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CENTRAL STIMULATION OF BREATHING MOVEMENTS IN FETAL LAMBS BY PROSTAGLANDIN SYNTHETASE INHIBITORS

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SUMMARY

1. In unanaesthetized fetal lambs at 125–135 days gestation *in utero* central acidosis caused by perfusion of the cerebral ventricular system with a solution containing < 1 mM-HCO_3^- (cerebrospinal fluid (c.s.f.) pH 6·98) or intravenous infusion of ammonium chloride (c.s.f. pH 7·1) produced an increase in the depth and frequency of episodic breathing but no change in electrocortical activity, heart rate or arterial pressure.

2. Administration of prostaglandin synthetase inhibitors, sodium meclofenamate (0·8–10 mg/kg I.V. or 0·6–2·6 mg/kg intracerebrally) or acetylsalicyclic acid (6·7 mg/kg I.V.) caused prolonged episodes of fetal breathing during low and high voltage electrocortical activity, with a large increase in breath amplitude. Blood gas values, heart rate, blood pressure, electrocortical activity and eye movements were not altered.

3. In fetuses whose brain stems had been sectioned in the upper pons or the inferior colliculus, sodium meclofenamate induced prolonged deep breathing. Intravenous prostaglandin E_2 abolished the continuous breathing induced by meclofenamate, but not breathing movements enhanced by hypercapnia or hypoxia.

4. It is concluded that the central chemoreceptors respond to acidosis in near-term lamb fetuses qualitatively as in adult animals. Secondly, the results suggest that prostaglandin E_2 and the inhibitors of prostaglandin synthesis also act centrally in the lower pons or medulla to modulate fetal breathing.

INTRODUCTION

Breathing movements in fetal lambs occur almost continuously until about 110 days gestation (Bowes, Adamson, Ritchie, Dowling, Wilkinson & Maloney, 1981). After that time breathing movements become episodic and upon differentiation of the electrocorticogram (at about 115 days) these breathing episodes normally occur during low voltage states associated with rapid eye movements (Dawes, 1984). However, breathing movements can be induced during high voltage states after intravascular

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infusion of prostaglandin synthetase inhibitors (Kitterman, Liggins, Clements & Tooley, 1979), 5-hydroxytryptophan (Quilligan, Clewlow, Johnston & Walker, 1981) or atropine (van der Wildt, 1982). Breathing also occurs during high voltage activity in fetal lambs with suprapontine transection of the brain stem (Dawes, Gardner, Johnston & Walker, 1980; Dawes, Gardner, Johnston & Walker, 1983).

The effect of prostaglandin synthetase inhibitors on breathing movements in fetal lambs is particularly interesting since, unlike 5-hydroxytryptophan or atropine, these inhibitors produce prolonged periods of breathing without altering the electrocorticogram. This suggests that inhibition of fetal breathing activity during high voltage states may be mediated through a prostaglandin mechanism.

Since fetal breathing activity is little affected by input from the peripheral arterial chemoreceptors (Dawes, Fox, Leduc, Liggins & Richards, 1972; Jansen, Ioffe, Russell & Chernick, 1981) the normal inhibition of fetal breathing movements during high voltage states most likely occurs through central mechanisms. Consequently, cerebral ventricular perfusion and transection of the brain stem were used in chronic fetal sheep experiments to determine the site of action of prostaglandin synthetase inhibitors in producing continuous breathing. These studies suggest that prostaglandin synthetase inhibitors produce continuous breathing movements during high voltage activity through effects on the fetal medulla. A brief account of this investigation has been published (Koos, 1982).

METHODS

Under halothane anaesthesia carotid arterial, jugular venous and tracheal catheters were inserted into twenty-five lambs at about 120 days gestation ($\sim$ 0·8 of term). Stainless-steel electrodes (Cooner Wire Co., CA, U.S.A.) were placed on the dura to record electrocortical activity and on the orbital ridge to record electro-ocular activity.

