Nitric oxide modulates spontaneous swallowing behavior in near-term ovine fetus

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El-Haddad, Mostafa A., Conrad R. Chao, Sheng-Xing Ma, and Michael G. Ross. Nitric oxide modulates spontaneous swallowing behavior in near-term ovine fetus. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R981–R986, 1999.—Human and ovine fetuses demonstrate an enhanced rate of swallowing, an activity critical for amniotic fluid regulation. Fetal swallowing may be modulated by both systemic and central factors. Nitric oxide (NO) is a central neuromodulator that has been localized to brain regions regulating thirst and swallowing. We sought to determine if NO contributes to the regulation of spontaneous ovine fetal swallowing. Six time-dated pregnant ewes with singleton fetuses (129 ± 1 day) were chronically prepared with fetal vascular and lateral ventricle catheters and electrocorticogram (ECoG) and esophageal electromyogram electrodes. After a 2-h control period, fetuses were given lateral ventricle injection of NO synthase inhibitor nitro-L-arginine methyl ester (L-NAME) and monitored for 2 h. NO precursor L-arginine was then injected into the lateral ventricle, and fetuses were monitored for a final 2 h. All fetuses received an additional control study of fetal swallowing before and after lateral ventricle injection of artificial cerebrospinal fluid (aCSF). Data were analyzed with repeated-measures ANOVA and paired t-test (P < 0.05). Suppression of a central NO with central L-NAME significantly reduced mean (±SE) spontaneous fetal swallowing (1.2 ± 0.1–0.6 ± 0.1 swallows/min low-voltage ECoG; P < 0.01). Restoration of central NO by L-arginine significantly increased fetal swallowing to pre-L-NAME levels (1.2 ± 0.1 swallows/min low voltage). There were no changes in fetal swallowing during the control study of aCSF. Fetal ECoG activity and blood pressure did not change during the study or control aCSF injection. We conclude that NO is an important neuromodulator of fetal swallowing activity. Central NO synthase activity may contribute to the heightened level of spontaneous fetal swallowing and thus amniotic fluid regulation.

thirst; amniotic fluid; drinking

IN PRECOCIAL SPECIES, fetal swallowing develops in utero, contributing importantly to fetal gastrointestinal growth and development and preparing for newborn ingestive behavior. By the last one-third of ovine gestation, fetal swallowing is modulated similar to that of the mature adult. Thus systemic stresses, including hypoxia and hypertension (32, 36), suppress fetal swallowing. Conversely, fetal swallowing may be stimulated by systemic and central dipsogens (hypertonicity, ANG II) (30, 31), demonstrating an intact dipsogenic neural pathway near term. Despite the similar modulation of near-term fetal and adult dipsogen-mediated swallowing, spontaneous fetal swallowing is markedly different from that of the adult. Whereas the early gestation human fetus swallows only 100 ml/day, near-term fetal swallowing may exceed 1,000 ml/day, accounting in part for the reduction in amniotic fluid volume at term (20). Fetal swallowing is highly influenced by fetal neurobehavioral state, with increased swallowing during low-voltage (LV) compared with high-voltage (HV) electrocortical activity (10, 16). Nevertheless, before electrocortical differentiation at ~120 days gestation and during both low- and high-voltage periods of late gestation, swallowing activity is significantly greater in the fetus than in the adult. Accordingly, when adjusted for fetal or adult weight differences, ovine and human fetuses swallow significantly greater volumes of fluid than do adults.

Nitric oxide (NO) and NO synthase (NOS) enzyme have been localized to diffuse areas of the adult brain (1–5, 40). NO likely acts in the brain as a neuromodulator rather than a conventional neurotransmitter, as it is synthesized on demand and not stored within synaptic vesicles (2). It is a gaseous molecule and has no electrical charge or cell receptors; rather, it diffuses readily through cell membranes affecting a large number of neighboring synaptic functions (9). Once inside the target presynaptic or postsynaptic neurons, NO may alter brain function by way of soluble guanylyl cyclase (35) or perhaps other signaling pathways such as cyclic adenosine diphosphate ribosyl cyclase (41).

