The Central Effects of Thyrotropin-Releasing Hormone on the Breathing Movements and Electrocardiac Activity of the Fetal Sheep

LAURA BENNET, PETER D. GLUCKMAN, AND BARBARA M. JOHNSTON

Department of Paediatrics, University of Auckland, Auckland, New Zealand

ABSTRACT. The fetal respiratory and electrocardiac effects of thyrotropin-releasing hormone (TRH) administered into the lateral cerebral ventricles, have been investigated in chronically catheterized unanesthetized fetal sheep at 125–140 days of gestation. Stimulatory effects on fetal breathing movements were seen at doses as low as a μg bolus. TRH given as a $5\text{-}\mu\text{g}$ bolus followed by a $10\text{ }\mu\text{g/h}$ infusion for 2 h induced a rapid switch to significantly faster, deeper, and continuous fetal breathing movements, while the electrocardiogram remained episodic. Fetal breathing movements did not stop during hypoxia. TRH given as a $2\text{-}\mu\text{g}$ bolus followed by a $4\text{ }\mu\text{g/h}$ infusion or as a $5\text{-}\mu\text{g}$ bolus followed by a $5\text{ }\mu\text{g/h}$ infusion induced the same stimulation of FBMs, but breathing essentially remained episodic, state related and inhibited by hypoxia. As hypothermia presumably induces a surge in TRH secretion at birth it is possible that TRH has some role in the switch from fetal to postnatal breathing patterns. (*Pediatr Res* 23: 72–75, 1988).

Abbreviations

TRH, thyrotropin-releasing hormone
CSF, cerebrospinal fluid
FBM, fetal breathing movements
ECOG, electrocardiogram
LV, low voltage
HV, high voltage
5-HTP, 5-hydroxytryptophan

TRH has been found to exist throughout the nervous system including areas of the brain stem known to contain nuclei involved in respiratory control (1–3). TRH administered centrally to the adult and preterm neonatal animal stimulates respiration (3–12). This stimulation results from an initial shortening of inspiratory time followed by an increase in respiratory rate due to a decrease in expiratory time (7). The effects of TRH on respiratory function appear to be mediated in the central nervous system, possibly at sites in the hindbrain (3, 6, 7). To investigate the central effects of TRH on both the episodic nature of fetal breathing movements and the apneic response to hypoxia, we infused TRH via an indwelling lateral cerebral ventricle cannula to fetal sheep late in gestation (13).

Received May 4, 1987; accepted September 16, 1987.

Correspondence P. Gluckman, Developmental Physiology Lab, Dept. of Paediatrics, University of Auckland, Private bag, Auckland, New Zealand.

Supported by The Medical Research Council of New Zealand and The Wellcome Trust. L.B. was a fellow of the New Zealand Federation of University Women.

METHODS

Animal preparation. Operations were performed on nine fetal sheep (Romney-Suffolk cross) at 120–130 days gestation under maternal halothane/oxygen anaesthesia, using sterile techniques. Catheters were implanted into a carotid artery, a jugular vein, the trachea, and the amniotic sac. Stainless steel electrodes were implanted bilaterally onto the parietal dura and the nuchal and diaphragm muscles (14). A lateral cerebral ventricle cannula was implanted as previously described (13). Fetal catheters were exteriorized through the maternal flank. Vascular catheters were also implanted into a maternal pedal artery and vein. These catheters were exteriorized on the maternal flank close to the fetal catheters. The postoperative care of the ewe and maintenance of the CSF catheter have been described previously (13).

EXPERIMENTAL PROCEDURES

Experiments were conducted at least 5 days postoperatively and were carried out only when the fetal arterial PO_2 was higher than 18.0 mm Hg and fetal arterial pH was more than 7.32. Only one experiment a day was conducted. The gestational age at which fetuses were studied ranged between 125 to 140 days.

