Adenosine A_{2A}-receptor blockade abolishes the roll-off respiratory response to hypoxia in awake lambs

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Koos, Brian J., Yoshikazu Kawasaki, Young-Han Kim, and Fanor Bohorquez. Adenosine A_{2A}-receptor blockade abolishes the roll-off respiratory response to hypoxia in awake lambs. *Am J Physiol Regul Integr Comp Physiol* 288: R1185–R1194, 2005. First published December 23, 2004; doi:10.1152/ajpregu.00723.2004.—Adenosine (ADO) receptor antagonists (aminophylline, caffeine) blunt the respiratory roll-off response to hypoxia in the newborn. This study was designed to determine the ADO receptor subtype involved in the respiratory depression. Chronically catheterized lambs of 7–16 days of age breathed via face mask a gas mixture with a fraction of inspired O_2 of 0.21 (normoxia) or 0.07 (hypoxia), while being infused intravascularly with 9-cyclopentyl-1,3-dipropylxanthine (DPCPX; ADO A_1-receptor antagonist, $n = 8$), ZM-241385 (ADO A_{2A}-receptor antagonist, $n = 7$), or vehicle. Ventilation was measured at 20°C by a turbine transducer flowmeter. In normoxia [arterial P_{O_2} (Pa_{O_2}) of ~83 Torr], infusion of vehicle did not alter cardiorespiratory measurements, whereas hypoxia (Pa_{O_2} of ~31 Torr, 15 min) elicited biphasic effects on mean arterial pressure (transient increase), heart rate (HR; diminishing tachycardia), and minute ventilation. In the latter, hypoxia increased ventilation to a peak value of ~2.5 times control within the first 3 min, which was followed by a significant ($P < 0.05$) decline to ~50% of the maximum increment over the subsequent 7 min. ZM-241385 abolished the hypoxic ventilatory roll-off and blunted the rate of rise in HR without affecting mean arterial pressure or rectal temperature responses. In normoxia, DPCPX increased ventilation and mean arterial pressure but did not change HR. Compared with vehicle, DPCPX did not significantly affect cardiorespiratory responses to hypoxemia (Pa_{O_2} of ~31 Torr, 10 min). It is concluded that *1*) ADO A_{2A} receptors are critically involved in the ventilatory roll-off and HR responses to hypoxia, and *2*) ADO A_1 receptors, which are tonically active in cardiorespiratory control in normoxia, appear to have little impact on hypoxic ventilatory depression.

brain; newborn; respiration; caffeine; thermoregulation

THE RESPIRATORY RESPONSE of the human newborn to hypoxia is biphasic: an initial stimulation followed by a decline toward control levels (3, 34). The hyperpnea is blunted in preterm infants and, in fact, is not evident during sleep in those weighing <1,500 g (1). The depression of ventilation in the second phase, which is more prominent in developmentally immature newborns (e.g., kittens, human neonates), is attenuated with greater neurological maturity, as in newborn lambs (7). A second-phase ventilatory decline can also be observed in conscious cats (40), goats (14), and human subjects (13), but adults of most other species display the roll-off only while anesthetized. Thus the magnitude of the reduction in ventilation in the second phase depends on the state of consciousness, species, maturity, and degree of hypoxia (2, 3).

Low P_{O_2} levels in the brain trigger the decline in ventilation, which may be accompanied by impaired integration of afferent input from the carotid sinus nerves into respiratory drive (3). Most evidence indicates that a time-dependent attenuation in peripheral arterial chemoreceptor activity is not involved. Reduced metabolic rate and other factors can also contribute to the respiratory roll-off (3, 34).

A rise in brain concentrations of adenosine may have a critical role in the second-phase response because the roll-off is generally attenuated by adenosine receptor blockade achieved with methylxanthines, such as aminophylline and caffeine. This blunting of the depressant phase has been demonstrated in anesthetized rabbit pups (35), piglets (10), and adult rats (39). Adenosine receptor antagonists have also reduced the ventilatory roll-off in conscious cats (28) and human subjects (13).

Central application of stable agonists highly selective for adenosine A_1 receptors depressed respiration in decerebrate rabbit pups and anesthetized rats and cats, a response that was blocked by nonselective adenosine receptor antagonists. Thus, of the four adenosine receptors (A_1, A_{2A}, A_{2B}, A_3), the adenosine A_1 receptor has been surmised to be the key subtype involved in hypoxic respiratory inhibition (3).

In unanesthetized fetal sheep (3), intravascular infusion of methylxanthines that effectively cross the blood-brain barrier also blunts the depressant effects of hypoxia on breathing movements (4, 7, 21, 26), and stable adenosine A_1-receptor agonists, injected intravenously or into the cisterna magna, inhibit breathing activity. These observations are consistent with the notion that adenosine A_1 receptors also mediate the hypoxic arrest of fetal breathing. Subsequent studies with an adenosine A_{2A}-receptor agonist and more potent, specific, and selective adenosine receptor antagonists, however, have shown that adenosine A_{2A} receptors (rather than A_1) are critically involved in hypoxic inhibition of fetal breathing (20, 25). These provocative results raise the question as to whether central adenosine A_{2A} receptors also depress respiratory responses to hypoxia in postnatal life. In this study, highly selective antagonists to adenosine A_1 and A_{2A} receptors were administered to developing lambs to determine whether they would blunt the second-phase decline in ventilation. These experiments were designed to test the hypothesis that the adenosine A_{2A}-receptor subtype is involved in the hypoxia-induced ventilatory roll-off in newborn lambs, as it is in the fetus.

EXPERIMENTAL PROCEDURES

All surgical and experimental procedures were carried out in accordance with the guidelines of the American Physiological Society and were approved by the UCLA Chancellor's Animal Research Committee. Upon completion of the studies, euthanasia of the lambs was achieved by intravenous injection of pentobarbital sodium (180 mg/kg iv).

Surgery

Fifteen lambs 3–5 days of age underwent aseptic surgery for placement of vascular catheters. Oxytetracycline (10 mg/kg), ampicillin (20 mg/kg), and atropine (0.1 mg/kg) were administered intramuscularly about 30 min before induction of 0.5% isoflurane anesthesia; buprenorphine (0.01 mg/kg im) and ketamine (3 mg/kg iv) were injected before intubation for general anesthesia. Polyvinyl catheters, which were implanted in the right external jugular vein and carotid artery, were advanced so that the catheter tips were within the superior vena cava and aorta, respectively. The catheters were exteriorized through a small incision on the right dorsolateral thorax and secured in a nylon pouch attached to the lamb's flank.