A nylon catheter (1·0 mm i.d., 1·25 mm o.d., 4 cm long) was inserted into the cisterna magna through a hole in the occipital bone above the insertion of the nuchal muscle (Radulovacki & Girgis, 1968). The catheter was connected by polyethylene tubing to the inverted barrel of a 5 ml syringe containing sterile Ringer solution. The barrel was held about 10 cm above the external auditory meatus so that a slow inflow of the cerebrospinal fluid (c.s.f.) distended the lateral ventricles, facilitating introduction of a ventricular probe (Pappenheimer, Heisey, Jordan & Downer, 1962). The cisternal catheter was then attached to a nylon outflow catheter (1·0 mm i. d., 1·25 mm o. d., 120 mm long).

Ventricular probes were constructed on stainless-steel tubing (0·58 mm i.d., 0·90 mm o.d.) with one end bent through 70 deg and the other sharpened to a point. A silver collar (3·4 mm diameter, 0·5 mm thick) was crimp-fitted to the stainless-steel shaft just below the bend in the tubing. A probe was attached to the end of a polyethylene inflow catheter (0·85 mm i.d., 1·25 mm o.d., 132 cm long) filled with Ringer solution and inserted through a hole in the parietal bone about 6 mm lateral to the mid line just caudal to the coronal suture (Evans, Reynolds, Reynolds & Segal, 1974). A free flow of fluid through the inflow tubing indicated that the catheter tip was within the lateral ventricle. If the probe failed to penetrate the ventricle, the next longest probe was used; the silver collar was then glued to the bone with cyanoacrylate ester adhesive.

Cerebral ventricular perfusions were normally started on the fourth post-operative day and repeated perfusions were carried out at 2–3 day intervals. A synthetic c.s.f. solution was infused at 0·1 ml/min with a Braun Melsungen pump while simultaneously c.s.f. was withdrawn from the cisternal catheter at the same rate (Arndt & Freye, 1979). Intraventricular pressure relative to that of amniotic fluid was continuously recorded. The perfusing solution had ionic concentrations approximating that of fetal sheep c.s.f. after 120 days gestation (Bradbury, Crowder, Desai, Reynolds, Reynolds, & Saunders, 1972) (mequiv/kg H_2O): Na^+, 154; K^+, 3·2; Ca^{2+}, 2·9; Mg^{2+}, 1·9;

Cl^-, 138; HCO_3^-, 24. This solution was used to administer prostaglandin synthetase inhibitors into the cerebral ventricles. Central acidosis was produced by perfusing a virtually HCO_3^--free solution (< 1 mM) in which HCO_3^- was replaced by Cl^-. Both solutions were equilibrated with 5% CO_2 and had an osmolality of 298 mosM, equal to that of the c.s.f. (298 ± 1) in six fetal lambs at surgery.

A gas-tight syringe (model 1001 TLL, Hamilton Bonaduz AG, Switzerland) was used to collect c.s.f. for P_{CO_2} and pH determinations (Bureau, Begin & Bethiaume, 1979). Mercury filled the dead space of the syringe. About 0·6 ml of perfusate was withdrawn at 6 ml/min from a sterile length of nylon tubing connected to the outflow catheter. After collecting the c.s.f., the syringe was positioned so that the mercury sealed the syringe outlet. The pH and P_{CO_2} of the c.s.f. sample were determined at 39 °C (Corning, model 165). HCO_3^- concentrations were calculated from the Henderson–Hasselbalch relation using constants for c.s.f. (Mitchell, Herbert & Carman, 1965). Repeated pH measurements were made without removing the syringe until consecutive pH values were within 0·005 units (Bureau *et al.* 1979).

The brain stem was transected in the upper pons or inferior colliculus in seven fetal lambs at 120 days gestation using a 3 mm wide spatula through a 4 mm trephine hole in the interparietal bone, halfway between the parietal and occipital bones (Dawes *et al.* 1983). Stainless-steel diaphragm electromyogram (e.m.g.) electrodes (Cooner Wire Co., CA, U.S.A.) were inserted through the ninth intercostal space on the right side (Dawes, Gardner, Johnston & Walker, 1982).

The fetal brains were fixed *in situ* by arterial perfusion with 10% (w/v) formalin in saline solution. The brains were placed in 10% formalin for an additional 2 weeks before they were cut in sagittal sections of 15 μm and examined to determine the site of transection. Three out of every 100 sections were stained with Cresyl Violet, Weil's or Luxolfast Blue.

