/kg body wt in the ovine fetus are markedly greater than adult values of 40–60 ml/kg. The periodicity of bouts of swallowing activity was consistent with the observations of Harding et al. (54) describing the association of laryngeal adductor muscle activity with fetal breathing and low-voltage electrocortical (ECoG) activity.

In addition to amniotic fluid, the fetus swallows a significant volume of lung fluid and perhaps additional salivary secretions. Fetal lung liquid production aver-
ages 60–100 ml·day⁻¹·kg fetal wt⁻¹ near term (86), with 50% of secreted lung liquid swallowed and 50% entering the amniotic cavity (13). The role of secreted fetal lung liquid in the stimulation of fetal swallowing activity remains uncertain. As reported by Harding et al. (54) and confirmed by our laboratory, breathing movements are invariably present during bouts of swallowing. As lung liquid is released through an open glottis during respiratory activity and pharyngeal fluid may stimulate fetal swallowing (20, 54), it is likely these mechanisms account in part for the ingestion of lung liquid. Although Harding et al. (54) were unable to demonstrate a change in fetal swallowing activity when tracheal fluid was diverted away from the pharynx, swallowed volume was not determined. Furthermore, as swallowed lung liquid may represent ≤20% of total swallowed fluid (13), it will be difficult to discern a change in swallowing activity or volume when this stimulus is altered.

Rhythmic changes between high- and low-voltage ECoG activities are a distinct characteristic of near-term (>120-day gestation) ovine fetal neurobehavior as well as adult sleep. We sought to determine the association of ovine fetal neurobehavioral state with spontaneous swallowing activity. Spectral analysis of 24 h of swallowing and ECoG activity was used for determination of oscillation frequencies in near-term ovine fetuses (67). Similar to previous studies (122, 123), fetuses were observed in low-voltage (high frequency) ECoG 48% of time, high-voltage (low frequency) ECoG 42% of time, and an intermediate ECoG 10% of time. We noted a 45-min oscillation in high- and low-voltage cycling, consistent with previous studies of the near-term ovine fetus (123). Fetal swallowing averaged 54 ± 5 swallows/h, with 75% of swallowing occurring in low-voltage ECoG periods. Swallowing further demonstrated a 40-min oscillation of activity patterns, consistent with cycling between high and low voltage. The association of swallowing with low-voltage ECoG activity is consistent with human studies, indicating stimulation of fetal swallowing with induced shifts from quiet to active sleep (98). Factors that impact the behavioral state of the fetus (particularly the relative proportion of low-voltage activity) may therefore influence swallowing activity.

It remains unknown if the state of fetal ECoG regulates fetal swallowing or if a central state generator independently regulates ECoG, swallowing, and other fetal behaviors. To differentiate the regulation of ECoG activity from swallowing activity, we examined fetal swallowing and ECoG activities in response to intravenous atropine sulfate-induced central and peripheral anticholinergic stimulation. Central anticholinergic stimulation produces a fetal behavioral state characterized by high-voltage, low-frequency oscillations in the ECoG (6). Atropine sulfate induced a significant decrease in low-voltage ECoG activity (56 ± 5 to 14 ± 4%), an increase in the time spent in high-voltage ECoG (40 ± 5 to 81 ± 5%), and no change in intermediate ECoG (4 ± 1 to 5 ± 1%). Fetal swallowing activity decreased from 46 ± 12 to 12 ± 2 swallows/h after atropine sulfate, although there was no significant change in the swallowing activity per minute of each ECoG state (low voltage 1.2 ± 0.2; high voltage 0.2 ± 0.2; Intermediate 8.6 ± 6.6 swallows/min). Peripherally acting atropine methyl nitrate had no discernible effect on fetal ECoG or swallowing activity. These studies confirmed that central cholinergic antagonism alters fetal ECoG activity, likely an effect mediated at the level of the rostral pontine medulla (31, 75). Furthermore, spontaneous swallowing activity is intimately linked to the state of central activation that is reflected by the low-voltage ECoG state.