After recovery from anesthesia, the lambs were housed with their ewes. Lamb weights were measured daily to document normal weight gain (≥ 0.3 kg/day). The catheters were kept patent by daily injections of heparinized saline (4 U/ml).

Experiments

Studies in lambs (7–16 days of age) were begun at least 2 days after surgery at the lower range of thermoneutrality (20°C), an ambient temperature that maintains body temperature in normoxia with minimal O_2 consumption (38). The unanesthetized lamb was placed in the prone position in a sling. Arterial pressure, which was measured with a pressure transducer (Argon Medical, Dallas, TX) and amplifier (Quadbridge amplifier, ADInstruments, Mountain View, CA), was referenced to the level of the right atrium. Heart rate was determined from the arterial pressure pulse. Arterial P_{O_2} (Pa_{O_2}), P_{CO_2} (Pa_{CO_2}), and pH were measured on a blood-gas machine (Instrumentation Laboratories, Lexington, MA) with measurements corrected to 39°C. Rectal temperature was measured with a Thermalert model TH-8 temperature probe (Physitemp, Clifton, NJ).

Ventilation was measured by a precision turbine transducer flowmeter (Interface Associates, Laguna Nigel, CA), which was attached to a malleable rubber face mask (Jorgensen Laboratories, Loveland, CO) that was bonded with hydrophilic vinyl polysiloxane (31) to the lamb's shaven snout. The analog-output signal was processed with an electronic ventilation measurement module (VMM-400, Interface Associates) to record the increment in lung volume over the time of each inspiration. The signal was reset to zero after peak inspiratory volume (i.e., tidal volume) was achieved. Breath interval was calculated from the time of the start of each breath. This volume transducer had a resolution of 0.5 ml and a linear accuracy of $\pm 1.5\%$ for volumes up to 3 l/s. The turbine transducer flowmeter had the advantages of stability of calibration and of no zero drift.

Gas mass flowmeters (302 series, Hastings Instruments, Hampton, VA) and a controller (model 400, Hastings Instruments) provided precision mixing of respiratory gases (air, O_2, CO_2, and N_2). The humidified gas mixture flowed (18 l/min) through flexible respiratory tubing (2.2 cm ID) that was attached via a Y-connector to the flowmeter and face mask. End-tidal O_2 fraction was measured (model 175818 oxygen analyzer, VacuMed, Ventura, CA) continuously from gas drawn from the face mask.

Heart rate, arterial pressure, rectal temperature, and respiratory volumes were sampled at 200 Hz, displayed on an electronic chart recorder (PowerLab/16 SP chart data recorder, ADInstruments), and recorded on the hard drive of a microcomputer.

Experimental protocol. The lambs breathed air from the respiratory circuit for at least 15 min before the onset of hypoxia (fraction of inspired $O_2 = 0.07$). An intravenous infusion of a selective adenosine receptor antagonist or its vehicle was started 10 min before induction of hypoxia. A total of 30 ml of each solution (at 20°C, room temperature) was infused for each experiment. One experiment was performed per day to minimize potential carryover effects of the adenosine receptor antagonists, and the order of infusion (drug, vehicle) was varied to control for order and/or time effects (25). Arterial blood-gas and pH levels were measured before infusion (control) and 1, 5, 10, and 15 min after the start of hypoxia. A recovery sample was taken 10 min after inspired O_2 fraction was restored to normal. Carbon dioxide (0.2–0.8 l/min) was added to the hypoxic gas mixture to minimize changes in Pa_{CO_2} that could confound interpretation of results (32). Adjustments in inspired CO_2 fraction were made according to measured Pa_{CO_2}.

Adenosine A_1-receptor antagonist. 9-Cyclopentyl-1,3-dipropylxanthine (DPCPX; Tocris Cookson, Ballwin, MO) was dissolved in 0.04 M 2-hydroxypropyl-β-cyclodextrin and 0.2 N NaOH (50:50, vol/vol) to a final drug concentration of 2.5 mg/ml. DPCPX was infused into the superior vena cava at 1.2 mg·min^{-1}·kg^{-1} over 10 min of normoxia and, subsequently, at 0.24 mg·min^{-1}·kg^{-1} for 15 min of hypoxia. During these studies, the duration of hypoxia was reduced from 15 to 10 min because lambs with adenosine A_1-receptor blockade were less tolerant of low Pa_{O_2}. The dose of this highly specific and selective adenosine A_1-receptor antagonist was based on our previous studies in fetal sheep (25). The extent of adenosine A_1-receptor blockade was determined by respiratory responses to hypoxia upon doubling the dose of DPCPX.

Adenosine A_{2A}-receptor antagonist. ZM-241385 (Tocris Cookson) was dissolved in polyethylene glycol at 400 and 0.1 N NaOH (50:50, vol/vol) and diluted with saline to a final concentration of 0.66 mg/ml. ZM-241385 was infused into the superior vena cava at 0.65 mg·min^{-1}·kg^{-1} for 10 min of normoxia and 0.056 mg·min^{-1}·kg^{-1} for 15 min of hypoxia. The dose of ZM-241385, a highly specific and selective blocker of adenosine A_{2A} receptors, was based on our previous work in fetal sheep (25). The adequacy of adenosine receptor blockade was determined by evaluating respiratory responses to hypoxia while administering twice the dose of the antagonist.

Data Analysis Methods

Mean values of heart rate, mean arterial pressure (MAP), temperature, tidal volume, inspiratory time (T_I), and respiratory period (total breath duration or T_{TOT}) were determined for sequential 20-s epochs with the use of PowerLab software (ADInstruments). Minute ventilation (l·min^{-1}·kg^{-1}) was calculated by multiplying tidal volume (l/kg) by 60 s/min with division of this value by respiratory period (s/breath). Measurements before the start of infusion were subtracted from values during the infusion and recovery to correct for baseline differences. These mean profiles over time were compared within and between groups using a two-way repeated-measures ANOVA model and the Tukey-Fisher's least significant difference post hoc comparison criterion (JMP version 5.1, SAS Institute, Cary, NC). The two fixed factors in the two-way repeated-measures analysis were time and infusion group (infusion of drug or vehicle).