All infusions were performed using a CSF pH, after dissolution of TRH, of 7.34–7.38 (13). The CSF catheter dead-space was 0.7 ml. The control experiments consisted of a 1.4 ml bolus followed by a 1.0 ml/h infusion of artificial CSF for 2 h, over a pH range of 7.34–7.38. The control for bolus alone experiments was the administration of a 1.4-ml bolus of CSF as previously described (13). These experiments were performed in seven fetuses. As an additional check the lateral ventricle cannula was flushed with filtered artificial CSF 1–2 h before each experiment.

TRH (1-pyroglyutamyl-L-histidyl-L-prolinamide, Sigma Chemical Co.) was diluted appropriately with artificial CSF and the pH checked and adjusted if necessary by bubbling 5% $\text{CO}_2/95\%$ O_2 gas mixture through the solution. This solution remained stable for up to 6 h. The TRH solution was then passed through a Millipore filter ($0.22\text{ }\mu\text{m}$) and administered as either a 2- or $5\text{-}\mu\text{g}$ bolus, in a volume of 1.4 ml, injected slowly over a 5-min period into the lateral ventricle cannula, and followed by a 2-h infusion of either 4, 5, or $10\text{ }\mu\text{g/h}$, in a volume of 1 ml/h. The same rate of infusion was used in both the control and TRH studies.

Preliminary studies were performed using a bolus alone administration of 1, 10, and $50\text{ }\mu\text{g}$. The TRH was administered as a bolus of 0.7 ml followed by a CSF flush of 0.7 ml (13). All control and TRH experiments were begun during HV ECOG activity, although previous preliminary TRH experiments showed that the effect of TRH administration on FBM during LV ECOG activity was not significantly different from that seen during HV ECOG activity.

The effect of hypoxia on the increased FBM induced by TRH was tested by allowing the ewe to breathe a gas mixture of 9% O₂, 2.5% CO₂, and balance N₂, for 20 min. The CO₂ was added to maintain isocapnia (15). Fetal arterial blood samples and gas analysis of the inspired air verified the degree of hypoxia and maintenance of isocapnia. In all experiments the hypoxemia was induced approximately 20 min into the infusion during a period of LV ECOG activity.

At the conclusion of experimentation the ewes were sacrificed by a barbiturate overdose and the fetus removed by caesarean section. The placement of the cannula in the lateral ventricle was confirmed by the injection of dye and postmortem examination of the brain. Fetal weights at postmortem ranged between 2.8 to 3.9 kg.

The effects of drug administration were measured by comparing the amplitude, rate, and duration of breathing movements, the duration of apnea episodes, and periods of HV and LV electrocortical activity with those observed in the 12 h prior to drug administration. When an experimental protocol was repeated on a fetus, the data from these experiments were meaned. The degrees of freedom represent the number of fetuses used in each protocol. Statistical analysis was carried out using the Student's paired *t* test. All results are presented as means $\pm$ SEM.

RESULTS

Artificial CSF infusion. In the control experiments (*n* = 7) in which artificial CSF was given as a 1.4-ml bolus alone or followed by a 1 ml/h infusion for 2 h, there was no effect on FBM ECOG activity (Table 1), and the normal fetal apneic response to hypoxia or fetal pH and blood gas tensions.

Preliminary bolus studies 1, 10, and 50 μ g TRH. In preliminary experiments a bolus dose alone of 1, 10, and 50 μ g of TRH was administered to three fetuses. All doses induced a switch to LV ECOG activity and stimulated the rate and amplitude of FBM 1–4 min after the end of the bolus administration. ECOG activity then cycled normally and FBM continued either entirely or partly through the next epoch of HV ECOG activity. The length of breathing movements appeared dose dependent, ranging from 24 to 53 min. Rate, but not amplitude, of FBMs also appeared dose dependent.

TRH 2 μ g bolus followed by a 4 μ g/h infusion. A 2 μ g bolus followed by a 4 μ g/h infusion for 2 h was administered to two fetuses. In both fetuses rapid, deep FBM (Table 1) started 3–4 min after the bolus administration began. These changes in FBM were associated with a simultaneous ECOG switch to LV activity. FBM and ECOG activity remained episodic (Table 1) although FBM were observed to occur during some shorter periods (<7 min) of HV ECOG episodes. This occurred 30–45 min into the

TRH infusion. FBM remained irregular for 25.5 $\pm$ 9.5 min after the infusion was discontinued and thereafter returned to normal values. There were no observable effects on nuchal activity, which remained associated with HV ECOG activity, or fetal pH and blood gas tensions.