Several lines of investigation suggest that NO contributes to the regulation of adult water ingestion activity (4, 13, 14, 18). NOS has been demonstrated within the dipsogenic neurons (i.e., supraoptic and paraventricular nuclei and circumventricular organs including the subfornical organ and organum vasculosum lamina terminalis) of adult rat brains (11, 29), indicating a potential role of NO in the regulation of drinking behavior. Moreover, NOS activity is upregulated in the rat hypothalmo-hypophysial system after chronic salt loading (15), and the functional activity (glucose utilization) of the dipsogenic neurons (subfornical organ and median preoptic nucleus) is significantly reduced after blockade of NOS activity (13). In view of existing data, indicating a role for NO in adult drinking activity, and the marked differences in fetal and adult swallowing activity together with the lack of any data regarding the role of NO in mediating fetal swallowing, we sought to determine the contribution of NO to the heightened level of spontaneous fetal swallowing activity.

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MATERIALS AND METHODS

Six time-dated pregnant ewes with singleton fetuses (129 ± 1 days gestation on the first study day) were studied. Animals were housed indoors in individual steel study cages and acclimated to a 12-h light-to-dark cycle. Surgical procedures and studies were approved by the Harbor-UCLA Animal Use Committee. Food (alfalfa pellets) and water were provided ad libitum, except for withholding of food for 24 h before surgery.

Surgical preparation. Anesthesia was induced with ketamine hydrochloride (15–20 mg/kg im). General anesthesia was maintained with isoflurane (1–2%) and O2 (1 l/min). The uterus was exposed by a midline abdominal incision, and a small hysterotomy was performed to expose a fetal hindlimb. Polyethylene catheters with inner diameter (ID) 0.04 in. and outer diameter (OD) 0.07 in. were placed in the fetal femoral vein and artery and threaded to the inferior vena cava and abdominal aorta, respectively. Surgical placement of bipolar electromyography (EMG) electrodes (thyrohyoid muscle, upper and lower nuchal esophagus) for determination of swallowing activity was performed as previously described (37).

Electrodes were also implanted on the parietal dura through two drilled burr holes (5 mm above the bregma and 10 mm from each side of the sagittal suture) for the determination of fetal electrocortical activity [by electrocorticogram (ECoG) activity]. An 18-gauge needle connected to polyethylene catheter (0.02 and 0.04 in. ID and OD, respectively) was inserted into the lateral ventricle, which was identified 20 mm above the lambdoid suture and 5 mm lateral to the sagittal suture. The lateral ventricle needle and the dural electrodes were immobilized with dental cement with the assistance of two stainless-steel screws fixed in the skull. An intratracheal catheter (0.125 and 0.25 in. ID and OD, respectively; Corometrics Medical Systems, Wallingford, CT) was inserted for anamnestic fluid pressure measurement. Uterine and maternal incisions were repaired, and all catheters and electrodes were externalized to the maternal flank and placed in a cloth pouch. Catheters also were placed in the maternal femoral vein and artery. Animals were allowed at least 5 days for postoperative recovery, which included catheter maintenance and antibiotic administration (7).

The study protocol was conducted over 2 days. Day 1 consisted of a control study of the effects of an intracerebroventricular (ICV) injection of aCSF (1 ml) consisting of (in ml) 145 Na, 3.3 K, 121 Ca, 2.2 Mg, 121 Cl, and 25 meq/l HCO3 (297 mosmol/kg) on spontaneous fetal swallowing activity. After a 2-h control period, fetuses were given a lateral ventricle injection of aCSF (aCSF-1) and monitored for 2 h; a second intracerebroventricular injection of aCSF (aCSF-2) was administered, and monitoring continued for an additional 2 h.