Minute ventilation was also analyzed by phase, in which *phase 1* was defined as the maximum ventilation for each lamb achieved during the first 5 min of hypoxia and *phase 2* or roll-off was defined as ventilation measured 10 and 15 min after the start of hypoxia. Ventilation was compared within and between groups by an ANOVA model and the Tukey-Fisher's least significant difference post hoc comparison criterion. Phase and the infusion group were the two fixed factors in the two-way repeated measures analysis.

For selected time periods of infusion (normoxia, hypoxia), slopes (rates) of temperature change for normoxia and hypoxia were determined for each animal by linear regression, and the mean slopes for ZM-241385, DPCPX, and vehicle were compared within and between

animals by two-way repeated measures ANOVA, where time period (not time in min) and drug were the fixed factors. Differences were considered statistically significant if $P < 0.05$. Values given are means ± SE.

RESULTS

Adenosine A_1-Receptor Blockade

DPCPX was infused intra-arterially to eight lambs at 10.1 ± 0.8 (DPCPX) and 10.4 ± 0.9 (vehicle) days of age. Mean lamb weights were 6.82 ± 0.49 and 6.87 ± 0.52 kg for DPCPX and vehicle experiments, respectively. In these experiments, the duration of hypoxia was shortened from 15 to 10 min because the DPCPX-treated lambs were less tolerant of acute falls in Pa_{O_2}.

Arterial blood gases and pH. DPCPX minimally affected blood gases and pH in normoxic lambs ($n = 3$). Before infusion, mean Pa_{O_2}, Pa_{CO_2}, and pH were 92.7 ± 0.6 Torr, 41.1 ± 1.7 Torr, and 7.438 ± 0.040, respectively. DPCPX infusion (~8 min) was associated with a Pa_{O_2} of 96.6 ± 2.1 Torr, a Pa_{CO_2} of 39.3 ± 1.8 Torr, and an arterial pH of 7.454 ± 0.011. Infusion of the vehicle had virtually no effect on Pa_{O_2} (preinfusion: 88.8 ± 5.5 Torr, infusion: 86.0 ± 6.7 Torr), Pa_{CO_2} (preinfusion: 41.4 ± 0.9, infusion: 41.9 ± 0.9 Torr), and pH (preinfusion: 7.428 ± 0.029, infusion: 7.439 ± 0.027).

During hypoxia, Pa_{O_2} was lowered to 30–33 Torr, whereas mean Pa_{CO_2} was kept within 2–4 Torr of control (Fig. 1A).

Hypoxia significantly decreased arterial pH only in lambs treated with the adenosine A_1-receptor blocker.

Cardiovascular responses. In control vehicle experiments ($n = 7$), hypoxia increased MAP by ~7 mmHg ($P = 0.07$), which was followed by a decline toward control levels (Fig. 2A). In contrast, DPCPX was associated with a progressive increase in MAP in normoxia that was further enhanced by ~7 mmHg ($P = 0.07$) within the first minute of hypoxia before falling back toward control values.

In vehicle-treated lambs, mean heart rate rapidly increased within 3 min of the onset of hypoxia, which was followed by a progressive decline from maximum rates during the last 6 min of hypoxia. Heart rate responses to hypoxia in DPCPX-treated lambs were similar except that the tachycardia persisted throughout the 10-min recovery period.

Temperature. Mean rectal temperatures under basal conditions before the start of infusion were 39.99 ± 0.10 and 40.12 ± 0.09°C, respectively, for DPCPX and vehicle experiments ($n = 6$). Mean rectal temperature declined by 0.022 ± 0.006°C/min during the 10-min vehicle infusion before hypoxia ($n = 4$), which was not significantly different from the −0.01 ± 0.006°C/min reduction with DPCPX. The temperature in vehicle-treated lambs continued to fall at the same rate (−0.017 ± 0.006°C/min) during hypoxia (Fig. 2A).

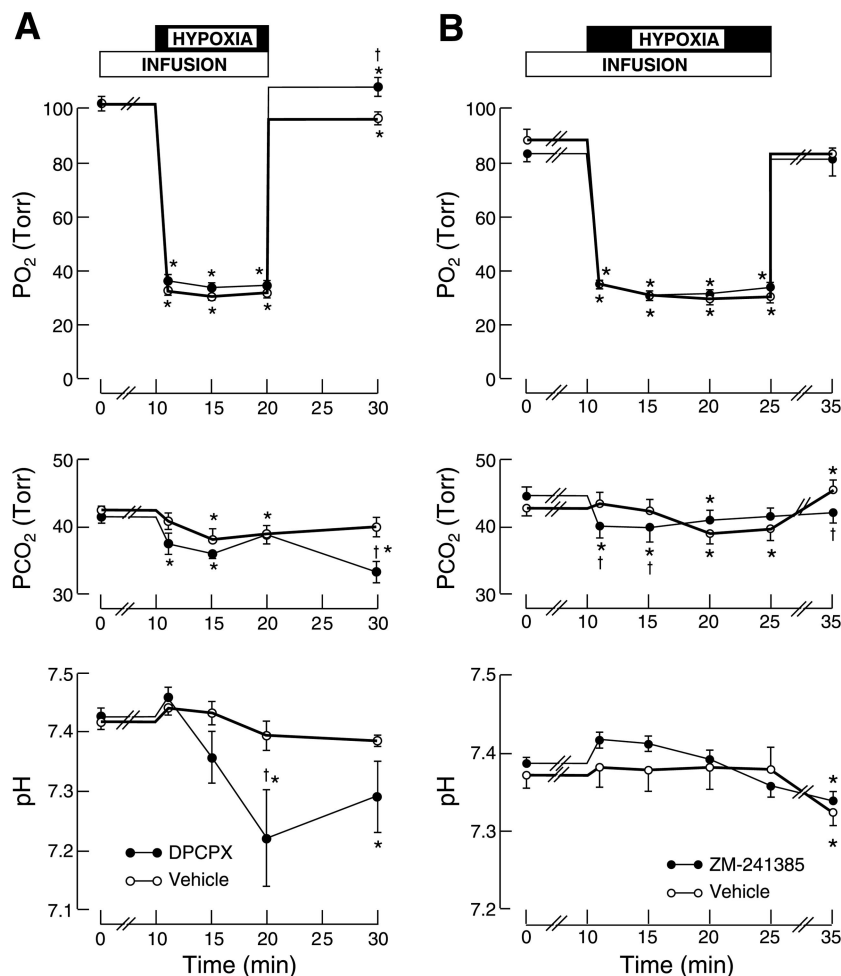Two distinctly different responses in core temperature occurred in lambs treated with DPCPX and hypoxia. In the first,