TRH 5 μ g bolus followed by a 5 μ g/h infusion. A 5- μ g bolus followed by a 5 μ g/h infusion for 2 h was administered to four fetuses. The effect of this dose on FBM and ECOG activity was the same as described above for the 2 μ g bolus plus 4 μ g/h infusion (Table 1). FBM remained both deep and fast 43 min $\pm$ 11.2 after the infusion was discontinued. There were no observable effects on nuchal activity, which remained associated HV ECOG activity, or fetal pH and blood gas tensions.

Hypoxia was induced in three fetuses approximately 20 min into the TRH infusion during LV ECOG activity. The fetal PO₂ fell from 20.8 $\pm$ 0.8 to 11.0 $\pm$ 0.5 mm Hg (*p* < 0.01). The PCO₂ did not change remaining at 40.8 $\pm$ 2.1 mm Hg compared to the control of 42.1 $\pm$ 3.5 mm Hg. Breathing movements stopped within 5–7 min and reappeared 4–22 min after the hypoxia was discontinued. The electrocorticogram switched to HV 6–9 min after the hypoxia began and HV activity continued until breathing movements resumed.

TRH 5 μ g bolus followed by a 10 μ g/h infusion. This dose was administered to six fetuses on eight occasions. Deep (*p* < 0.001), rapid (*p* < 0.01), continuous FBM (FBM associated with apnea of less than 10 s) (*p* < 0.001) (Table 1) started 2–4 min after the bolus administration began. In each study these FBM were accompanied by a simultaneous ECOG switch to LV activity (Fig. 1). Respiratory rate was on average faster in the first h of infusion compared to the second h (Table 1). Throughout the infusion the ECOG remained cyclic; however, the duration of both LV and HV episodes were significantly shorter (*p* < 0.05, Table 1). There was no consistent difference in rate of breathing or amplitude between LV and HV ECOG episodes during the experiments.

These faster and deeper FBM continued for 27.3 $\pm$ 8.1 min after the infusion was discontinued, thereafter returning to normal within 1 h in seven of eight experiments. Convulsive ECOG activity occurred in three of the experiments, starting 65–84 min into the infusion, but did not change the TRH-induced fetal breathing movement pattern. Nuchal activity remained normally associated with HV ECOG activity except in one experiment in which there was 1 h of very active nuchal activity after 83 min of infusion; this was accompanied by a further increase in amplitude of FBM.

Hypoxia was induced in four fetuses (Fig. 2). The fetal PO₂ fell from 19.5 $\pm$ 0.5 to 10.6 $\pm$ 1.0 mm Hg (*p* < 0.01). There were no significant changes in Ph or PCO₂. PCO₂ during the experiment was 40.3 $\pm$ 2.3 mm Hg compared to 37.5 $\pm$ 1.5 mm

Table 1. Effect of different doses of TRH on fetal breathing movements and electrocortical activity in fetal sheep (means $\pm$ SEM)

Dose	Longest apnea episode (min)	Longest breathing episode (min)	Longest HV episode (min)	Longest LV episode (min)	Amplitude of breathing (mm Hg)	Rate of breathing (breaths/min)
0* (<i>n</i> = 7)	22.0 $\pm$ 2.0	38.4 $\pm$ 3.0	20.7 $\pm$ 2.0	40.0 $\pm$ 2.6	5.0 $\pm$ 0.5	61.1 $\pm$ 2.7
2- μ g bolus + 4 μ g/h infusion (<i>n</i> = 2)	22.0 $\pm$ 2.0	35.0 $\pm$ 1.0	24.0 $\pm$ 4.0	37.0 $\pm$ 0.7	11.0 $\pm$ 1.0†	114.0 $\pm$ 13.0†
5- μ g bolus + 5 μ g/h infusion (<i>n</i> = 4)	21.0 $\pm$ 2.1	32.3 $\pm$ 3.0	26.5 $\pm$ 2.5	25.5 $\pm$ 2.3	17.0 $\pm$ 1.7‡	110.0 $\pm$ 6.3‡
5- μ g bolus + 10 μ g/h infusion (<i>n</i> = 6)	3.0 $\pm$ 0.3§	144.0 $\pm$ 7.5§	18.0 $\pm$ 2.0	24.1 $\pm$ 3.3	26.0 $\pm$ 1.6§	114.5 $\pm$ 12.7‡ 78.0 $\pm$ 2.6¶

* Artificial CSF administered alone.

