Stimulatory Effect of Theophylline on Regulation of Fetal Breathing Movements

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Summary

We have performed the fetal CO₂ response test on eight mature lambs *in utero* and have found that theophylline is a respiratory stimulant over a wide range of PaO₂ and pHa. Theophylline produces significant increase in slope of P₁₀₀, P_{max}, and VEq response curves and significant increase in P₁₀₀, P_{max}, f, and VEq responses at PaCO₂, 65 torr. It also produces significant lowering of the naturally high fetal absolute threshold to CO₂, *i.e.*, PaCO₂ at which breathing starts during the CO₂ test. "Offset" threshold, *i.e.*, PaCO₂ when breathing stops after the test, is the same with and without theophylline and equal to absolute threshold with theophylline. Our studies indicate that: (1) theophylline lowers fetal threshold and increases sensitivity to CO₂; (2) increased sensitivity is expressed mainly by heightened P_{max} (*i.e.*, the equivalent of tidal volume) with some contribution of f particularly at high PaCO₂; (3) decreased CO₂ threshold is best assessed by determination of absolute threshold rather than the conventional x-axis intercept method; (4) the low offset threshold is consistent with our understanding that arousal is a requisite for generation and maintenance of fetal breathing; (5) theophylline is as effective a respiratory stimulant during fetal acidemia and hypoxemia as it is under normal conditions; and (6) a number of serious reservations must be held regarding use of theophylline during low pHa, low PaO₂ states.

Speculation

Theophylline, which is shown to be an effective respiratory stimulant in the ovine fetus, activates central respiratory units by a process of recruitment analogous to that of other inputs, *e.g.*, somatosensory and chemoreceptor afferents. Changes in the state of respiratory center arousal, including its return to natural quiescence with apnea when inputs are withdrawn, can be assessed by the fetal CO₂ test and bear on the phenomena of onset of breathing at birth, neonatal apnea, and sudden infant death.

Rationale for the use of theophylline to reduce incidence and severity of apnea of prematurity (3, 17, 19) is its presumptive effect on activation of the central nervous system and excitation of physiologic respiratory reflexes (9). However, few controlled studies have been attempted to quantify the effect of theophylline on respiratory regulation, and some results have been contradictory. Whereas it is reported that theophylline increases the response to hypoxia but has no effect on CO₂ response in adults (10), it has also been suggested that CO₂ sensitivity increases and CO₂ threshold is lowered when theophylline is given to babies (7).

We have examined the effect of theophylline on regulation of breathing in our lamb fetal model (11), which offers the following advantages. Because the fetus is normally apneic for extended periods of time, one can assess directly the effect of theophylline on the onset of fetal breathing and on absolute threshold to CO₂. In addition, the fetal CO₂ test (11) can be used to evaluate the effect of theophylline on sensitivity and extrapolated threshold to CO₂ from the CO₂ response curves. At the serum levels achieved in this study, theophylline did not initiate fetal breathing move-

ments before elevation of PaCO₂. However, it did increase sensitivity to CO₂ and lower the naturally high CO₂ threshold of the mature fetus *in utero*.

MATERIALS AND METHODS

The animal preparation and method for estimation of respiratory control in the fetus have been described in detail in a previous report (11). In summary, experiments were carried out on eight pregnant sheep near term (140 to 144 days). Under local anesthesia, an endotracheal tube was inserted through a tracheostomy in the ewe and was used for CO₂ rebreathing. The uterus was exteriorized through an abdominal incision under spinal anesthesia and was kept moist and warm throughout the experiment. The fetal neck was marsupialized to the uterine wall under local anesthesia, and the fetal trachea and carotid artery were cannulated for measurement of intratracheal pressure (ITP), for recording of fetal arterial pressure and heart rate and measurement of pHa, PaCO₂, and PaO₂. The fetal tracheal cannula was attached directly to a Statham P23d transducer (closed, liquid-filled system) from which ITP was recorded directly on a Narco BioSystems eight channel polygraph (sensitivity <0.5 cm H₂O pressure). There was virtually no loss of amniotic fluid during the marsupialization procedure, and the fetus remained inside the uterus throughout the study.