Fig. 1. Effects of hypoxia on arterial blood gases and pH. *A*: intravenous infusion of 9-cyclopentyl-1,3-dipropylxanthine (DPCPX; ●) or vehicle (○) in 8 lambs. *B*: intravenous administration of ZM-241385 (●) or vehicle (○) in 7 lambs. *P < 0.05 compared with 0 min. †P < 0.05 compared with vehicle at same time.
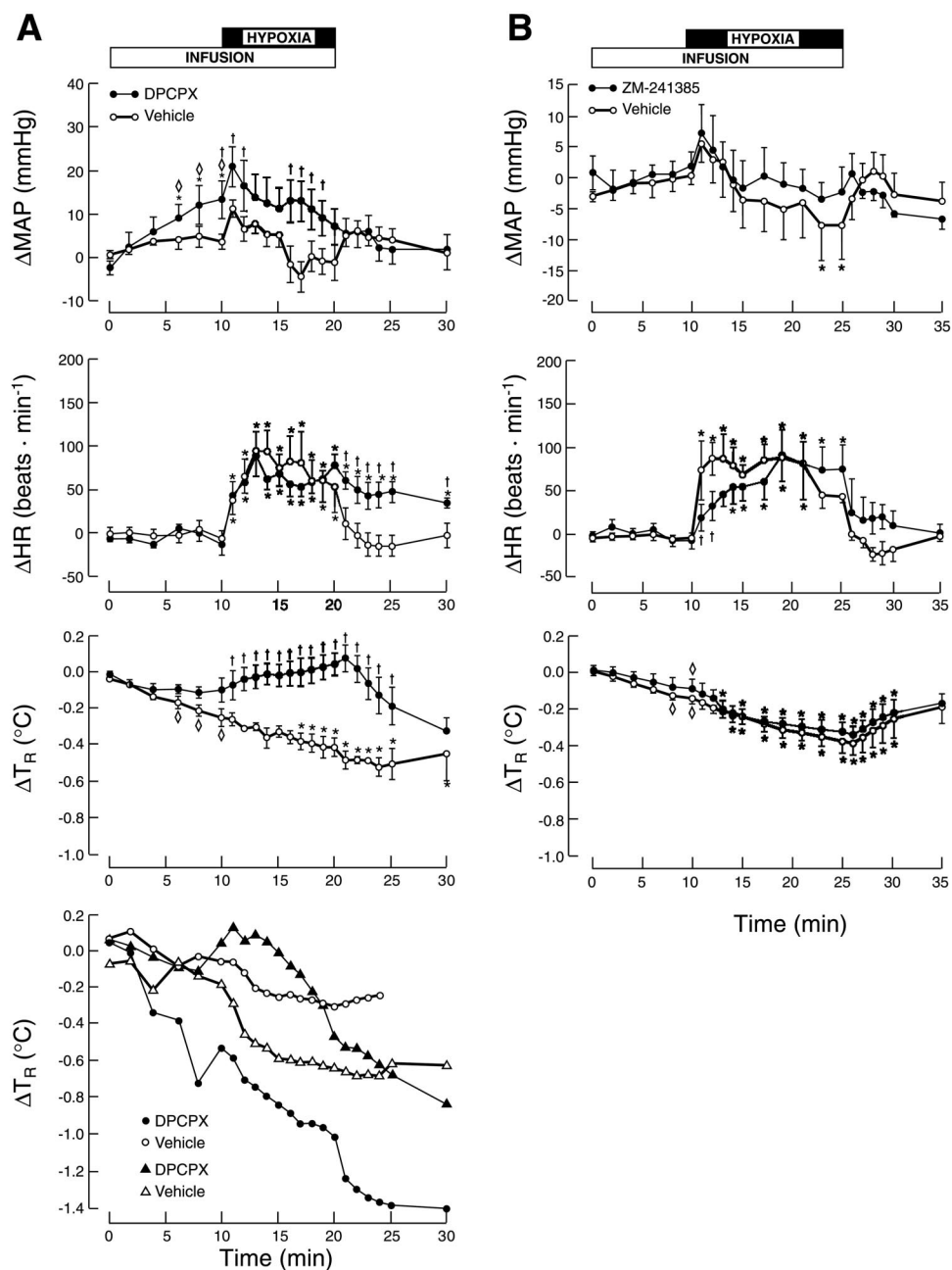
Fig. 2. Changes (Δ) in mean arterial pressure (MAP), heart rate (HR), and rectal temperature (T_R). *A*: administration of adenosine A_1-receptor blocker DPCPX ($\bullet$) or vehicle ($\circ$) in 7 (MAP, HR) and 6 lambs (T_R). *Panel 3* shows changes in rectal temperature in 4 lambs, and *bottom panel* depicts results in 2 additional lambs [no. 301 (circles), no. 351 (triangles)] that had atypical temperature responses to hypoxia with DPCPX infusion. *B*: infusion of adenosine A_{2A}-receptor blocker ZM-241385 ($\bullet$) or vehicle ($\circ$) in 5 lambs. *$P < 0.05$ compared with 10 min. †$P < 0.05$ compared with vehicle. $\Diamond P < 0.05$ for normoxia measurements compared with *time 0*.

mean core temperature did not fall with hypoxia in four lambs (8–13 days of age), which resulted in rectal temperatures significantly greater than vehicle-treated lambs during virtually the entire 10 min of hypoxia (Fig. 2*A*). In these DPCPX-treated lambs, the slope of the change in rectal temperature as a function of time for hypoxia ($0.011 \pm 0.006°C/min$) was significantly greater than for normoxia. In the second, mean rectal temperature in two lambs (7 and 9 days of age) declined by $0.049°C/min$ during hypoxia with DPCPX infusion (Fig. 2*A*, *bottom*).

Inspiratory time. In vehicle studies, T_I was unaltered by normoxia but was reduced by nearly 40% by hypoxia (Fig. 3*A*). In DPCPX-treated lambs, T_I progressively fell in normoxia but was not decreased further by hypoxia. During recovery, T_I

remained decreased in DPCPX-treated lambs compared with vehicle-infused animals.