Sodium meclofenamate, a water soluble prostaglandin synthetase inhibitor kindly supplied by Parke-Davis Co., was dissolved in the synthetic c.s.f. solution having a normal HCO_3^- concentration. Cerebral ventricular perfusions were carried out with a solution containing 0·7 mg/ml. Acetylsalicylic acid (Sigma Chemical Co.) was dissolved in saline and infused intravenously. Plasma salicylate levels were determined by a modified fluorimetric method (Schachter & Manis, 1958). A 10 μg/ml solution of prostaglandin E_2 (Upjohn) was infused into the jugular vein at 1 μg/kg . min (Kitterman, Liggins, Fewell, & Tooley, 1983).

Maternal and fetal blood gases were altered by having the ewe breathe a gas mixture from a polyethylene bag (Boddy, Dawes, Fisher, Pinter & Robinson, 1974). The ewe breathed 5% CO_2 with 18% O_2 in N_2 for isoxic fetal hypercapnia and 8·5% O_2 with 3% CO_2 in N_2 for isocapnic fetal hypoxia. Arterial blood gases were determined on a Corning 165 instrument.

The electrocorticogram, eye movements, blood pressure, heart rate, tracheal pressure, and cerebral ventricular pressure were continuously recorded on a Schwarzer polygraph. All pressures were referred to that of amniotic fluid. The diaphragm e.m.g. signal was filtered (30 Hz, high pass), passed through a 'leaky' integrator (time constant of 30 ms), and displayed on the polygraph.

During experiments the tracheal pressure and/or diaphragm e.m.g. were recorded on an FM tape recorder. The tracheal pressure signal was amplified, inverted, filtered (5 Hz, low pass), digitized (10 ms intervals) and analysed by a PDP 11/34 computer. The program identified the time and pressure values of the start and maximum amplitude of each breath and placed values on disk in files of up to 1024 breaths. In transected fetuses the integrated diaphragm e.m.g. was used for breath identification and timing (Dawes *et al.* 1982). Tracheal pressure amplitude, inspiratory time and breath interval were determined off-line, and their frequency distributions were displayed on an *X–Y* plotter. Arithmetic means were used, except for breath interval where log determinations were employed since the arithmetic distribution was typically skewed to the left.

Comparisons between two groups of similar data were made using Student's *t* test. Analysis of variance and mutiple range testing (Dunnett's test) were used to identify significant differences within multiple data groups. Results are expressed as means ± S.E. of means.

RESULTS

Central acidosis

Cerebral ventricular perfusion. Cerebral ventricular perfusion was carried out successfully twenty-four times in eight fetal lambs for up to 13 days after surgery.

In two fetuses the effects of central acidosis on breathing movements were determined by perfusion with solutions having normal or reduced HCO_3^- concentrations.

In control experiments (one perfusion in each of two fetuses), 4 h perfusion with a solution having a $[HCO_3^-]$ similar to that of c.s.f. in normal fetal sheep (24 mmol/kg H_2O) produced no significant change in the c.s.f. pressure, arterial blood gas values, mean heart rate or arterial blood pressure. Good electrocortical and ocular records

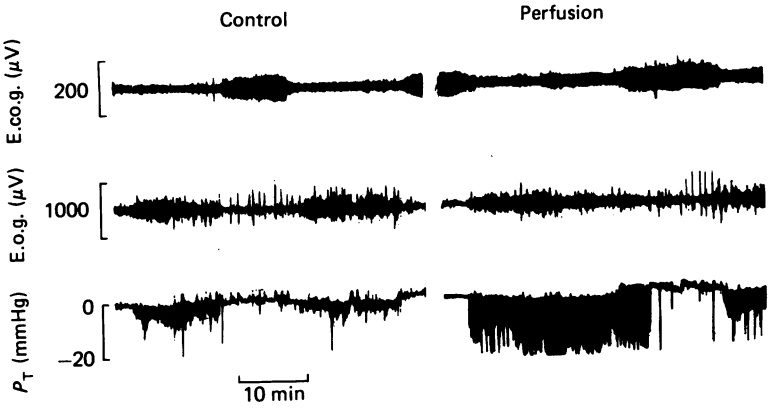

Fig. 1. Electrocorticogram (e.co.g.), electro-oculogram (e.o.g.), and breathing movements as indicated by changes in tracheal pressure (P_T) during central acidosis produced by cerebral ventricular perfusion with synthetic c.s.f. having a $[HCO_3^-] < 1$ mmol/kg H_2O compared to a control period before the perfusion.