† Insufficient data for statistical analysis.

‡ *p* < 0.01 by paired *t* test to control.

§ *p* < 0.001 by paired *t* test to control.

|| *p* < 0.05 by paired *t* test to control.

¶ Second h of the 2-h infusion.

Fig. 1. The effect of a 5- μ g bolus followed by 10 μ g/h infusion of TRH on breathing [tracheal pressure (T.P.)], ECG, and neck muscle (*nuchal*) activity in a 130 days of gestation fetal sheep. The start of the TRH administration into the lateral ventricle cannula is indicated by the arrow.

Fig. 2. The effect of a 20-min isocapnic hypoxia on the breathing movements [diaphragm electromyogram (EMG) and tracheal pressure (T.P.)] induced by a 5- μ g bolus followed by 10 μ g/h infusion of TRH in a 128 days of gestation fetal sheep. The first arrow indicates the start of the TRH administration.

Hg ($p > 0.05$) observed during the control. Breathing movements remained continuous (FBM associated with apnea of less than 10-s duration) throughout the hypoxia period. The respiratory rate began to fall around 8 min into the hypoxia period reaching rates similar to those seen in the control period during LV ECG activity. During the last 10 min of hypoxia the respiratory rate was significantly less than the rate during the 5 min prehypoxia ($p < 0.01$) and first 10 min of hypoxia ($p < 0.05$). However, apnea of more than 10 s was not observed at any time (Fig. 3). There was no significant effect of hypoxia on the amplitude of FBM.

DISCUSSION

The pattern of late gestation fetal breathing movements differs from postnatal breathing in two important respects. First, FBM are episodic, occurring only during periods of LV ECG activity, the alternating periods of HV being almost entirely apneic (16). Second, the fetal respiratory response to hypoxia is apnea in contrast to the ventilatory response seen after birth (15). Central administration of a sufficient dose of TRH to the late gestation fetal lamb had a considerable effect on the fetal breathing pattern. Breathing movements became continuous, while the electrocorticogram remained episodic and the fetus continued breathing during isocapnic hypoxia.

Our results are qualitatively similar to those reported in the adult and neonate (3–12). The onset of breathing was rapid and the rate increased significantly. In addition, breathing movements occurred during both LV and HV ECG activity (17). We also observed an increase in tracheal pressure amplitude, whereas an increase in the depth of breathing was not reported in the adult.

Experiments with fetal lambs following brain stem transections or lesions demonstrate that the generation of apnea during hypoxia and HV ECG is dependent on pathways in the rostral lateral pons (18–20). It would appear that the fetus needs a sufficiently high level of TRH (in this study a 5 μ g bolus followed by a 10 μ g/h infusion) to either override the inhibitory neural mechanisms operative during HV ECG activity and during hypoxia or, since FBMs were also stimulated during LV activity, to stimulate directly the medullary respiratory centers. At lower doses fetal breathing movements remained episodic with only partial if any breathing movements into HV ECG periods and during hypoxia breathing movements stopped. The higher dose of TRH, however, was probably also sufficient to cause the convulsive activity in three experiments.