**PRETERM SPONTANEOUS FETAL SWALLOWING**

As noted above, human fetal studies show that the volumes swallowed are less in early compared with late gestation. We evaluated spontaneous swallowing activity and swallowed volume in preterm ovine fetuses (114 days gestation) monitored for a 12-h period (0700–1900). As expected, at this early gestational age there was no visual or spectral analysis differentiation of high- and low-voltage fetal ECoG activity. Fetal swallowing activity averaged 50 ± 9 swallows/h, a rate similar to that noted in near-term fetuses. However, swallowed volume averaged only 7 ± 1 ml·h⁻¹·kg⁻¹, volumes significantly less than in near-term fetuses, even when adjusted for fetal body weight. Despite the lack of differentiation in ECoG activity, swallowing activity was not continuous, exhibiting an apparent 30-min
REGULATION OF ADULT DIPSOGENESIS

Systemically, there are two principal mechanisms of adult body water regulation. Small increases in extracellular fluid osmolality (~2%) stimulate arginine vasopressin (AVP) secretion and resulting urinary antidiuresis and thirst. Somewhat larger decreases (~10%) in circulating volume initiate AVP-mediated antidiuresis and likely ANG II-mediated thirst. Physiological and neuroanatomic studies in several adult species indicate that both systemic osmotic and ANG II dipsogenic effects are mediated within brain circumventricular organs (CVOs), small midline organs positioned on the surface of the cerebral ventricles and interfacing with the subarachnoid, ventricular, and vascular spaces (Fig. 3). Having fenestrated capillaries, the CVOs are in a unique position in the central nervous system to function as receptor sites to central neural signals, cerebrospinal fluid (CSF) signals, and transduction of blood-borne signals to neural signals. CVOs are known to participate in central control of salt and water balance and are likely to regulate autonomic, endocrine, and even behavioral functions of fluid homeostasis.

Putative systemic dipsogens (hypertonicity, ANG II) initiate responses via CVO “osmoreceptors” or ANG II receptors. Consistent with the location of CVOs, CSF hypertonicity or ANG II also evokes dipsogenic responses, although the site of action remains unclear. The precise role of the CVOs has been delineated in adult animals through a series of directed cerebral microinjections and selective nuclei ablations. In sheep and rats, the organum vasculosum of the lamina terminalis (OVLT) as well as the subfornical organ (SFO) respond to osmotic stimuli, whereas the SFO appears to be a putative site for ANG II-induced ingestive behavior (18, 81, 120). Neural efferents from both the OVLT and SFO evoke thirst and AVP release, and it is thought many of these efferents first synapse in the median preoptic nucleus (MnPO). The OVLT and SFO are intimately connected with one another and with the immediately adjacent MnPO, which lies inside the blood-brain barrier. The extensive neural interconnectivity of the OVLT, MnPO, and the SFO suggests a highly active local circuit for processing information from inputs derived from the rostral sensory CVOs. Furthermore, anatomic evidence indicates the supraoptic nucleus (SON) and both the parvicellular and magnocellular components of the paraventricular nucleus (PVN) receive afferents from each of the sensory CVOs as well as from neuroendocrine “modulators” within the blood-brain barrier (e.g., MnPO, parabrachial nucleus, and nucleus of the solitary tract), with extensive projections and interconnections. Efferents from the OVLT, SFO, and MnPO project to several preoptic hypothalamic regions and neurosecretory nuclei implicated in the control of ingestive behavior and fluid homeostasis (63).

The SFO is a well-established ANG II target site characterized by a highly dense ANG II receptor population (120). In addition, SFO ANG II-containing neural cells project to the MnPO, OVLT, SON and PVN nuclei (47, 48, 124, 142), medial septum, and anterior hypothalamus. The OVLT is ventral to the SFO, lying immediately dorsal to the optic chiasm. Both CVOs have efferent projections to the PVN and SON, lateral hypothalamus, arcuate nucleus, central gray, and the locus ceruleus, along with angiotensinergic pathways synapsing in the MnPO (47). In adult sheep and rats, lesions of the anteroventral third ventricle region (AV3V) result in a period of adipsia (4, 62, 76, 80, 82) and impaired AVP secretion (61). Ablation of any single nucleus does not evoke the degree of adipsia associated with complete AV3V lesions, indicating integrated circuits and potential roles of other nuclei as osmolality/sodium sensors. In adult sheep, lesions of the OVLT impair drinking responses to intracarotid hypertonic saline but not in response to ANG II administration (81). Similar OVLT lesions in adult dogs impair both hypertonic saline and ANG II dipsogenic responses, illustrating both species variation as well as possible neural pathways linking the SFO and OVLT (127). Similarly, ablation of ovine SFO, OVLT, and MnPO severely reduces AVP secretion in response to hypertonicity (80).