TABLE 1. Breathing movements during cerebral ventricular perfusion with a solution having an $[HCO_3^-] < 1$ mmol/kg H_2O. Experimental measurements were taken during the third hour of perfusion

	Fetus no.	Control	Experimental
Longest episode (h)*	1	0·25†	0·45
	2	0·32	0·25
Incidence (% time)*	1	42†	48 ± 11
	2	37 ± 4·0	42 ± 2·7
Tracheal pressure amplitude (mmHg)	1	4·7 ± 0·1	9·0 ± 0·1
	2	4·8 ± 0·1	7·9 ± 0·1
Inspiratory time (T_I, s)	1	0·35 ± 0·006	0·32 ± 0·004
	2	0·34 ± 0·008	0·33 ± 0·005
Breath interval (T_{TOT}, s)	1	1·18	0·99
	2	1·11	0·90

* 4 h before (control) or during (experimental) the perfusion.
† 2 h preceding the perfusion.

were obtained in one fetus. The average low : high voltage ratio and the incidence of rapid eye movements did not change significantly during the perfusion from those beforehand. Breathing movements were largely unaffected, though breathing occurred during one transitional period (between low and high voltage electrocortical activity); and the mean tracheal pressure amplitude was marginally increased (by 1 mmHg or less).

Central acidosis was produced by cerebral ventricular perfusion in two fetal lambs (one experiment in each fetus) of which one had good electrocortical records. Perfusions for 2 h with a solution having a $[HCO_3^-] < 1$ mM reduced the mean outflow c.s.f. $[HCO_3^-]$ of 21·5 mmol/kg H_2O to new steady-state values of 12·0 mmol/kg H_2O. C.s.f. P_{CO_2} remained about 57 mmHg, while the outflow pH decreased on average from 7·219 to 6·981. During this time the arterial pH decreased slightly (−0·028 units) with

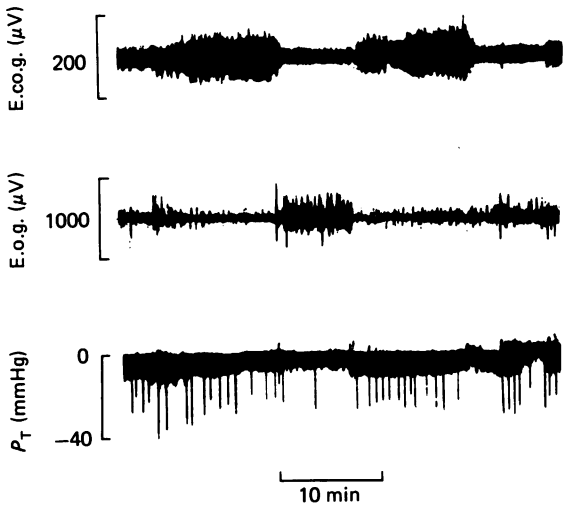

Fig. 2. Electrocorticogram (e.co.g.), electro-oculogram (e.o.g.), and breathing movements as measured by changes in tracheal pressure (P_T) after cerebroventricular administration of sodium meclofenamate.

no consistent change in plasma $[HCO_3^-]$. The central acidosis did not significantly affect heart rate or arterial pressure, the mean low:high electrocortical voltage ratio or the incidence of eye movements.

During the first 2 h of perfusion with acidic c.s.f., breathing movements occurred at times during transitional periods between low and high voltage states and for 1–3 min in episodes of high voltage electrocortical activity (Fig. 1). During the third and fourth hours of perfusion breathing movements were confined to low voltage periods.

The longest breathing episode (0·35 h) and breathing incidence (45 %) throughout the perfusion differed little from values (0·29 h and 40 % respectively) during the 4 h before the perfusion (Table 1). The mean tracheal pressure amplitude increased during the first hour of the perfusion, and after 2–3 h it was almost 80 % greater than the previous value (Table 1, Fig. 1). There was an average decrease in mean breath interval of 0·20 s, while inspiratory time was little changed (Table 1).