Respiratory period. In control vehicle studies, T_{TOT}, which was unaltered by normoxia, was reduced nearly 50% by hypoxia, with a subsequent return to control values upon normalization of fetal Pa_{O_2}. DPCPX lowered T_{TOT} in normoxia, an effect that persisted in hypoxia and recovery.

Tidal volume. In normoxia, DPCPX administration was associated with an ~30% rise in tidal volume that did not reach statistical significance ($P = 0.08$) with the number of animals studied. Hypoxia rapidly increased tidal volume by more than two-fold, which was followed by a gradual decline over the remainder of hypoxia.
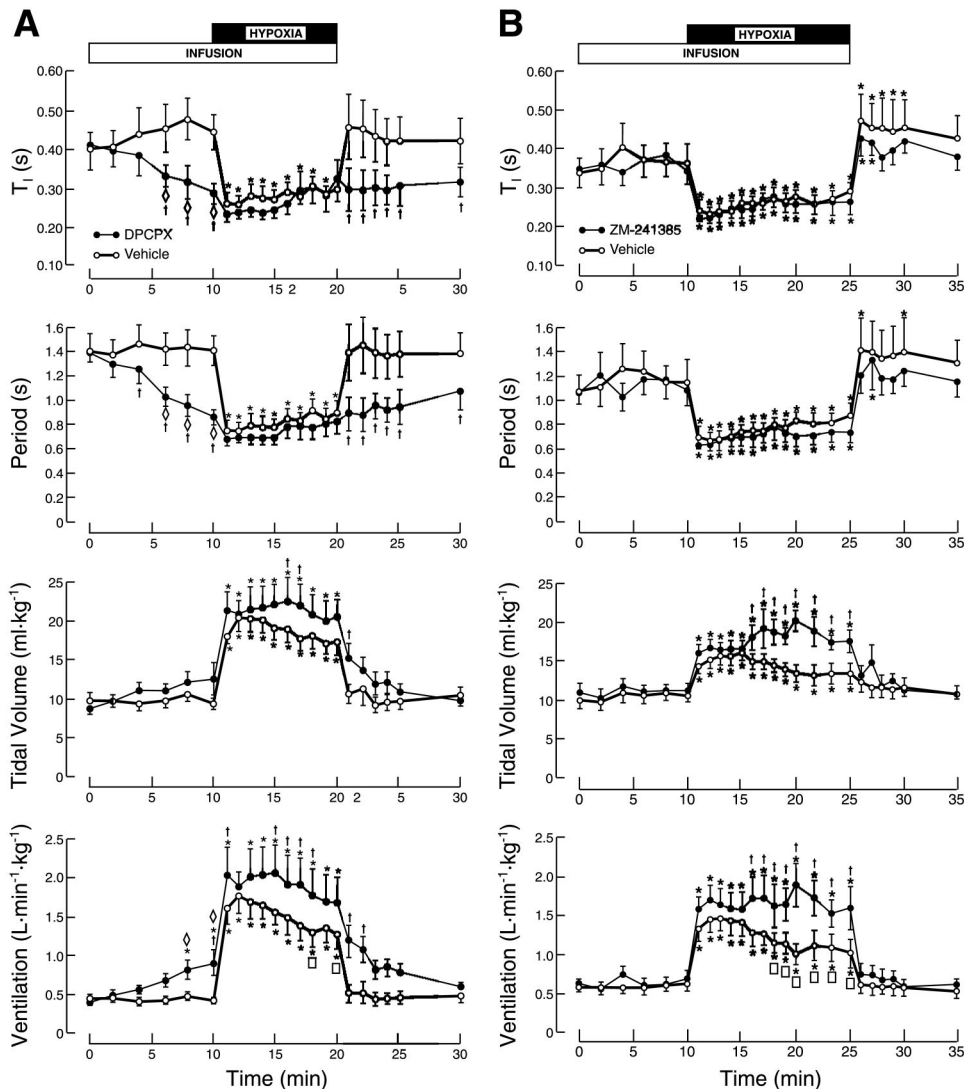
Fig. 3. Effects of selective antagonism of adenosine A_1- or A_{2A}-receptor subtypes on inspiratory time (T_I), respiratory period, tidal volume, and minute ventilation. *A*: DPCPX (●) or vehicle (○) in 8 lambs. *B*: ZM-241385 (●) or vehicle (○) in 7 lambs. °$P < 0.05$ for normoxia measurements compared with *time 0*. *$P < 0.05$ compared with 10 min. †$P < 0.05$ compared with vehicle. □$P < 0.05$ compared with peak minute ventilation at 12–13 min.

Minute ventilation. Vehicle-treated lambs displayed a clear biphasic respiratory response to hypoxia. Ventilation, which was unchanged in normoxia, rose nearly four-fold within 2 min of the start of hypoxia but subsequently fell ($P < 0.05$) to ~60% of the peak increment by the end of hypoxia.

DPCPX significantly increased minute ventilation by over two-fold in normoxia. Because of this change in baseline, ventilation reached significantly higher levels than those achieved in control vehicle experiments. Doubling the dose of DPCPX did not alter the respiratory response.

Baseline shifts in DPCPX-treated lambs have been taken into account in Fig. 4, which shows changes in respiratory variables in hypoxia relative to measurements taken immediately before hypoxia. In this analysis, the maximum stimulatory response to hypoxia in DPCPX-treated lambs appears blunted compared with vehicle-treated lambs, although the difference was not significant ($P = 0.08$) with the number of lambs studied. Otherwise, ventilatory responses were virtually identical for vehicle- and DPCPX-treated animals.

Ventilatory responses to hypoxia according to phase are shown in Fig. 5A. In vehicle-treated lambs, ventilation measured 10 min after the start of hypoxia was 37% lower ($P <$ 0.004) compared with peak *phase 1* values (*top*) or 46% lower ($P < 0.001$) than the *phase 1* increment (*bottom*). This ventilatory roll-off was not significantly altered by DPCPX.

Adenosine A_{2A}-Receptor Blockade

Respiratory responses to ZM-241385 and hypoxia were determined in seven lambs at 10.9 ± 0.7 (ZM-241385) and 10.3 ± 0.8 (vehicle) days of age. Mean weights of the lambs were 6.29 ± 0.57 and 6.30 ± 0.61 kg for experiments involving ZM-241385 and vehicle, respectively.