Despite extensive light and electron microscopic studies of the OVLT and SFO (69, 79, 83), limited studies have characterized the MnPO, a cluster of neurons within the rostral periventricular preoptic nucleus and caudal to the OVLT (64). Studies in the rat indicate the MnPO projects to both the OVLT and SFO (55), with a sparse projection to the medial part of the parvicellular division of the PVN and a more substantial projection to the magnocellular paraventricular and supraoptic nuclei. The MnPO receives major efferent projections from the SFO (72, 88), OVLT (19), and from the nucleus of the solitary tract (103). Lesions that encompass the MnPO but leave the OVLT and SFO intact abolish ANG II-induced thirst elicited by either systemic or central...
administration (64, 141). Because both anatomic and functional evidence indicates that the OVLT and SFO are reciprocally tied to components of the AV3V, particularly the MnPO, it is reasonable to believe that substantial processing of central input derived through the lamina terminalis-associated CVOs occurs within the MnPO and other periventricular nuclei surrounding the optic recess (63).

Recent studies using expression of the immediate early gene, c-fos, have confirmed the role of the CVOs, MnPO, and AVP-containing hypothalamic neurosecretory neurons in osmoregulation. Early reports indicated both c-fos mRNA and Fos were induced in the SON and PVN and lamina terminalis in response to intraperitoneal or subcutaneous injection of hypertonic saline in adult rats (44, 49, 57, 117). This expression of Fos occurs in magnocellular neurons, indicating that osmoregulatory neural pathways subserving the secretion of vasopressin (and oxytocin in the rat) may have been activated (44, 57). However, injection of hypertonic saline into the peritoneum or subcutaneously is a painful procedure, and the stress associated with the injection may have influenced the results (117). Recently, Fos levels have been shown to increase in certain neurons in response to systemic hypertension (44, 50, 96) or chronic hypertonic saline loading (117). Systemic hypertension in rats resulted in a concentration-related increase in the intensity of Fos expression in the OVLT, MnPO, SFO and SON, and PVN (96).

Systemic infusion of ANG II also increases Fos expression in neurons throughout the lamina terminalis and in neurosecretory sites in the hypothalamus (49, 78, 95). These studies have provided a means of identifying, through histochemical methods, cerebral nuclei that specifically participate in osmoregulatory mechanisms.

Despite the recognition of the CVOs as a critical regulatory site for fluid homeostasis, there remain controversies regarding the role of sodium versus osmoreceptors, as well as the location and sensitivity of receptors in relationship to the blood-brain barrier. Differences among species have contributed to the controversy. In adult sheep, a dual system of central nervous system osmoreceptors and sodium sensors likely mediates dipsogenic responses to hypertonic sodium chloride. Osmoreceptors, rather than sodium receptors, have been postulated to be predominant in the dog (126). Osmoreceptors are apparently more sensitive to systemic versus central hypertonicity, although this may relate more to the distance from systemic vessels versus the ventricular wall (126). Alternatively, an integrated system of a more-sensitive plasma osmoregulatory system, which responds to potentially rapid systemic changes, and a less sensitive central regulatory system, combining both sodium- and osmolality sensitive cells, may provide the optimal homeostatic control.

Controversy persists as well in the role of systemic versus central ANG II, further clouded by the mixture of central ANG II thirst and sodium appetite effects (58). Although there is evidence that systemic ANG II is an important mediator of thirst in rats and dogs, this remains controversial (97), because several studies have failed to demonstrate increased ingestive behavior after exogenous ANG II (97, 106). In comparison, only central ANG II appears to stimulate dipsogenic responses in sheep (1). Suppressive effects of exogenous ANG II-induced hypertension may obscure evidence of dipsogenic stimulation under experimental conditions, however (33). Notably, ANG II may contribute to dipsogenic stimulation primarily under conditions of reduced systemic pressure or volume. Evolutionary forces, including access to fresh water and sodium sources, may have been etiologic in species differences.