Fetal CO₂ responsivity was established by the fetal CO₂ test (11), in which fetal breathing response is measured during increasing fetal PaCO₂, which is brought about by CO₂ rebreathing in the ewe. All fetuses in this study were apneic before fetal CO₂ tests. Generated fetal breathing movements were made against a closed trachea so that flow of normal fetal pulmonary fluid was not a factor during the study. In addition, because the lungs are normally filled with pulmonary fluid at about functional residual capacity and because fluid volume is not expected to change significantly during episodes of induced fetal breathing (11, 15), we conclude that the lungs were at resting volume during the tests and that fluid compression was not a factor (11).

One-half to 1 hr after completion of surgical preparation and observation of the fetus to ensure normal blood pressure and heart rate, the ewe was made to rebreathe 7% CO₂-93% O₂ in our modification (11) of the Read rebreathing method (13, 14). The fetal breathing response to CO₂ was quantified as follows: (1) P₁₀₀ (torr) was defined as the ITP deflection during the first 100 msec of an inspiratory effort and was considered to represent respiratory drive; (2) P_{max} (torr) was defined as the maximal ITP per breath and was considered equivalent to tidal volume; (3) breathing frequency (f, min⁻¹), and (4) ventilation equivalent (VEq) was defined as the sum of peak intratracheal pressure efforts for 1 min (Σ torr min⁻¹). After the control fetal CO₂ test and when fetal PaCO₂ had returned to pretest levels, theophylline was administered to the ewe at a dose of H5 mg/kg aminophylline (according to estimated body weight, ± 5 kg, of the prestudy pregnant ewe) by IV infusion for 20 min. Serum theophylline levels were determined immediately before the CO₂ test, both in the ewe and fetus, by the theophylline-specific enzyme mediated immunoassay (Syva, Palo Alto, CA). Sensitivity of the assay in our laboratory

Table 1. Effects of theophylline on fetal heart rate and blood pressure and on P_{aCO_2} measurements during fetal CO_2 tests

	Heart rate (min^{-1})	Mean arterial pressure (torr)	P_{aCO_2} ¹ (torr)	ΔP_{aCO_2} ² (torr)
Control ($n = 8$) ³	176.3 ± 13.3 ⁴	62.0 ± 2.5	46.1 ± 4.4	$+21.4 \pm 3.2$
Theophylline ($n = 11$)	173.6 ± 14.9	59.9 ± 2.6	44.8 ± 3.3	$+18.8 \pm 1.6$
Difference ⁵	NS	NS	NS	NS

¹ Fetal P_{aCO_2} 1 min after the start of fetal CO_2 test.² Increase of fetal P_{aCO_2} during fetal CO_2 test.³ n = number of measurements.⁴ Mean $\pm$ S.E.⁵ By paired comparison statistical analysis and Student's t test; NS = not significant ($P > 0.05$).

is 2.5 to 20 $\mu\text{g}/\text{ml}$ with an intra-assay coefficient for variation of 4%. In one fetus (fetus 8), the CO_2 test was repeated once and in another (fetus 4), it was repeated twice after P_{aCO_2} had returned to control after the preceding test. P_{100} , P_{max} , f , and VEq were used to describe fetal breathing response to P_{aCO_2} both when CO_2 was the sole respiratory stimulus and when it was combined with theophylline. Results were expressed in terms of P_{100} - PCO_2 , P_{max} - PCO_2 , f - PCO_2 , and VEq - PCO_2 response curves, from which slopes and x-axis intercepts as well as specific values at P_{aCO_2} of 65 torr were used for statistical analysis. P_{aCO_2} 65 torr, for fetuses that did not achieve this level during the CO_2 test, was determined by extrapolation from the slope of the CO_2 response. This assumes that the fetus' response would continue along the linear plot if P_{aCO_2} had been allowed to increase to 65 torr.