Day 2 explored the effects of NOS inhibition and subsequent return of NOS activity on spontaneous fetal swallowing activity. After a 2-h control period, fetuses were given a lateral ventricle injection of 1 ml (1 mg/kg) of a nitric oxide synthase inhibitor nitro-l-arginine methyl ester (l-NNAME; Sigma, St. Louis, MO) and monitored for 2 h. l-Arginine (NO precursor; 1 ml, 2 mg/kg) was then injected into the lateral ventricle, and fetuses were monitored for an additional 2 h. In both aCSF and l-NNAME studies, fetuses were monitored for a total time of 6 h.

Before each study day, an equilibration period included preparation of the animals for the experiments and confirmation of starting criteria (fetal arterial pH was >7.30). Throughout the 2 days of the experiment, amniotic pressure and fetal arterial blood pressure, fetal heart rate, fetal swallowing, and fetal ECoG activities were monitored continuously. Maternal arterial blood pressure and heart rate were measured continuously. Maternal and fetal blood samples were collected at 60 and 120 min of the control and study periods.

Analytical methods. Fetal body weight was estimated by the formula of Robillard et al. (28). Maternal and fetal blood samples were collected in iced tubes containing 10 U/ml heparin. Blood aliquots were assessed for hematocrit, pH level, P O2, and P CO2; the remaining blood was centrifuged, and plasma osmolality and sodium, chloride, and potassium concentrations were measured. Maternal and fetal plasma aliquots were stored at −20°C until assayed for arginine vasopressin (AVP). Fetal blood samples were replaced with an equivalent volume of heparinized maternal blood withdrawn before the study, and maternal blood samples were replaced with an equivalent volume of 0.15 M saline. Blood Po2, P CO2, and pH were measured at 39°C with a Nova stat 3 blood-gas analyzer system (Nova Biomedical, Waltham, MA). Plasma osmolality was measured by freezing point depression on an Advanced Digimatic osmometer (model 3 M O, Advanced Instruments, Needham Heights, MA). Plasma sodium, potassium, and chloride concentrations were determined by a Nova 5 electrolyte analyzer (Nova Biomedical). Fetal and maternal blood pressures and amniotic pressures were measured by means of World Precision Instruments (Sarasota, FL) signal conditioners and transducers. Fetal blood pressure was corrected for amniotic cavity pressure. For the analysis of plasma AVP, the samples were collected in an ice-chilled glass tube containing 10 IU heparin and 500 IU aprotinin per milliliter of blood. Samples were extracted by a modification of the procedure of LaRochelle et al. (17), and AVP levels were determined by radioimmunoassay (38).

Fetal ECoG and swallowing analysis. Digitization of all signals was performed at a rate of 75 Hz. EMG and ECoG signals were directed into a Grass physiological recorder and a WinAQ (DataQ Instruments, Akron, OH) analog-to-digital system.

Fetal ECoG was assessed by visual analysis and was divided into LV high-frequency and HV low-frequency periods. Periods of ECoG activity that did not clearly belong to either LV or HV activities were considered intermediate ECoG activity. Intermediate ECoG activity constituted <5% of the total ECoG activity and was not considered in the analysis of the data.

An EMG-propagated swallow, representing a coordinated laryngeal-esophageal contraction, was defined by a time sequence of integrated EMG signals from the thyrohyoid muscle to the upper and lower nuchal esophagus (37). Total swallowing activity was counted and expressed as swallows per minute. The percentage of swallows associated with each ECoG state was then calculated. To normalize swallowing activity for the amount of each ECoG state, the number of swallows occurring in each state was divided by the time spent in that state for each animal and expressed as swallows per minute ECoG state.

Statistical analysis. For each animal, swallowing rates per minute, percentage of time spent in each ECoG state, and percentage of swallowing in each state was calculated for the control and drug exposure periods. Comparisons were made using one-way repeated-measures ANOVA with Dunnett’s test for post hoc analysis. Friedman one-way repeated-measures ANOVA on ranks was used when normality test failed. All values are presented as means ± SE; P < 0.05 was considered statistically significant.