**REGULATION OF FETAL DIPSOGENESIS**

The finding of spontaneous in utero swallowing indicates the distal steps in dipsogen-mediated swallowing (motoneuron and ingestion muscle integration) are intact before birth. However, the timing of development of proximal dipsogenic mechanisms (i.e., osmotic and ANG II-sensing mechanisms, neural pathways from the OVLT, SFO, and MnPO to motoneurons) is unknown. Despite the demonstrated high rates of spontaneous fetal swallowing, it remains unclear whether swallowing may be stimulated in utero. Human embryos demonstrate taste buds by 7 wk of gestation (16), and earlier experiments suggested increased human fetal swallowing after intra-amniotic injection of saccharin (28) and decreased human fetal swallowing in response to a noxious substance (70); similar aversion to bitter substances has been noted in newborn, but not fetal sheep (89).

In the neonatal rat, an altricial species, dipsogenic mechanisms apparently develop after birth. Wirth and Epstein (136) demonstrated a consistent pattern of development of neonatal thirst mechanisms, with responses to cellular dehydration developing at 2 days, hypovolemia at 4 days, and β-adrenergic stimulation at 6 days of life. Similarly, responses to central (intracerebroventricular) administration of putative dipsogens display a pattern of development with increased drinking after central ANG II at 2 days, carbachol at 4 days, and norepinephrine at 9 days (32). Thus rats develop responsiveness to thirst stimuli at least 2 wk before the time before water is normally encountered and long before it is necessary for survival.

One may initially interpret these data to suggest that dipsogenic mechanisms do not develop until after birth. However, 4-h-old rats will ingest increased liquid in response to elevated skin temperature, although this response abates between 1 and 4 days of age (136). Furthermore, nearly all neonatal rats show some response to ANG II, with statistical significance demonstrated on day 4 (136). Certainly, the marked endocrine and cardiovascular changes involved in parturition and neonatal transition may have modulatory influences on dipsogenic responses, because both hypertension (33) and hypotension (35, 114) suppress thirst stimulation. Thus functional dipsogenic mechanisms may develop in the fetal rat, although not be exhibited until after the immediate neonatal period. Nevertheless, if one ac-
cepts Epstein’s conclusions (136), the demonstration of spontaneous swallowing in early gestation fetuses and the sequential development of ANG II, osmotic, and adrenergic dipsogenic stimulation later in gestation suggest the critical or final link in the development of dipsogen-stimulated swallowing is a proximate step in the neural pathway. This critical step may involve the appearance of ANG II receptors or sensitivity of osmoreceptor cells, including postreceptor cellular signals, transmission via efferent neurons, and/or postsynaptic sensitivity. Although not truly precocial, the ovine and human fetus demonstrate a greater degree of in utero development compared with the fetal rat. Thus it is possible dipsogenic responses develop in utero in these species, potentially to provide thirst stimulation for appropriate water intake during the immediate neonatal period.

We sought to determine the ovine fetal swallowing response to the putative dipsogens ANG II and plasma hypertonicity. Intravenous infusion of ANG II (100 ng·kg⁻¹·min⁻¹) to near-term fetuses resulted in a significant increase in fetal systemic arterial blood pressure. Plasma ANG II concentrations were likely increased to ~400 pg/ml (105), a level well above the proposed 200 pg/ml plasma dipsogenic threshold in adult rats and dogs (60). However, there was no change in swallowing activity (0.85 swallows/min, 0.31 ml·min⁻¹·kg⁻¹) (113). These results are consistent with the lack of plasma ANG II dipsogenic stimulation in adult sheep, although the lack of swallowing stimulation also may result in part from the suppressive effects of hypertension (106). It is of interest that central, although not peripheral, ANG II stimulates thirst in adult sheep. As noted above, central ANG II likely stimulates thirst sensation by binding to CVO sites, particularly the SFO. Whether the ovine SFO or entire blood-brain barrier has a reduced permeability to systemic ANG II is unknown. However, renin-releasing stimuli (e.g., hypotension) may theoretically contribute to dipsogenic stimulation in sheep as a result of increased plasma and central angiotensin I, with central ANG II likely to dipsogenic stimulation in sheep as a result of increased plasma and central angiotensin I, with central conversion of angiotensin I to ANG II (29).