RESULTS

Serum levels of theophylline at the time of the fetal CO_2 tests were $11.6 \pm 2.0 \mu\text{g}/\text{ml}$ ($\bar{x} \pm \text{S.E.}$) in the ewe and $5.9 \pm 2.1 \mu\text{g}/\text{ml}$ in the fetus ($n = 11$). As shown in Table 1, theophylline did not result in significant changes of fetal heart rate and blood pressure. One min after the start of the test, fetal P_{aCO_2} was 46.1 ± 4.4 torr at control and 44.8 ± 3.3 torr after theophylline infusion; the difference is not significant. P_{aCO_2} increased during the test by 21.4 ± 3.2 torr at control and by 18.8 ± 1.6 torr after theophylline; the difference between the two is not significant. These results are summarized in Table 1.

The relationship between fetal P_{aO_2} and pH_a 1 min into the CO_2 test is shown in Figure 1. The correlation coefficient (0.76) is significant ($P < 0.01$), whereas the differences in each parameter between control and theophylline are not. The variation of P_{aO_2} and pH_a among fetuses was related both to their individual response to the surgical procedure and to the time after preparation at which the CO_2 tests were done. However, heart rate and rhythm and blood pressure were normal at the levels given in Table 1; these parameters did not change during the course of the experiments. Fetal P_{aO_2} increased significantly ($P < 0.01$) during the CO_2 test by 4.4 ± 1.0 torr (control) and by 3.9 ± 0.7 torr (theophylline), and the difference between the two is not significant. Because of the wide range of P_{aO_2} - pH_a among fetuses, we compared the CO_2 responses of the acidemic subjects ($pH_a < 7.2$) with those of fetuses with $pH_a > 7.24$, as shown in Figure 1 (boxed groups). Although responses of the acidemic group tended to be slightly lower, there was no significant difference between these two groups. There was no relationship between the level of theophylline and the magnitude of fetal breathing response in individual experiments.

Theophylline alone did not initiate breathing movements in any of the fetuses before and between the CO_2 tests. The effects of theophylline on P_{100} , P_{max} , f , and VEq during the fetal CO_2 test are shown in Figure 2 in which the response curves from a single experiment are presented. The effects of theophylline on slope, x-axis intercept (*i.e.*, extrapolated CO_2 threshold), and response at P_{aCO_2} 65 torr are summarized in Table 2 as the differences between responses at control and after theophylline. Theophylline produced a significant increase both in the slopes of the P_{100} - PCO_2 , P_{max} - PCO_2 , and VEq - PCO_2 response curves and in the P_{100} , P_{max} ,

Fig. 1. The relationship between fetal P_{aO_2} and pH_a 1 min after the start of fetal CO_2 test with and without theophylline. Fig. 2. Respiratory drive (P_{100} ; A), intratracheal pressure (P_{max} ; B), breathing frequency (f ; C) and ventilation equivalent (VEq ; D) responses to fetal P_{aCO_2} in response to CO_2 alone and to CO_2 combined with theophylline. Data are from a single experiment (see text).

Table 2. Effects of theophylline on CO₂ response in fetal lambs in utero

	S ¹	I	65
P ₁₀₀	0.3 ± 0.1 ² P < 0.025	1.5 ± 9.7 NS	10.4 ± 2.9 P < 0.005
P _{max}	0.9 ± 0.4 P < 0.050	0.6 ± 6.4 NS	22.1 ± 3.7 P < 0.001
f	1.1 ± 0.7 NS	-18.4 ± 20.4 NS	50.7 ± 18.9 P < 0.025
VEq	77.2 ± 22.7 P < 0.010	-2.4 ± 7.3 NS	1799.3 ± 582.4 P < 0.025

¹ S = slope, i.e., CO₂ sensitivity in torr·PCO₂ torr⁻¹ for P₁₀₀ and P_{max}, in min⁻¹·PCO₂ torr⁻¹ for f, and in torr/min·PCO₂ torr⁻¹ for VEq; I = x-axis intercept, i.e., CO₂ threshold in torr PaCO₂; 65 = a selected fetal PaCO₂ level at which P₁₀₀, P_{max}, f, and VEq were calculated specifically.