Arterial blood gases and pH. During hypoxia, mean Pa_{O_2} was reduced to ~31 Torr from the control of ~83 Torr (Fig. 1*B*). Hypoxia was associated with relatively small changes in Pa_{CO_2} (<3 Torr) with significantly lower values in the first 5 min of hypoxia in the group that received the adenosine A_{2A}-receptor antagonist. For both vehicle and ZM-241385 experiments, mean arterial pH was not significantly reduced until 10 min after hypoxia was terminated.

Cardiovascular responses. A transient increase ($P = 0.06$) in MAP of 5–6 mmHg occurred within the first 2 min of hypoxia in lambs treated with either vehicle or ZM-241385
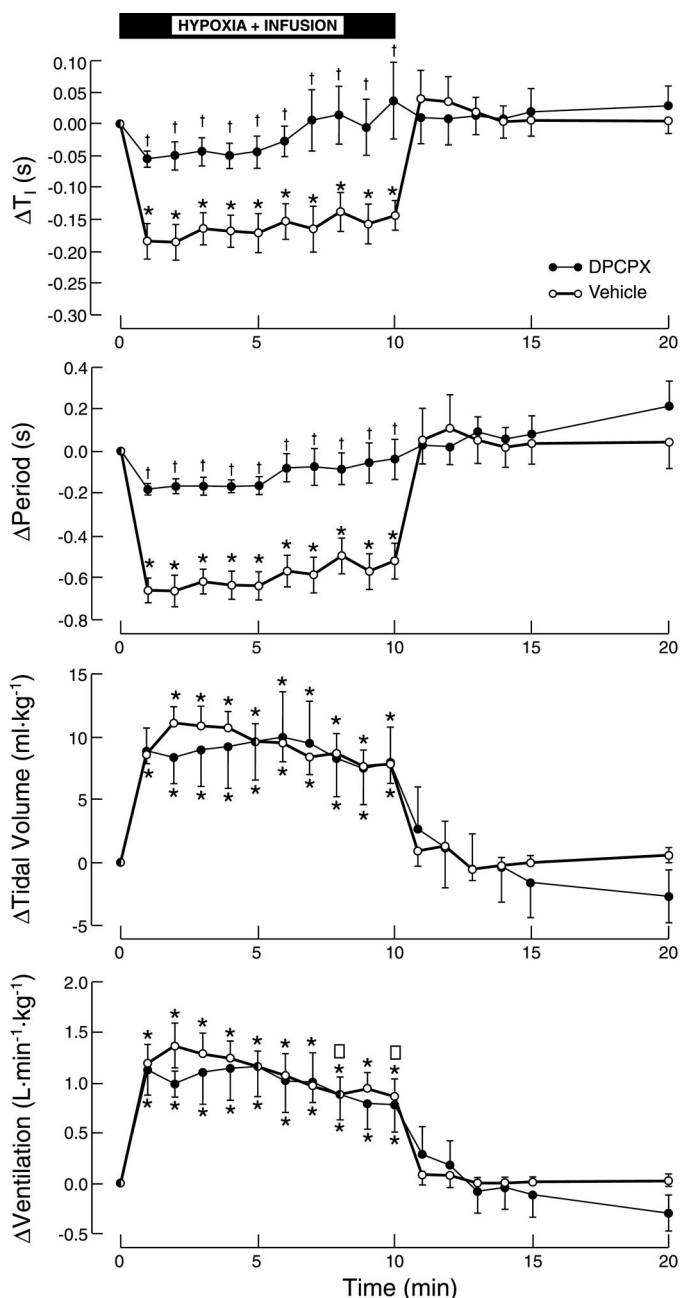
Fig. 4. Changes in T_I, respiratory period, tidal volume, and minute ventilation during hypoxia with intravenous infusion of DPCPX (●) or vehicle (○). *$P < 0.05$ compared with *time 0*. †$P < 0.05$ compared with vehicle. □$P < 0.05$ compared with peak ventilation at 2 min for vehicle infusions.

(Fig. 2B). In both groups, MAP fell after 2 min, which became significantly less than prehypoxic values for the vehicle group after 12 min of hypoxia.

In vehicle-treated lambs, hypoxia induced a tachycardia, which declined over the last 5 min of hypoxia. ZM-241385 significantly blunted the hypoxia-induced rise in heart rate; however, the maximum rate, which was similar in magnitude to vehicle, was sustained throughout latter stages of hypoxia.

Temperature. Mean rectal temperatures under basal conditions before the start of infusion were 39.83 ± 0.17 and $39.64 \pm 0.12°C$ for lambs treated with ZM-241385 and vehi-