The site of drug administration is important to the interpretation of the physiological effect of TRH. We observed stimulation of fetal breathing movements with considerably lower doses

Fig. 3. The effect of isocapnic hypoxia on the rate of breathing movements during a 5- μ g bolus followed by a 10 μ g/h infusion of TRH. The values shown are means $\pm$ SEM. The bar indicates the period of hypoxia. The rate during the last 10 min was significantly less than the rate during the 5-min prehypoxia period ($p < 0.01$) and the first 10 min of hypoxia ($p < 0.05$). Apnea was not observed at any time.

than those reported by Umans *et al.* (17) (0.5–5.0 mg) using intravenous and limited intracisternal administration in the fetal lamb. Indeed in preliminary studies of the effect of a centrally administered bolus of TRH to the fetus we observed stimulation of fetal breathing movements at a dose as low as 1 μ g. TRH would appear to act centrally because these very low doses of TRH given intracerebroventricularly stimulate respiration. The specific site of TRH respiratory action in the adult is debated and different sites of action are possibly species specific (7). TRH-positive fibers have been found to exist throughout the medulla-pons region of the rat brain (1–3). The major effects observed in the present study at a dose three orders of magnitude less than that used by Umans *et al.* (17) demonstrates the utility of the chronically instrumented catheterized lateral ventricle model in fetal pharmacology.

In the fetus a number of drugs have been shown to induce prolonged fetal breathing movements (see Ref. 21 for review) although only a few, *e.g.* the GABA antagonist picrotoxin (14), the prostaglandin synthetase inhibitors indomethacin and meclofenamate (22), and 5-HTP (23), induce breathing movements during both HV and LV ECG activity. Hypoxia has, however, inhibited pharmacologically induced FBM, except during one 5-HTP experiment on one animal (23) and during the administration of apomorphine where FBM were briefly stimulated (24). Thus the data we present herein on TRH where breathing movements were maintained throughout hypoxia are unique.

The possibility that TRH is acting in conjunction with other neurotransmitter agents has not been investigated herein but should also be considered. TRH for example is known to coexist with both 5-hydroxytryptophan and substance P in medullary neurons (2). TRH is also known to enhance the turnover of norepinephrine (25) and the metabolism of dopamine (26) and to antagonize the actions of endogenous opiates (27). Thus the respiratory effects described after TRH infusion herein may in fact be due to a number of other TRH-induced neuropharmacological actions rather than to a direct effect by TRH.

There is a rise of TSH, and thus a presumed but not documented rise in neural TRH release, at birth. This rise in TSH has been demonstrated to be dependent on the fall in environmental temperature experienced at birth (28). In view of our observation that TRH is effective at very low doses (within the physiological range) it is possible that TRH is involved in the switch from fetal to postnatal patterns of respiration at birth.

Acknowledgments. The authors thank T. Mekkelholt for surgical assistance.