Intravenous injection of hypertonic sodium chloride to near-term ovine fetuses resulted in a marked increase in plasma osmolality (292 to 306 mosmol/kH₂O) and an acute stimulation of fetal swallowing activity (Fig. 4) (113). Although plasma osmolality remained elevated to levels 3–4% above basal osmolality, swallowing was stimulated only briefly. As noted previously, this degree of plasma hypertonicity typically induces more prolonged ingestive behavior in adult animals (139). Although these were the first studies to demonstrate an intact, functional osmotic dipsogenic mechanism in the developing fetus, the results suggested a relative insensitivity of the fetus to osmotic dipsogens, compared with the adult.

To further explore osmotic dipsogenic mechanisms, a model of intracarotid hypertonic sodium chloride injections was developed. Repeated injections (0.15 ml/kg body wt) of increasing hypertonic saline concentrations were administered, with a threshold concentration defined as the lowest saline concentration to stimulate swallows after four of five injections. Studies in near-term fetuses demonstrated a mean sodium chloride concentration threshold of 0.56 M (107), with increased plasma AVP demonstrated at a lower saline concentration than that required for stimulation of swallowing (107). Identical injections of equiosmolar hypertonic urea resulted in increased fetal plasma AVP, although no change in swallowing activity. Together, these results are consistent with a model of discrete fetal osmoreceptors for thirst and AVP secretion and a heightened osmotic sensitivity for AVP secretion compared with swallowing. Although the relative osmotic threshold for adult human "thirst" sensation versus AVP secretion remains controversial (125), the higher threshold thirst is consistent with most studies (104). Furthermore, it must be recognized the ovine studies assess swallowing, rather than "thirst" sensations. Teleologically, it would be desirable for nonconscious activities, such as AVP-mediated renal antidiuresis, to respond to mild dehydration before conscious, behavior-modifying water seeking and ingestive activities resulting from thirst.

In a recent study of near-term ovine fetuses, we demonstrated that induced fetal plasma hypotonicity (295 ± 2 to 278 ± 3 mosmol/kH₂O) significantly increased the intracarotid saline concentration required for stimulation of swallowing (0.77 to 1.03 M) (unpublished observation). On the assumption of a linear extrapolation of swallowing versus plasma osmolality, we calculated an effective plasma osmolality of 338 mosmol/kH₂O for stimulation of fetal swallowing. Consistent with this threshold, chronic fetal plasma osmolality increases of 10 mosmol/kH₂O (due to maternal dehydration) do not stimulate fetal swallowing (115).
One may speculate on a teleological explanation for the increased fetal plasma osmolality thresholds for thirst stimulation. Notably, human and rat maternal plasma osmolality decreases by 8–10 mosmol/kgH2O during pregnancy, accompanied by an equivalent resetting (lower) of maternal plasma osmolality thresholds for thirst as well as AVP secretion. Fetal plasma osmolality is regulated by maternal levels; thus maternal hypotonicity results in fetal plasma hypotonicity. If one postulates that fetal plasma osmolality thresholds for thirst and AVP secretion are not reset (in relationship to the neonate), then a far greater increase in fetal osmolality must occur to induce thirst and hence swallowing. This mechanism would serve to protect the amniotic fluid volume from intermittent maternal dehydration, although additional fluid resorption may occur via the intramembranous pathway in response to increasing osmolar gradients. Although maternal plasma hypertonicity would result in fetal plasma hypertonicity and a fetal-to-maternal water transfer, maternal thirst and antidiuretic responses would be evoked before fetal swallowing and renal responses, preserving amniotic fluid volume. However, previous studies indicate that maternal sheep do not lower plasma osmolality during pregnancy (10). Furthermore, the ruminant may tolerate prolonged periods of water restriction associated with dehydration and plasma hypertonicity. To preserve normal amniotic fluid volume under these circumstances, it would be advantageous if the ovine fetus maintained a relatively high threshold for dipsogenic stimulation. Whether human fetuses similarly display an elevated osmotic threshold is unknown.