cle, respectively. In normoxia, the decline in rectal temperature ($n = 5$) was similar for vehicle- and ZM-241385-treated lambs (slope: $-0.017 \pm 0.004°C/min$ for vehicle, $-0.013 \pm 0.004°C/min$ for ZM-241385). The slopes of temperature fall during hypoxia, which were $-0.015 \pm 0.004°C/min$ for vehicle and $-0.015 \pm 0.004°C/min$ for ZM-241385, were virtually identical to those for normoxia. In both cases, rectal tempera-
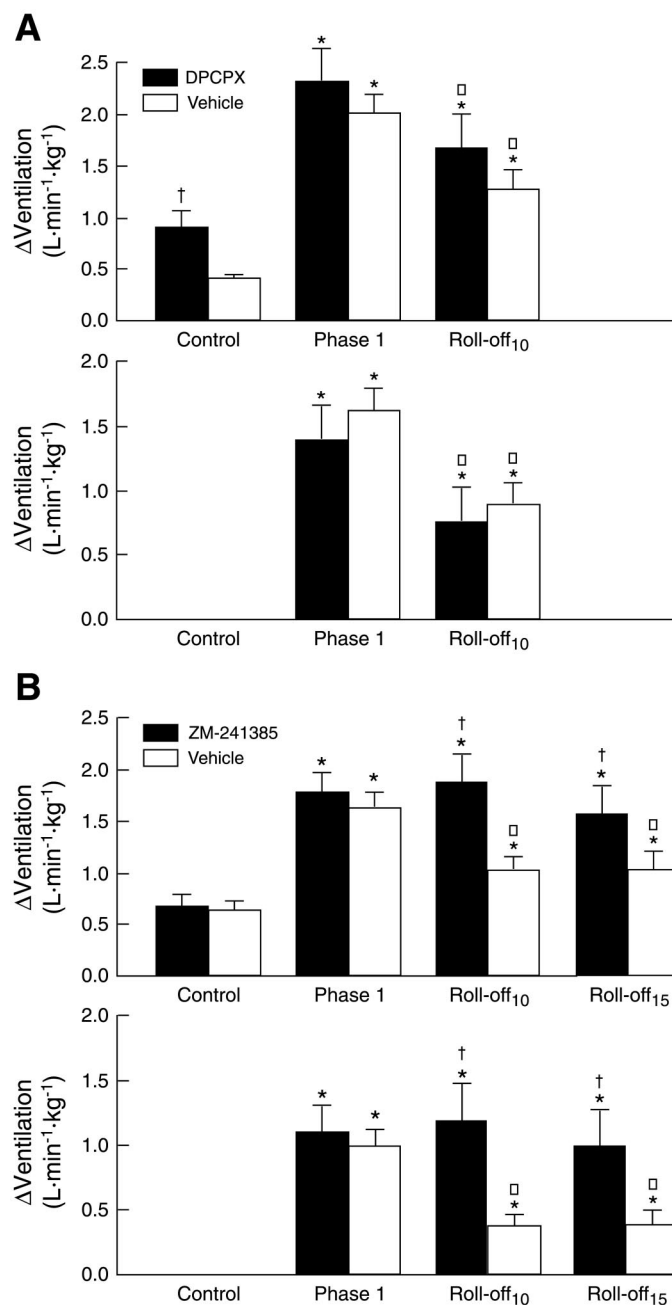
Fig. 5. *A, top*: effects of DPCPX or vehicle on maximum hypoxic stimulation of ventilation (*phase 1*) in each lamb and on ventilation 10 min (roll-off$_{10}$) and 15 min (roll-off$_{15}$) after the start of hypoxia. *Bottom*: changes in ventilation from control for *phase 1* and for roll-off$_{10}$ and roll-off$_{15}$. *B, top*: effects of ZM-241385 or vehicle on the maximum stimulatory effects of hypoxia on ventilation (*phase 1*) in each lamb and at roll-off$_{10}$ and roll-off$_{15}$. *Bottom*: changes in ventilation from control for *phase 1* and for roll-off$_{10}$ and roll-off$_{15}$. *$P < 0.05$ compared with control. †$P < 0.05$ compared with vehicle. □$P < 0.05$ compared with *phase 1*.

ture rose toward control values on termination of the infusion and hypoxia.

Inspiratory time. T_I, which was not altered by infusion of ZM-241385 or vehicle in normoxia, was significantly reduced by hypoxia in both cases (Fig. 3*B*). T_I increased rapidly after Pa_{O_2} was returned to normal, with the initial recovery value significantly greater than that at *time 0*. The effects of adenosine A_{2A}-receptor blockade with hypoxia on T_I were similar to those observed for vehicle.

Respiratory period. The adenosine A_{2A}-receptor antagonist did not significantly alter T_{TOT} in normoxic lambs (Fig. 3*B*). Hypoxia reduced mean T_{TOT} by about one-third, which approximated the response observed with the vehicle alone.

Tidal volume. In vehicle-treated lambs, the hypoxia-induced rise in tidal volume was not sustained as ventilation fell over the last 10 min of hypoxia. In ZM-241385 experiments, tidal volume rose within the first minute of hypoxia, an increment that was either maintained or augmented through the remainder of hypoxia.

Minute ventilation. Neither the vehicle nor ZM-241385 changed minute ventilation in normoxia. Vehicle-treated lambs displayed a biphasic respiratory response to hypoxia that was characterized by an initial 2.3-fold rise in ventilation followed by ~50% retracement from peak values. ZM-241385 abolished the depressant response: the initial 2.6-fold rise in ventilation was sustained or augmented throughout the 15 min of hypoxia. Doubling the dose of ZM-241385 did not significantly alter the monophasic respiratory response.

Analysis according to phase response (*phase 1* and roll-off responses 10 and 15 min after the start of hypoxia) revealed that, in vehicle-treated lambs, ventilation measured 10 or 15 min after the start of hypoxia was 37% less ($P < 0.002$) than the maximum value of *phase 1* (Fig. 5*B*, *top*). Relative to control, maximum *phase 1* ventilation was increased by ~1 $l \cdot min^{-1} \cdot kg^{-1}$, which was followed by a 60% decline ($P < 0.002$) for ventilation measured 10 and 15 min after the start of hypoxia (Fig. 5*B*, *bottom*). In contrast, ventilation was maintained throughout the 15 min of hypoxia in lambs treated with ZM-241385.

DISCUSSION

This study shows that activation of adenosine A_{2A} receptors mediates the respiratory roll-off response to hypoxia in lambs; adenosine A_1 receptors do not appear to be significantly involved. That adenosine A_{2A} receptors are crucial modulators of the depressant respiratory effects of hypoxia in the fetal sheep (25) and developing lambs indicates that a central adenosinergic inhibitory pathway remains functional as respiratory control changes at birth.

Hypoxia-Induced Responses During Vehicle Infusion

Cardiorespiratory and temperature changes to vehicle infusion were measured to establish control responses to normoxia and hypoxia. These experiments were used to compare lamb responses to infusion of the adenosine A_1- or A_{2A}-receptor antagonist.

Normoxia. Infusion of vehicle for DPCPX or ZM-241385 did not significantly alter mean heart rate, MAP, or respiration, although both infusions were associated with a small decline in

rectal temperature. The latter was likely due to the infusion of a solution (20°C) cooler than body temperature (~39.7°C).

Hypoxia. Biphasic cardiovascular changes in hypoxia included *1*) an initial rise and then fall in MAP and *2*) an increase followed by a modest retracement in mean heart rate, as previously reported (37). Chemoreflex-mediated systemic vasoconstriction accounted for the initial rise in arterial pressure, whereas vasodilatation that was partly secondary to hyperventilation produced the decline in MAP (9, 37). The tachycardia resulted from stimulation evoked from several sources, including pulmonary reflexes (via hyperpnea), aortic chemoreceptors, central nervous system, and probably other elements (9, 37). The carotid chemoreceptors were not directly involved because bradycardia is the primary cardiac response to stimulation of the carotid bodies in mammals (9).