Chemical acidosis. The relation between breathing movements and electrocortical activity was also determined during central acidosis produced by another method (Molteni, Melmed, Sheldon, Jones & Meschia, 1980). Ammonium chloride (30·6 ± 4·9 mmol/kg) was infused intravenously over 6 h on five occasions in three fetal lambs. The mean arterial pH fell from 7·316 ± 0·006 to a steady value of 7·085 ± 0·019 by 2 h. In one lamb where c.s.f. samples were available the c.s.f. pH fell from 7·26 to 7·09, reaching a stable figure after 4 h. The incidence of breathing was much

TABLE 2. Heart rate, mean arterial pressure, and breathing movements in intact fetuses given sodium meclofenamate by intravenous infusion (I.V.) or cerebral ventricular perfusion (C.V.P.), or in transected fetal lambs after intravenous administration. The control period is the hour preceding the infusion, and the experimental period is the third hour after starting drug administration

	I.V. intact (6)		C.V.P. intact (5)		I.V. transected (5)	
	Control	Experimental	Control	Experimental	Control	Experimental
Sodium meclofenamate (mg/kg)	—	8·3±2·0	—	2·6±0·7	—	4·5
Heart rate (beats/min)	150±10 (4)	149±8·4	169±7·8	194±14	158±11	194±17
Blood pressure (mmHg)	49±3·6	46±3·8	46±2·5	49±3·6	49±4·3	50±5·2
Breathing movements						
Start of longest episode after drug infusion (h)	—	2·20±0·31	—	1·93±0·64	—	0·41±0·17
Longest episode (h)*	0·45±0·06	2·7±1·0	0·53±0·14	2·9±1·1	0·82±0·26	5·4±2·3
Tracheal pressure amplitude (mmHg)	4·6±0·9 (5)	8·6±1·4 (5)†	4·1±0·6	9·9±2·4†	4·8±0·7	8·9±1·5†
Inspiratory time (s)	0·35±0·05 (5)	0·24±0·05 (5)	0·27±0·03	0·26±0·04	0·56±0·03 (4)	0·53±0·06
Breath interval (s)	1·14±0·17 (5)	0·64±0·08 (5)	0·84±0·10	0·73±0·08	1·24±0·09 (4)	1·22±0·18

Figures in parentheses refers to number of fetuses.
* During 6 h before (control) or after (experimental) starting the drug administration.
† $P < 0·05$

reduced during the first 2 h but returned to approximately normal values (about 40 %) thereafter. Mean tracheal pressure amplitude always increased significantly. Breathing remained episodic and did not occur during high voltage electrocortical activity.

Prostaglandin synthetase inhibitors

Administration of meclofenamate either intravenously (six lambs at 124–135 days gestation: 8·3 ± 2·0 mg/kg) or by cerebral ventricular perfusion (five lambs:

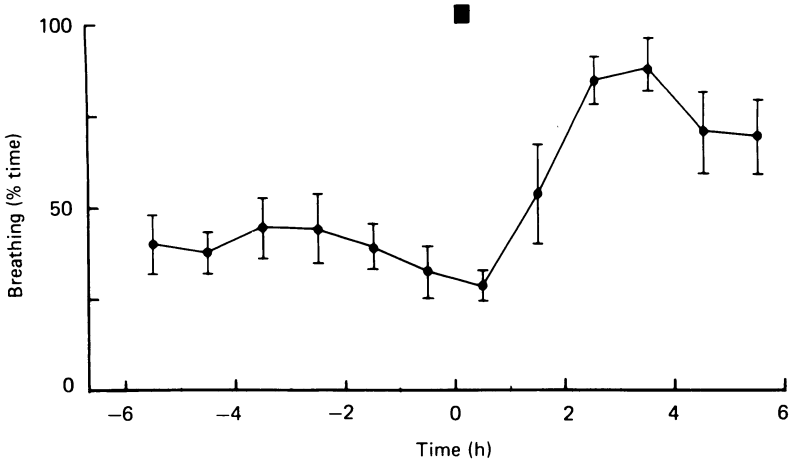

Fig. 3. Incidence of breathing movements after intravenous infusion of sodium meclofenamate (8·3 ± 2·0 mg/kg) in six fetal lambs. The bar shows the time of drug administration.