Alternatively, one can interpret the available data to suggest that fetal osmoreceptor cells are perhaps less sensitive to prolonged (minutes) or chronic (hours to days) hypertonicity. Osmoreceptors, believed to be located in the OVLT, are likely to respond to anisosmotic conditions with acute cellular volume changes. Under hypertonic conditions, cell regulatory volume changes may minimize osmotic stimulation by uptake of inorganic ions and a more gradual accumulation of organic solutes (85). Possibly, fetal osmoreceptor cells have enhanced volume regulatory processes (or relatively leaky membranes) such that compensatory processes modify responses after acute hypertonic stimulation.

To assess whether dipsogenic responses to central stimuli are functional in utero, central ANG II injections into the lateral ventricle of near-term fetuses induced a nearly threefold increase in swallowing activity (1.2 ± 0.1 to 3.3 ± 0.6 swallows/min of low-voltage ECoG) and a marked increase in plasma AVP (108) (Fig. 5). As noted previously, the stimulation of swallowing in response to central, although not systemic, ANG II is consistent with studies in adult sheep. Studies in the rat have demonstrated two different angiotensin receptor subtypes in peripheral tissues: AT1 and AT2. AT1 receptors have been localized to brain areas outside (e.g., SFO, OVLT) and inside (e.g., periventricular nucleus of the hypothalamus) the blood-brain barrier and are related to fluid and electrolyte homeostasis, blood pressure regulation, and AVP secretion (132). Recent studies have indicated a primary role of AT1 receptors in the regulation of adult drinking responses, with a potential inhibitory role of AT2 receptors (7, 30). Although the fetal brain has not been specifically studied, the developing rat fetus transiently expresses significant numbers of mesenchymal AT2 receptors, which decrease after birth (46). Whether AT2 receptors contribute to the inhibition (i.e., elevated thresholds) of fetal ingestive responses to osmotic dipsogens is unknown.

To confirm central osmotic dipsogen-mediated swallowing, hypertonic sodium chloride in artificial CSF was infused into the lateral cerebral ventricular cavity in near-term fetal sheep (109). As would be expected from swallowing responses to systemic hypertonicity, fetal swallowing activity significantly increased from 1.4 ± 0.4 to 2.9 ± 0.5 swallows/min of low-voltage ECoG, and plasma AVP increased (9.1 to 24.2 pg/ml) during the control period in response to intracerebroventricular injection of artificial cerebrospinal fluid (aCSF) and in response to intracerebroventricular injections of increasing angiotensin II (ANG II) concentrations in aCSF (0.1–500 ng/kg). Plasma AVP samples were obtained 15 min after each intracerebroventricular injection. *P < 0.05 vs. control period. [Modified from Ross et al. (108).]

**Fig. 5.** Ovine fetal swallows per minute of low-voltage (LV) electrocorticogram (top) and plasma arginine vasopressin (AVP) levels (bottom) during the control period in response to intracerebroventricular injection of artificial cerebrospinal fluid (aCSF) and in response to intracerebroventricular injections of increasing angiotensin II (ANG II) concentrations in aCSF (0.1–500 ng/kg). Plasma AVP samples were obtained 15 min after each intracerebroventricular injection. *P < 0.05 vs. control period. [Modified from Ross et al. (108).]
The near equivalence of the preterm saline threshold surrounding 114 days of ovine gestation. Further during the last one-third of gestation, in the systemic osmotic dipsogenic mechanism becomes functional near-term fetuses. These findings suggest that the threshold for stimulation in the responding fetuses was similar to that noted in the term fetuses suggests that dipsogenic mechanisms develop acutely, with an abrupt turn-on, rather than by a gradual increase in osmotic sensitivity.