² Mean ± S.E. of the difference between responses to CO₂ alone and to CO₂ combined with theophylline (n = 11). Statistics are based on paired comparison analysis and student's *t* test.

and VEq responses at PaCO₂ 65 torr. Although the slope of the f-PCO₂ response was higher than control after theophylline, the change was not significant, in contrast to the significant increase of f at PaCO₂ 65 torr.

Whereas in the experiment shown in Figure 2 the x-axis intercepts of P₁₀₀, P_{max}, f, and VEq curves are shifted to the left with theophylline, these extrapolations were variable among experiments, so that the overall result was that theophylline did not change the intercept significantly (Table 2). Because determination of CO₂ threshold by this method is indirect, we also measured fetal PaCO₂ directly near the time at which the fetus started breathing during the CO₂ test. This value may be taken as an index of absolute ("onset") fetal threshold to CO₂ (11). We found that, at control, the fetuses started breathing at PaCO₂ of 55.9 ± 2.5 torr (n = 8), and after theophylline, they started breathing at PaCO₂ of 46.4 ± 2.9 torr (n = 11). When calculated by paired comparison analysis, the difference between absolute thresholds to CO₂ is significant (P < 0.005).

All fetuses stopped breathing within 1 to 3 min after the CO₂ test, at which time fetal blood samples were drawn and PaCO₂ threshold for offset of breathing was determined. At control, this threshold was 46.8 ± 3.1 torr (n = 4), and with theophylline, it was 45.7 ± 2.4 torr (n = 5). The difference between the two is not significant, nor is the difference between these offset thresholds and the absolute (onset) threshold with theophylline.

DISCUSSION

Our studies show that theophylline increases central responsiveness to CO₂ and thus may be classified as a respiratory stimulant of the fetus over a wide range of Pao₂ and pHa. However, this designation of theophylline should include the following qualifications: (1) because theophylline is a general metabolic stimulant that accelerates cerebral metabolism, it may be particularly harmful in the presence of fetal hypoxemia (18); (2) because methylation to caffeine is a significant pathway of theophylline metabolism in the perinatal subject (2, 4), the cumulative effect of the two drugs may increase toxicity; (3) although theophylline is effective at low pH, the combined effects of methyl xanthines and acidemia on cerebral, cardiac, and gastrointestinal function need to be taken into account. Observation of heart rate, blood pressure, and amniotic fluid appearance during the course of our studies gave no sign of toxic response to theophylline. Clearly, more direct and extended assessment is needed.

Cosmi (6) has reported that theophylline infusion to the ewe results in generation of fetal breathing movements; the quantities administered were two to three times larger than those of our experiments. In addition, a number of investigators have reported successful treatment of apnea of prematurity with theophylline infusion to the infant (1, 3, 7-9, 17, 19). The serum levels achieved in infants, when reported, were comparable to or larger than those

produced in our fetuses. In contrast to our fetuses *in utero*, however, the patients were subject to the usual environmental stimuli of the nursery, which are important activators of perinatal breathing (5, 16). Thus, we would suggest that theophylline levels in our studies were too low to induce spontaneous breathing movements in the previously apneic fetus, but that respiratory control was affected to the extent that CO₂ threshold and responsiveness were facilitated. As PaCO₂ was increased during the CO₂ test, the lowered threshold and increased sensitivity became apparent. This state would correspond to stage 1 in the activation of central respiratory units of the apneic fetus, in which recruitment of respiratory neurons has not yet reached the level needed for activation of inspiratory-facilitatory neurons, as discussed previously (5). Because we have no data on possible conversion of theophylline to caffeine in the lamb fetus, we cannot speculate on the possible role of the latter in stimulating respiratory control.