REFERENCES

1. Eskay RL, Long RT, Palkovits M 1983 Localization of immunoreactive thyrotropin releasing hormone in the lower brainstem of the rat. *Brain Res* 277:159–162
2. Johanson O, Hokfelt T, Pernow B, Jeffcoate SL, White N, Steinbusch HWM, Verhofstad AAJ, Emson PD, Spindel E 1981 Immunohistochemical support for three putative transmitters in one neuron: coexistence of 5-hydroxytryptamine, substance P- and thyrotropin releasing hormone-like immunoreactivity in medullary neurons projecting to the spinal cord. *J Neurosci* 6:1857–1882
3. McCown TJ, Hedner JA, Towle AC, Breese GR, Mueller RA 1986 Brainstem localization of a thyrotropin-releasing hormone-induced change in respiratory function. *Brain Res* 373: 189–196
4. Hedner J, Jonason J, Lundberg D 1981 Central respiratory stimulant effect by thyrotropin releasing hormone in the rat. *Neurosci Lett* 25:317–320
5. Hedner T, Hedner J, Jonason J, Lundberg D 1982 Respiratory effects of TRH in preterm rabbits. *Pediatr Res* 16:543–548
6. Hedner I, Hedner T, Wessberg P, Lundberg D, Jonason J 1983 Effects of TRH and TRH analogues on the central regulation of breathing in the rat. *Acta Physiol Scand* 117:427–437
7. Holtman JR, Buller AL, Hamosh P, Gillis RA 1986 Central respiratory stimulation produced by thyrotropin-releasing hormone in the cat. *Peptides* 7:207–212
8. Homma I, Oouchi M, Ichikawa S 1984 Facilitation of inspiration by intracerebroventricular injection of thyrotropin releasing hormone in rabbits. *Neurosci Lett* 44:265–269
9. Horita A, Carino AM, Smith JR 1976 Effects of TRH on the central nervous system of the rabbit. *Pharmacol Biochem Behav* 5:111–116
10. Koivusalo F, Paakkari J, Leppaluoto J, Karppanen H 1979 The effect of centrally administered TRH on blood pressure, heart rate and ventilation in rat. *Acta Physiol Scand* 106:83–86
11. Paakkari I, Nurminen M-L, Siren A-L 1986 Cardioventilator effects of TRH in anaesthetized rats: role of the brain stem. *Eur J Pharmacol* 122:131–134
12. Yamamoto Y, Lagercrantz H, vonEuler C 1981 Effects of substance P and TRH on ventilation and pattern of breathing in newborn rabbits. *Acta Physiol Scand* 113:541–543
13. Bennet L, Johnston BM, Gluckman PD 1986 The central effects of morphine on fetal breathing movements in the fetal sheep. *J Dev Physiol* 8:297–305
14. Johnston BM, Gluckman PD 1983 GABA-mediated inhibition of breathing in the late gestation sheep fetus. *J Dev Physiol* 5:353–360
15. Boddy K, Dawes GS, Fisher R, Pinter S, Robinson JS 1974 Fetal respiratory movements, electrocortical and cardiovascular responses to hypoxia and hypercapnia in sleep. *J Physiol* 243:599–618
16. Dawes GS, Fox HE, Leduc BM, Liggins GC, Richards RT 1972 Respiratory movements and rapid eye movement sleep in the fetal lamb. *J Physiol* 229:119–143
17. Umans JG, Umans HR, Szeto HH 1986 Effects of thyrotropin-releasing hormone in the fetal lamb. *Am J Obstet Gynecol* 155:1266–1271
18. Gluckman PD, Johnston BM 1987 Lesions in the upper lateral pons abolish the hypoxic depression of breathing in unanaesthetized fetal lambs in utero. *J Physiol* 382:373–383
19. Dawes GS, Gardner WN, Johnston BM, Walker DW 1980 Breathing patterns in fetal lambs after mid-brain transection. *J Physiol* 308:29P
20. Dawes GS, Gardner WN, Johnston BM, Walker DW 1983 Breathing in fetal lambs: the effect of brain stem transection. *J Physiol* 335:535–553
21. Gluckman PD, Bennet L 1986 Neuropharmacology of fetal and neonatal breathing. In: *Respiratory Control and Lung Development in the Fetus and Newborn. The Scientific Basis of Clinical Practice. Reproductive and Perinatal Medicine, Vol. III.* Perinatology Press, Ithaca, NY, pp 250–272
22. Kitterman JA, Liggins GC, Fewell JE, Tooley WH 1983 Inhibition of breathing movements in fetal sheep by prostaglandins. *J Appl Physiol* 54:687–692
23. Quilligen EJ, Clewlow F, Johnston BM, Walker DW 1981 Effect of 5-hydroxytryptophan on electrocortical activity and breathing movements of fetal sheep. *Am J Obstet Gynecol* 141:271–275
24. Bamford OS, Dawes GS, Ward RA 1986 Effects of apomorphine and haloperidol in fetal lambs. *J Physiol* 377:37–47
25. Keller HH, Bartholini G, Pletscher A 1974 Enhancement of cerebral noradrenaline turnover by thyrotropin releasing hormone. *Experientia* 30:1182–1183
26. Kalivas PW, Stanley D, Prange AJ 1986 Interaction between thyrotropin releasing hormone and the mesolimbic dopamine system. *Neuropharmacology* 26:33–38
27. Tache Y, Lis M, Collu R 1977 Effects of thyrotropin releasing hormone on behavioural and hormonal changes induced by β -endorphin. *Life Sci* 21:841–846
28. Sack J, Beaudry MA, DeLamater PV, Oh W, Fisher DA 1976 Umbilical cord cutting triggers hypertriiodothyroninemia and nonshivering thermogenesis in the newborn lamb. *Pediatr Res* 10:169–175