The concept of an abrupt turn-on of dipsogenic responsiveness is consistent with the previously discussed hypothesis that the critical or final developmental pathway of dipsogen-specific stimulated swallowing is a proximate step in the neural pathway, potentially the sensitivity or intracellular signaling of osmoreceptor cells, transmission via efferent neurons, and/or the development of postsynaptic mechanisms.

We have recently initiated studies exploring the development of neural mechanisms central to fetal dipsogenesis (unpublished data). In view of the previously discussed finding that the fetus may have a reduced sensitivity to osmotic stimuli compared with the adult, we examined ovine fetal and adult central neural pathways for osmotic stimulation and responses. Nonpregnant ewes and near-term (130 days) fetuses were chronically prepared with vascular and intraperitoneal catheters. After recovery, intraperitoneal infusions of hypertonic (1.5 M) sodium chloride were administered to nonpregnant ewes (18 ml/kg) and fetuses (6 ml/kg). Animals were killed and brain tissue obtained 60 min after intraperitoneal NaCl, after which Fos immunocytochemistry was performed to identify neurons activated by hypertonicity and AVP counterstaining was used to identify the subpopulation of activated neurons in the SON and PVN. Despite a lesser increase in fetal versus adult plasma sodium concentration (140 to 145, 147 to 163 meq/l, respectively), hypertonic saline-treated fetuses exhibited similar patterns of intense Fos activation in the OVLT, SFO, MnPO, SON, and both the parvocellular and magnocellular subdivisions of the PVN. In both ewes and fetuses, AVP counterstaining revealed that 65-70% of AVP-containing neurons in the SON and PVN were activated by hypertonic challenge. These results indicate intact fetal neural pathways from the CVOs to the SON and PVN and suggest that the relative fetal insensitivity to osmotic dipsogenic stimuli is not due to reduced neuronal populations.

DEVELOPMENT OF FETAL DIPSOGENSE

Human and animal studies confirm the relatively high rates of fetal spontaneous swallowing activity and volume during the second half of gestation, and our studies demonstrated dipsogenic responsiveness in the near-term ovine fetus. However, there was no information as to the timing or mechanism for the development of osmotic dipsogenic responses. In recognition that the ovine gestational period of 110–120 days was marked by maturation of several fetal endocrine systems and evidence of fetal electrocortical differentiation into low- and high-voltage periods, we elected to study preterm (114 days gestation) fetal swallowing responses to intracarotid sodium chloride injections. Despite injection concentrations far exceeding those administered to term fetuses, swallowing stimulation was demonstrated in only two of five preterm fetuses (68). Of note, in the responding fetuses the threshold for stimulation of swallowing (0.75 M) was similar to that noted in near-term fetuses. These findings suggest that the systemic osmotic dipsogenic mechanism becomes functional during the last one-third of gestation, in the period surrounding 114 days of ovine gestation. Furthermore, the near equivalence of the preterm saline thresh-
able to the fetus appears to impact to a greater degree on the volume swallowed than on the spontaneous swallowing activity. The reduction in fetal swallowing may be of value in the preservation of amniotic fluid volume during conditions of amniotic fluid loss or reduced fetal fluid production.

In utero, fetuses may be commonly exposed to hypoxic conditions, which have previously been demonstrated to alter fetal ECoG activity (shift to predominantly high-voltage ECoG) and induce a paradoxical decrease in fetal breathing activity. Hypoxia similarly has been demonstrated to effect an acute dose-dependent decrease in fetal swallowing activity and volume swallowed, correlating with the decrease in breathing activity (119) (Fig. 7). Prolonged (24 h) fetal hypoxia induced by maternal hypoxia or reduced uterine blood flow results in a suppression of swallowed volume (13, 14). In association with a return to normal pH, swallowing inhibition diminishes during the prolonged hypoxia (14). As noted above, spontaneous swallowing occurs predominantly during low-voltage ECoG activity and transitions between high- and low-voltage periods. Whether hypoxia-induced suppression of swallowing is secondary to the alteration in ECoG activity or directly affects neural centers regulating ingestive behavior is unknown. It is notable, however, that a reduction in swallowing activity and swallowed volume would tend to increase amniotic fluid volume. However, intrauterine growth retardation, often attributed to chronic fetal hypoxia, is generally associated with reduced amniotic fluid volume (36). These results suggest that amniotic fluid volume is more affected by hypoxia or growth retardation-related reductions in fetal urine flow than by reduced fetal swallowing. Nevertheless, much remains to be learned of the association of altered swallowing and amniotic fluid volume regulation. Similar to hypoxia, fetal hypotension induces a near-complete suppression of swallowing activity (114), consistent with the suppression of food and fluid intake in adult rats under hypotensive conditions. As central neurobehavioral changes also likely occur with hypotension, the alterations in spontaneous fetal swallowing activity may be secondary to regulatory influences by neural pathways other than those controlling dipsogenesis.