RESULTS

Day 1: aCSF study. During the control period, fetal swallowing occurred primarily during LV ECoG activity (1 ± 0.1 total swallows/min; 1.2 ± 0.2 swallows/min LV, and 0.4 ± 0.1 swallows/min HV). The two injections
of aCSF into the lateral ventricle did not significantly change fetal swallowing activity (aCSF-1, 0.9 ± 0.1 total swallows/min, 1.2 ± 0.1 swallows/min LV, and 0.4 ± 0.1 swallows/min HV; aCSF-2, 0.9 ± 0.1 total swallows/min, 1.1 ± 0.1 swallows/min LV, and 0.6 ± 0.1 swallows/min HV; P = 0.2; Fig 1). Fetal LV neurobehavior activity during the 2-h control period (60 ± 4% of time) did not significantly change as a result of aCSF-1 (66 ± 2%) or aCSF-2 (61 ± 3%) injection into the lateral ventricle (P = 0.2; Fig 1).

Fetal mean blood pressure, heart rate, hematocrit, PO2, PCO2, plasma osmolality, sodium, chloride, and AVP did not significantly change as a result of aCSF injection (Table 1). There was a small yet significant decrease in fetal arterial pH at 60 min post-L-NAME and post-L-arginine. Plasma potassium also slightly but significantly decreased at the end of the study. The lower values for both pH and potassium remained within normal physiological range. Maternal values did not significantly change from basal values (mean blood pressure 102 ± 3 mmHg, heart rate 111 ± 4 beats/min, hematocrit 26 ± 1%, pH 7.46 ± 0.01, PO2 113 ± 3 mmHg, PCO2 35 ± 2 mmHg, plasma osmolality 299 ± 1 mosmol/kg, sodium 146 ± 0 meq/l, chloride 112 ± 1 meq/l, potassium 4.1 ± 0 meq/l, and AVP 1.2 ± 0.1 pg/ml).

Day 2: L-NAME and L-arginine injection into the fetal lateral ventricle. During the control period, fetal swallowing occurred primarily during LV ECoG activity (1 ± 0.1 total swallows/min, 1.2 ± 0.2 swallows/min LV, 0.4 ± 0.1 swallows/min HV). Injection of L-NAME into the lateral ventricle decreased basal fetal swallowing activity by ~50% (0.5 ± 0.1 total swallows/min, 0.6 ± 0.1 swallows/min LV, P < 0.001, and 0.3 ± 0.1 swallows/min HV; P = 0.2; Fig 2). Restoring central NO activity by L-arginine (NO precursor) injection into the lateral ventricle resulted in restoration of both LV and HV ECoG spontaneous fetal swallowing activity to a normal baseline level (0.9 ± 0.1 total swallows/min, 1.2 ± 0.2 swallows/min LV, and 0.4 ± 0.1 swallows/min HV; Fig 2). Fetal LV neurobehavior activity during the 2-h control period (67 ± 2% of time) did not significantly change as a result of l-NAME (65 ± 3%) or L-arginine (63 ± 3%) injection into the lateral ventricle (P = 0.3; Fig 2).

Table 1. Fetal arterial values during the control period and in response to aCSF-1 and aCSF-2 injections