We have described in a previous report (11) the ovine animal model for study of generation and regulation of fetal breathing. This model offers the advantages of: (1) producing linear increase of fetal PaCO₂ during maternal rebreathing; (2) assessment of respiratory drive from measurements of ITP; (3) determination of ventilation analogue (VEq) from measurements of P_{max} and f; and (4) avoiding the complications of changes in lung mechanics during the test period. Whereas Pao₂ did not change significantly during the fetal CO₂ test in our previous study (11), it did increase in the present experiments. The increase, however, was the same both with and without theophylline so that Pao₂ can be excluded as a cause for the increased CO₂ responsiveness with the drug.

The effect of theophylline on fetal breathing may be quantified in several ways. It is evident from the changes in slope of P₁₀₀-PCO₂, P_{max}-PCO₂, and VEq-PCO₂ response curves that theophylline does increase CO₂ sensitivity. Because the apparent increase of f-PCO₂ slope was not significant, we may conclude that augmentation of VEq-PCO₂ slope was due primarily to increased P_{max}, which is the equivalent of tidal volume (11). However, facilitation of f cannot be excluded because, for a given PaCO₂ (e.g., 65 torr), theophylline produces a significant increase of P₁₀₀, P_{max}, VEq, and f. Whether or not such changes in f are related to recruitment of inspiratory-expiratory units by mechanisms similar to those described in another report (5) remains speculative at present.

The most complicated aspect of the breathing response to theophylline is assessment of its effect on threshold to CO₂. Inasmuch as the fetuses of this study were apneic before the CO₂ test, we were able to determine CO₂ threshold both by extrapolation of the response curves to the x-axis (extrapolated threshold) and by direct measurement of PaCO₂ near the time that breathing movements began (absolute threshold). We compared absolute threshold at control (before theophylline) to both absolute and extrapolated thresholds of apneic fetuses in our previous study (11) and found no difference among any of these; mean values ranged from PaCO₂ 52.7 to 55.9 torr. This confirms our conclusion (11) that CO₂ threshold in the apneic fetus is high and that normal fetal PaCO₂ is in itself not a stimulus to breathing. In contrast, the absolute threshold with theophylline was significantly lower than all CO₂ thresholds of previously apneic fetuses, but it was not different from the extrapolated threshold of fetuses that had been breathing spontaneously before the CO₂ test (11). This indicates that, in terms of CO₂ responsiveness, the effect of theophylline is analogous to that of other stimuli (5, 11) that may produce arousal and activation of central respiratory neurons.

Our finding that theophylline effect was not apparent by comparison of extrapolated thresholds before and after infusion of the drug reflects possible errors inherent in the x-axis intercept method when applied to the previously apneic subject. It suggests that the rate of increase of respiration from the state of apnea has at least two slopes which may be concealed in standard analysis. For spontaneously breathing fetuses, the difficulty is the same as that encountered when the test is applied to air-breathing subjects, *viz.*, the curve may not in fact intercept the x-axis from a linear plot (12). A real limitation is the number of PaCO₂ determinations that can be made during the test. Continuous recording would be

preferred, and it is clear that, under any circumstances, "curves" based on only two determinations (7) may compromise reliability of the test. However, when the fetus is apneic, absolute threshold, which is determined easily during the CO₂ test, should be taken as the definitive threshold.

The offset CO₂ threshold before and after theophylline was the same as both the theophylline-induced absolute (onset) threshold and the extrapolated (onset) threshold of spontaneously breathing fetuses of our previous study (11). Because all these thresholds were lower than the absolute (onset) threshold of control apneic fetuses, it is conceivable that chemical and pharmacologic stimuli (*i.e.*, CO₂ and theophylline) act similarly to natural arousal (*i.e.*, spontaneous breathing *in utero*) in exerting an excitatory effect on the respiratory control system located in the brainstem. The high absolute threshold to CO₂ of control apneic fetuses reflects a less excited state of respiratory control which may be of relevance to clinical conditions, such as apnea of prematurity and sudden infant death syndrome. Additional studies are required, including monitoring of cortical activity and function of central respiratory units.

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