In view of the extremely high (relative to newborn or adult) basal fetal swallowing rates and the demonstration of hypertonicity-induced fetal swallowing, we postulated that a level of tonic dipsogenic stimulation is present in utero. To determine if plasma hypertonicity would suppress spontaneous fetal swallowing, we used a model of maternal and fetal plasma hypertonicity induced by maternal 1-desamino-8-D-Arg-vasopressin and oral water. In response to a 20-mosmol/kgH₂O decrease in plasma hypertonicity, near-term fetal swallowing decreased from 56 to 33 swallows/h (110). The suppression of spontaneous swallowing by plasma hypertonicity suggests that the increased basal rate of spontaneous swallowing activity in utero may be regulated, in part, by tonic osmotic stimulation.

**REGULATION OF FETAL SWALLOWING ACTIVITY: IMPRINTING**

Imprinting of physiological systems is increasingly recognized as a long-term effect of alterations of the in utero environment. Imprinting of several endocrine systems, including osmoregulation (AVP and thirst) (39, 52) and thyroid regulation (26, 101), has been well demonstrated in the fetal or neonatal rat. Recent human studies have provided support for the association of prenatal nutrition and adult-onset hypertension [i.e., Barker hypothesis (8)]. The demonstration of intact central and systemic dipsogenic mechanisms in utero has important implications for the imprinting of dipsogenic sensitivities. Prenatal sodium deprivation of pregnant rats results in increased water drinking of the adult offspring (90). Similarly, prenatal sodium deprivation results in reduced amniotic fluid in utero and increased water drinking of male offspring (24), whereas extracellular dehydration of rats results in an increased sodium appetite of adult offspring (93). Neonatal rat exposure to AVP results in a long-lasting decrease in renal AVP responsiveness (51) due to a reduction in AVP binding sites in the adult kidney (52). Vasopressin administration during the first month of life increases 24-h water intake and decreases urine osmolalities in diabetes insipidus rats 6 wk after cessation of treatment (140). These findings indicate that altered osmotic environments may modulate not only swallowing activity in utero but also the development of adult sensitivities for thirst and AVP secretion and...
AVP responsiveness. Thus the normal development of maternal, and thus fetal, plasma hyposmolality during human pregnancy (27) and the potential intermittent exposure to maternal dehydration, as a result of hyperemesis, thermal exposure, or exercise-induced water loss, may have important implications for fetal and adult dipsogenic regulation.

Perspectives

In summary, fetal swallowing activity contributes importantly to fetal and amniotic fluid homeostasis and fetal somatic and gastrointestinal development. Human and ovine fetal swallowing increases throughout gestation, resulting in swallowed volumes markedly greater (relative to body weight) than newborns or adults. Although the regulation of swallowing activity in early gestation is unknown, intact central and systemic dipsogenic mechanisms have been demonstrated during the last third of ovine gestation. Recent studies suggest that swallowing behavior may be modulated in accordance with neurobehavioral state changes and influenced by hypoxia, hypotension, and plasma osmolarity changes. Whether fetal swallowing also is regulated by the development of appetite sensation, salt appetite, or the development of taste is uncertain. Nevertheless, in precocial species, swallowing behavior develops in utero with potentially dramatic influences on the maternofetal pregnancy environment on the imprinting of regulatory mechanisms controlling ingestive behavior.

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REFERENCES

However, the text seems to be incomplete and contains many numbers and references that do not form a coherent document. It appears to be a page from a scientific journal with a list of authors and references, but the content is not legible due to the format and presentation.