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Control 60</th>
<th>Control 120</th>
<th>aCSF-1 60</th>
<th>aCSF-1 120</th>
<th>aCSF-2 60</th>
<th>aCSF-2 120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal BP, mmHg</td>
<td>48 ± 1</td>
<td>48 ± 2</td>
<td>47 ± 2</td>
<td>44 ± 2</td>
<td>45 ± 3</td>
<td>46 ± 2</td>
</tr>
<tr>
<td>Fetal HR, beats/min</td>
<td>169 ± 4</td>
<td>171 ± 5</td>
<td>161 ± 7</td>
<td>157 ± 5</td>
<td>160 ± 6</td>
<td>164 ± 5</td>
</tr>
<tr>
<td>Hct, %</td>
<td>31 ± 2</td>
<td>30 ± 2</td>
<td>30 ± 2</td>
<td>31 ± 2</td>
<td>31 ± 2</td>
<td>30 ± 1</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 ± 0.01</td>
<td>7.37 ± 0.01</td>
<td>7.36 ± 0.01*</td>
<td>7.37 ± 0.01</td>
<td>7.35 ± 0.01*</td>
<td>7.37 ± 0.01</td>
</tr>
<tr>
<td>PO2, mmHg</td>
<td>21 ± 1</td>
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<td>22 ± 1</td>
<td>22 ± 0</td>
<td>22 ± 1</td>
</tr>
<tr>
<td>PCO2, mmHg</td>
<td>51 ± 2</td>
<td>52 ± 2</td>
<td>51 ± 2</td>
<td>50 ± 2</td>
<td>52 ± 3</td>
<td>50 ± 2</td>
</tr>
<tr>
<td>Osmolality, mosmol/kg</td>
<td>296 ± 1</td>
<td>295 ± 1</td>
<td>295 ± 2</td>
<td>296 ± 3</td>
<td>296 ± 1</td>
<td>296 ± 2</td>
</tr>
<tr>
<td>Sodium, meq/l</td>
<td>140 ± 0</td>
<td>140 ± 0</td>
<td>140 ± 1</td>
<td>140 ± 1</td>
<td>140 ± 1</td>
<td>141 ± 1</td>
</tr>
<tr>
<td>Chloride, meq/l</td>
<td>104 ± 1</td>
<td>105 ± 1</td>
<td>104 ± 0</td>
<td>104 ± 1</td>
<td>105 ± 1</td>
<td>105 ± 1</td>
</tr>
<tr>
<td>Potassium, meq/l</td>
<td>3.7 ± 0.1</td>
<td>3.7 ± 0.1</td>
<td>3.7 ± 0.2</td>
<td>3.6 ± 0.2</td>
<td>3.6 ± 0.2*</td>
<td>3.6 ± 0.2*</td>
</tr>
<tr>
<td>AVP, pg/ml</td>
<td>1.7 ± 0.5</td>
<td>1.3 ± 0.1</td>
<td>1.7 ± 0.5</td>
<td>1.2 ± 1.2</td>
<td>2.7 ± 0.9</td>
<td>1.8 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 ewes. aCSF, artificial cerebrospinal; aCSF-1, first injection of aCSF; aCSF-2, second injection of aCSF; BP, blood pressure; HR, heart rate; Hct, hematocrit; AVP, arginine vasopressin. *P < 0.05 compared with control.
Fetal blood pressure, hematocrit, \( \text{PO}_2 \), \( \text{PCO}_2 \), plasma osmolality, sodium, chloride, and AVP did not significantly change as a result of \( \text{L-NAME} \) or \( \text{l-arginine} \) injection (Table 2). However, fetal heart rate decreased significantly at 120 min post-\( \text{L-NAME} \) and at 60 min post-\( \text{l-arginine} \). There was a small yet significant decrease in fetal arterial \( \text{pH} \) at 60 and 120 min post-\( \text{l-arginine} \). Plasma potassium also slightly but significantly decreased post-\( \text{L-NAME} \) and post-\( \text{l-arginine} \). However, arterial \( \text{pH} \) and plasma potassium remained within normal physiological ranges. Maternal values did not significantly change from its basal values (blood pressure 101 ± 4 mmHg, heart rate 112 ± 4 beats/min, \( \text{pH} \) 7.46, \( \text{PO}_2 \) 112 ± 2 mmHg, \( \text{PCO}_2 \) 34 ± 2 mmHg, plasma osmolality 302 ± 1 mosmol/kg, sodium 145 ± 6 meq/l, chloride 111 ± 1 meq/l, and AVP 1.2 ± 0.1 pg/ml). Maternal hematocrit decreased from 26 ± 1% during the control period to 25 ± 1% at the end of the study (\( P < 0.01 \)).