2·6 ± 0·7 mg/kg) induced prolonged periods of fetal breathing (Fig. 2), preceded by more regular and deeper episodic breathing movements. The onset of the longest continuous breathing episode varied from 0·78 to 3·05 h (mean 2·20 ± 0·31) after intravenous infusion and 0·17 to 3·82 h (mean 1·93 ± 0·64) after starting cerebral ventricular perfusion (Table 2). The longest episode after intravenous infusion (2·7 ± 1·0 h) was similar to that after cerebral ventricular perfusion (2·9 ± 1·1 h); both were greater than the longest episodes before drug administration (0·45 ± 0·06 and 0·53 ± 0·14 h, respectively). Both intravenous (Fig. 3) and intraventricular (Fig. 4) administration more than doubled the incidence by the third hour (to 85 ± 6 % and 84 ± 10 % respectively: $P < 0·05$).

Breathing movements which continued during both low and high voltage electrocortical activity were also produced by intravenous infusion of low doses of meclofenamate (0·77–0·84 mg/kg) on three occasions in two fetuses, or by cerebral ventricular administration of the drug (0·63 mg/kg). Lower intravenous (0·25–0·74 mg/kg) or intraventricular (0·23 mg/kg) doses failed to increase the incidence of breathing but did cause an increase in breath amplitude during low voltage activity. The increase in the depth of breathing movements was independent of drug dose over the range studied.

The pattern of breathing was similar after intravenous or intraventricular administration of sodium meclofenamate. Inspiratory time and the mean log breath interval were not significantly affected (Table 2). But breathing became more regular, and

the mean tracheal pressure amplitude was almost doubled. The electrocortical state (high or low voltage activity) had no significant effect on the mean inspiratory time, breath interval or tracheal pressure amplitude; yet inspiratory time and breath interval were less variable during high voltage activity.

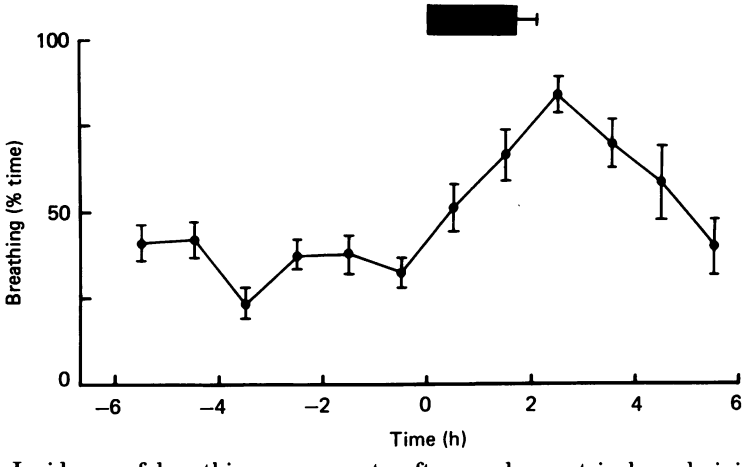

Fig. 4. Incidence of breathing movements after cerebroventricular administration of sodium meclofenamate (2·6 ± 0·7 mg/kg) in five fetal sheep. The bar shows the mean time of drug administration.

Some variation in responsiveness to intravenous administration of meclofenamate was observed when a dose was repeated within less than 2 days. Occasionally, the response as described was delayed or attenuated.

The blood gas values (P_{a,O_2} 23 ± 2 mmHg, P_{a,CO_2} 46 ± 2 mmHg and pH 7·34 ± 0·01), arterial pressure (49 ± 3·6 mmHg) and heart rate (150 ± 10 beats/min) were not altered significantly by administration of meclofenamate intravenously or into the cerebral ventricles. Likewise, the mean low:high voltage ratio of electrocortical activity was not changed significantly by intravenous (five lambs: 1·5 ± 0·5 control, 2·8 ± 0·4 experimental) or intraventricular (mean ratio in two lambs: 1·9 control, 1·3 experimental) administration of sodium meclofenate. The incidence of rapid eye movements (about 35 %) was also not significantly affected by meclofenate in either group.

Intravenous infusions of the less potent prostaglandin synthetase inhibitor acetylsalicylic acid (6·7 ± 1·3 mg/kg in two fetuses) also produced prolonged episodes of deep breathing which persisted during high voltage electrocortical activity. The plasma salicylate concentration was 89–115 μM in one such experiment. Prolonged breathing episodes were not observed (five infusions in two fetuses) with lower doses (3·0 ± 1·6 mg/kg); but, as with meclofenamate, such low doses caused an increase in the depth of breathing during low voltage electrocortical activity. None of these infusions caused a significant change in the blood gases, blood pressure, heart rate or electrocortical activity.

Hypoxia. Isocapnic hypoxia was induced for 15 min in three lambs in which continuous breathing for more than 1 h had been caused by intravenous administration of meclofenamate (an injection of 4·5–8 mg/kg followed by an infusion of 1 mg/kg . h).

The fetal arterial P_{O_2} fell from a mean value of 21 to 11 mmHg, and breathing ceased within 10 min. When the ewe was then given air to breathe, episodic fetal breathing returned within 20 min, and in two of four experiments it became continuous once more, persisting during high voltage electrocortical activity.

Brain stem transection. Successful brain stem sections were carried out in five lambs, of which two survived for seven days, and the remaining three were terminated

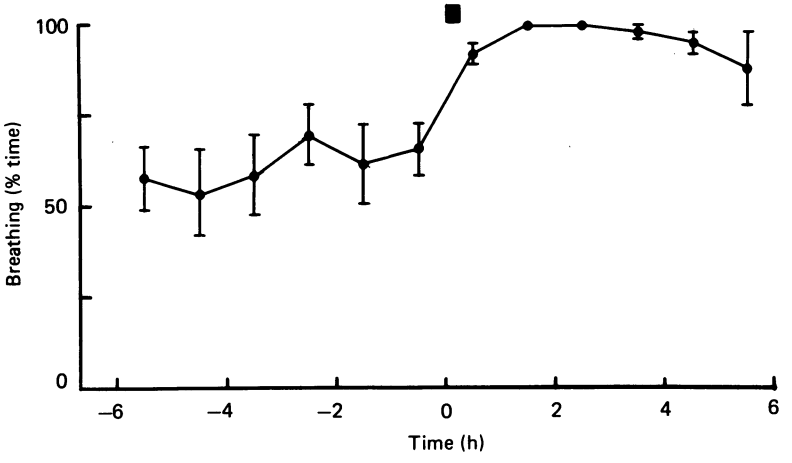

Fig. 5. Incidence of breathing movements in brain-stem transected fetuses after intravenous infusion of sodium meclofenamate (4·5 mg/kg). Bar indicates period of infusion.

15–24 days after operation. The brain stem was transected between the colliculi in one lamb, and within the pons in four fetuses, with variable necrosis in the mid-brain and rostral pons. One fetus had complete degeneration from the superior colliculus to almost the pontomedullary junction; less than 10% of the pons remained. Electrocortical activity was recorded in all five fetuses. Fetal breathing movements and electrocortical activity were dissociated in three lambs in which transection was complete. Hypoxia did not arrest breathing but induced an increase in the amplitude of breathing in all four lambs tested.

Intravenous infusions of sodium meclofenamate (4·5 mg/kg) induced prolonged deep breathing in fetal lambs with sectioned brain stems (Table 2, Fig. 5) without affecting significantly the low voltage:high voltage ratio of electrocortical activity ($1·6 \pm 0·2$ control, $2·4 \pm 0·6$ experimental). The effect differed in several respects from that in intact lambs. Continuous deep breathing began sooner, $0·41 \pm 0·17$ h after starting the infusion. The episodes of breathing were longer, with the mean longest ($5·4 \pm 2·3$ h) about twice that in intact fetuses. As in intact lambs the responses were variable when doses were repeated within less than 24 h.

The effects of hypoxia or hypercapnia were also studied after intravenous injection of meclofenamate (4·5 mg/kg) had induced continuous breathing of higher amplitude. Isocapnic hypoxia always increased the depth of breathing still further, with a small increase in frequency in three of four fetuses. Isoxic hypercapnia caused an increase in the depth of breathing in two of three lambs, with no significant change in frequency.