**DISCUSSION**

In comparison to the adult, near-term fetal swallowing activity occurs at a significantly higher rate. The increased volume of swallowed fluid may be important for regulation of amniotic fluid volume (7), fetal gastrointestinal development (22), and perhaps somatic growth (27). Yet, little is known regarding the regulation of fetal swallowing. Notably, fetal dipsogenic and osmoregulatory mechanisms may be imprinted in utero, with permanent effects on adult dipsogenic and AVP secretory sensitivities (8).

We investigated the role of NO in the regulation of spontaneous ovine fetal swallowing activity. We sought to remove potential tonic stimulatory effects of NO on spontaneous fetal swallowing using NOS inhibitor \( \text{L-NAME} \). Administration of central \( \text{l-NAME} \) has been shown to diminish brain NOS activity in dose- and time-dependent manners, with a duration of effect of at least 6 h (39), thus permitting an analysis of swallowing activity during a 120-min period in the present study. Consistent with a study in adult rats (18), we administered an \( \text{L-NAME} \) dose of 1 mg/kg and an \( \text{l-arginine} \) dose of 2 mg/kg to reverse the \( \text{L-NAME} \) effects.

### Table 2. Fetal arterial values during the control period and in response to \( \text{L-NAME} \) and \( \text{l-arginine} \) injections

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Control</th>
<th>L-NAME</th>
<th>L-Arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>120</td>
<td>60</td>
<td>120</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>48 ± 1</td>
<td>49 ± 2</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>170 ± 5</td>
<td>168 ± 4</td>
<td>158 ± 2*</td>
</tr>
<tr>
<td>Hct, %</td>
<td>31 ± 2</td>
<td>32 ± 3</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 ± 0.00</td>
<td>7.36 ± 0.01</td>
<td>7.35 ± 0.01*</td>
</tr>
<tr>
<td>( \text{PO}_2 ), mmHg</td>
<td>21 ± 1</td>
<td>22 ± 1*</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>( \text{PCO}_2 ), mmHg</td>
<td>50 ± 2</td>
<td>51 ± 1</td>
<td>49 ± 1</td>
</tr>
<tr>
<td>Osmolality, mosmol/kg</td>
<td>296 ± 2</td>
<td>298 ± 2</td>
<td>298 ± 3 *</td>
</tr>
<tr>
<td>Sodium, meq/l</td>
<td>140 ± 0</td>
<td>135 ± 6</td>
<td>141 ± 1</td>
</tr>
<tr>
<td>Chloride, meq/l</td>
<td>104 ± 1</td>
<td>105 ± 1</td>
<td>106 ± 1</td>
</tr>
<tr>
<td>Potassium, meq/l</td>
<td>3.97 ± 0.12</td>
<td>3.79 ± 0.12*</td>
<td>3.74 ± 0.13*</td>
</tr>
<tr>
<td>AVP, pg/ml</td>
<td>1.0 ± 0.0</td>
<td>1.2 ± 1.2</td>
<td>2.1 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 6 \) ewes. \( \text{L-NAME} \), nitro-\( \text{l-arginine} \) methyl ester. *\( P < 0.05 \) compared with control.
During the basal period, fetal cardiovascular and arterial values were within normal limits. Similarly, swallowing activity rates and association with ECoG were consistent with previous studies of near term ovine fetuses (10, 33). The present study represents the first demonstration that central NO activity modulates the heightened level of spontaneous fetal swallowing activity. Central NO inhibition with L-NAME resulted in >50% reduction in spontaneous fetal swallowing, with subsequent restoration to normal levels by increasing availability of central NO with l-arginine. Our results are consistent with some, though not all studies in the adult rat. Liu et al. (18) and Zhu and Herbert (42) demonstrated that intracerebroventricular injection of L-NAME inhibits water intake in response both to systemic hypertonicity and central ANG II, respectively. Kadekaro et al. (13) and Kannan et al. (14) provided further evidence that an NOS inhibitor attenuates water intake induced by dehydration and hypertonicity, respectively. Conversely, Roth and Rowland (34) and Calapai and Caputi (4) suggested that increased central NO activity by intracerebroventricular l-arginine inhibits water intake induced by dehydration and central ANG II. The reason for the discrepancies in effects of NOS on rat water intake is unknown. However, NO demonstrates both stimulatory (21) and inhibitory (23) actions on nerve synapses. Thus local neuronal activation, sites of action, and dosage may contribute to response variation.