Prostaglandin E_2

In two fetuses with transected brain stems, intravenous infusion of prostaglandin E_2 virtually abolished meclofenamate-induced breathing within about 10 min after starting the prostaglandin infusion. This decreased incidence of breathing movements lasted for 36 min following a 15 min prostaglandin E_2 infusion, or 90 min following a 30 min prostaglandin E_2 infusion, and was not associated with significant changes in blood gases. On the other hand, prostaglandin E_2 in these two fetuses did not abolish breathing movements stimulated by hypercapnia ($\Delta P_{a,CO_2} = 9$ mmHg) or hypoxia ($\Delta P_{a,O_2} = -13$ mmHg).

DISCUSSION

Intravenous infusion of the prostaglandin synthetase inhibitor sodium meclofenamate produced breathing movements of increased amplitude and regularity during low and high voltage electrocortical activity as previously reported (Kitterman *et al.* 1979). The present study shows that fetal breathing movements were similarly stimulated with administration of the drug into the cerebral ventricles, suggesting that meclofenamate affects breathing through central mechanisms. This is supported by observations that the fetal breathing response to prostaglandin synthetase inhibitors is not altered by vagotomy and/or carotid body denervation (D. T. Murai & J. A. Kitterman, personal communication).

These studies extend the work of Dawes *et al.* (1983) and show that hypoxia stimulates breathing movements in fetuses with brain stem transection as low as the caudal pons. Furthermore, these studies show that prostaglandin synthetase inhibitors most likely produce continuous breathing by abolishing sleep-related inhibition within the medulla and that these inhibitors do not affect the pathways mediating hypoxic or pentobarbitone depression of breathing activity in normal fetal lambs (Dawes *et al.* 1983).

Prostaglandin synthetase inhibitors might act on the medulla in two categories of mechanisms, one non-specific and the other specific. For instance, prostaglandin synthetase inhibitors might stimulate breathing movements by uncoupling oxidative phosphorylation or by altering some other metabolic pathway (Flower, 1974). This seems unlikely since continuous breathing movements occurred in these experiments with free plasma salicylate levels about three times lower than those associated with such metabolic effects *in vitro* (Smith & Dawkins, 1971). On the other hand, these observations are consistent with breathing movements being stimulated as a result of inhibition of prostaglandin synthetase. The exact product(s) of this enzyme responsible for these breathing effects is unknown, although prostaglandins themselves may modulate breathing activity. For instance, intravenous prostaglandin E_2 or $F_{2\alpha}$ virtually abolished breathing activity in normal fetal sheep (Kitterman *et al.* 1983), and the present study suggests that this inhibition occurs within the medulla.

How prostaglandins and their inhibitors affect respiratory control is not known. They could alter vascular resistance centrally, and the breathing effects of these agents could result from a change in the metabolism/blood flow ratio within the brain stem (Pickard, MacDonell, MacKenzie & Harper, 1977; Sakabe & Siesjö, 1979).

Another possibility is that these agents affect prostaglandin modulation of neuronal activity (Wolfe & Coceani, 1979) related to respiratory control within the medulla.

Central acidosis produced by cerebral ventricular perfusion or by intravenous administration of ammonium chloride increased the amplitude of fetal breathing movements. This indicates that the central chemoreceptors in the fetal lamb may respond to changes in brain interstitial fluid pH as suggested by others (Hohimer, Bissonnette, Richardson & Machida, 1983). Central acidosis induced by cerebral ventricular perfusion also produced breathing movements for short periods (3 min or less) in high voltage states during the first 2 h of perfusion as previously reported (Hohimer *et al.* 1983); however, the present results differ in that breathing movements were confined entirely to low voltage states during the third and fourth hours of the experiment.

I am grateful for the interest and advice by Professor G. S. Dawes, surgical assistance by Dr D. Walker, plasma salicylate determinations by Dr J. McVittie, and histological examination of the brains by Dr T. Powell and Dr J. Sloper. This research was carried out under a Postdoctoral Fellowship from the United States Public Health Service and was assisted by a grant from the Medical Research Council.

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