Notably, no prior study has demonstrated that excess or deficient NO activity alters spontaneous adult swallowing activity (4, 14). However, spontaneous adult swallowing may differ from fetal swallowing in both rate and regulation. Thus the comparatively high rates of spontaneous ovine fetal swallowing activity may be similar to stimulated adult swallowing rates. Consistent with this hypothesis, ovine fetal plasma hypertonicity suppresses swallowing activity, suggesting that spontaneous fetal ingestive behavior is regulated, in part, via tonic dipsogenic stimulation (24).

Although NO inhibition suppressed spontaneous fetal swallowing, it did not alter fetal neurobehavior activity or the association of swallowing and fetal ECoG. As demonstrated previously, 75% of fetal swallowing occurs in LV ECoG activity (16). It is unlikely that L-NAME altered fetal swallowing by inhibition of appetite-mediated swallowing behavior, as NO does not affect food intake in the adult rat (34). Significant increases (33) or decreases (32) in systemic blood pressure may reduce fetal or adult swallowing. However, in the present study there was no significant effect of L-NAME on fetal blood pressure. Similar to ingestive behavior, intracerebroventricular L-NAME has been reported to increase (25) or not change arterial blood pressure (3) and to either stimulate (26) or suppress (19) AVP secretion. We demonstrated no effect of central inhibition of NO on fetal plasma AVP levels.

As NO has been shown to regulate cerebral blood flow (12), we cannot rule out the possibility that the changes in ovine fetal swallowing were secondary to the effect of L-NAME on regional cerebral blood flow. As a nonselective NOS inhibitor, L-NAME blocks both cerebral endothelial NOS as well as neuronal NOS (nNOS). Thus L-NAME may increase cerebrovascular resistance and reduce local cerebral blood flow (6), potentially altering swallowing behavior. Studies of selective nNOS inhibitors, regional blood flow, or neuronal glucose uptake in future research may clarify the specific role of nNOS in modulating fetal drinking behavior.

In conclusion, the present results indicate that central NO inhibition reduces spontaneous ovine fetal swallowing. As NO may affect long-term neuronal potentiation, we speculate that central NO tonic action imprints the regulation of spontaneous and potentially dipsogenic-mediated fetal swallowing.

**Perspectives**

Fetal swallowing activity, important for amniotic fluid volume regulation and fetal gastrointestinal development, has unique characteristics. The markedly elevated rate of spontaneous fetal swallowing, relative to adult levels, suggests a tonic level of stimulation. However, stimulation of fetal swallowing requires a higher level of systemic osmolality. Although fetal dipsogenic and osmoregulatory mechanisms may be imprinted in utero, there is little understanding of the regulation of fetal ingestive behavior. NO is a well-established neuromodulator, with a pivotal role in regulating several brain functions. This study is the first report to demonstrate a role of central NO in the regulation of spontaneous fetal swallowing. As NO does not affect spontaneous adult drinking activity, these results suggest that tonic NO may account, in part, for the elevated rates of spontaneous fetal swallowing. NO may contribute to the imprinting of dipsogenic neuron synaptogenesis of the fetus.

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