Respiratory responses to hypoxia in both control groups involved increased tidal volume and respiratory frequency (decreased T_{TOT}), which resulted in hypoxic augmentation (*phase 1*) of ventilation, as reported previously (3, 7). The magnitude of this rise in ventilation depends on input of the peripheral arterial chemoreceptors, which increases with postnatal age and developmental maturity, and probably also to the extent of excitatory input from the posterior hypothalamus (3).

After 2–4 min of hypoxia, ventilation declined significantly over the remainder of the study due to a fall in respiratory rate and tidal volume. The mechanisms underlying this roll-off involve a complex interaction of several factors, including central respiratory inhibition as well as the central modulation of excitatory afferent traffic from the carotid bodies (3). The depressant effects of steady-state hypoxia on respiration represent a dynamic adjustment in respiratory regulation because it can be rapidly abolished by further reductions in Pa_{O_2} (7).

Hypoxia, which increases oxygen delivery by eliciting hyperpnea, can also lower O_2 consumption through a regulated decrease in body temperature (2, 30). The latter would reduce CO_2 production and thus decrease excitatory input to respiratory drive. In the present studies, the rate of fall in rectal temperature for vehicle-treated lambs was virtually the same for normoxia and hypoxia, indicating that the decline during hypoxia resulted from infusion of a room-temperature solution rather than from a regulated lowering of metabolic rate and core temperature in response to low Pa_{O_2}. Thus hypoxia-induced hypometabolism with a resulting fall in CO_2 production would not appear to be a major factor in the roll-off ventilatory response to hypoxia in these lambs.

Adenosine A_1-Receptor Blockade

Normoxia. DPCPX increased the lambs' MAP results by ~7 mmHg, which presumably reflected increased systemic vascular resistance and/or cardiac output. These results are contrary to our observations in unanesthetized fetal sheep in which DPCPX did not significantly affect MAP (23, 24). In both the fetus and newborn, mean heart rate was not altered by adenosine A_1-receptor blockade.

DPCPX stimulated ventilation in normoxic lambs by primarily reducing inspiratory and expiratory durations. These results, which show that adenosine A_1 receptors tonically suppress respiratory drive, are consistent with our previous studies in unanesthetized fetal sheep (24) and in anesthetized cats with peripheral arterial chemodenervation (36). Thus

blockade of brain adenosine A_1 receptors is the likely mechanism involved in the well-recognized respiratory stimulation evoked by nonselective adenosine receptor antagonists, such as caffeine and theophylline. The depressant respiratory effects of central adenosine A_1-receptor activation probably involve presynaptic inhibition of neurotransmitter release, postsynaptic hyperpolarization, inhibition of intracellular cAMP production, and activation of K_{ATP} channels (29, 36).

Hypoxia. Compared with vehicle, MAP and heart rate responses to hypoxia were qualitatively similar for DPCPX-treated lambs. However, the posthypoxia tachycardia in the animals with adenosine A_1-receptor blockade was not observed in vehicle-treated lambs.

Systemic blockade of adenosine A_1 receptors failed to significantly alter breathing responses to hypoxia, which counters the notion that adenosine A_1 receptors are major participants in the second-phase decline in ventilation. The results are surprising because DPCPX administration not only antagonized central adenosine A_1 receptors that inhibit breathing but also resulted in conditions (increased body temperature, metabolic acidemia) that normally stimulate respiration. On the other hand, these respiratory effects in the newborn are consistent with our previous studies in fetal sheep in which hypoxic arrest of breathing was not blunted by DPCPX (25).

Adenosine A_1-receptor blockade by DPCPX does not alter hypoxia-induced cerebrovasodilation (6). Thus the respiratory effects of DPCPX appear not to be significantly affected by drug-induced alterations in brain blood flow and O_2 delivery.

In contrast to our studies in fetal sheep (25), hypoxia with adenosine A_1-receptor blockade produced a rapidly progressive metabolic acidemia that restricted the duration of hypoxia in lambs with DPCPX. At the end of hypoxia, core body temperature in DPCPX-treated lambs was ~0.33°C greater than in vehicle-infused animals, which is consistent with an increase in their metabolic rate. However, factors other than increased O_2 consumption are likely involved in their reduced tolerance to hypoxia because a significant metabolic acidemia with a decline in rectal temperature occurred in two lambs.

In four of the six lambs in which rectal temperature was measured, DPCPX abolished the infusion-associated fall in rectal temperature. The anterior hypothalamus, which triggers sympathetic activation of brown fat metabolism (2, 27), would be a likely site of action, although peripheral effects of adenosine A_1-receptor blockade on thermogenesis cannot be excluded.

For the two lambs in which DPCPX did not blunt the decline in rectal temperature during hypoxia, the temperature data are shown separately (Fig. 2*A*, *bottom*) because the results did not represent the predominant response. In contrast to temperature, cardiorespiratory measurements in these two animals did not differ significantly from the group means depicted in Figs. 2*A*, 3*A*, 4, and 5*A*. These two lambs were studied on days of age 7 (no. 351) and 9 (no. 301), whereas the four lambs mentioned above were 8–13 days of age. Further experiments should be conducted to determine whether the modulation of core temperature by A_1 receptors changes developmentally, with inhibitory effects emerging 7–9 days postnatally.

Adenosine A_{2A}-Receptor Blockade

Normoxia. ZM-41385 did not significantly affect MAP, even though A_{2A}-receptor blockade increased MAP by 3–5 mmHg

in near-term fetal sheep (23, 24). As in unanesthetized fetal sheep (24), ZM-241385 did not change respiratory period or tidal volume, which indicates that adenosine A_{2A} receptors are not tonically involved in respiratory regulation.

Hypoxia. ZM-241385 attenuated the rate of rise in heart rate in newborn lambs, which suggests that peripheral and/or brain adenosine A_{2A} receptors modulate afferent chemoreceptor stimuli and/or their central integration. That ZM-241385 did not significantly alter MAP responses to hypoxia compared with vehicle indicates that adenosine A_{2A} receptors are not significantly involved in this cardiovascular adaptation. This is contrary to fetal sheep in which reflex autonomic effects of hypoxia on systemic arterial pressure are modulated by peripheral adenosine A_{2A}-receptor activity (23).

The initial hyperpnea in lambs treated with ZM-241385 was similar to that observed for vehicle. Thus adenosine A_{2A} receptors associated with the peripheral arterial chemoreceptors or brain have little impact on the *phase 1* respiratory responses to hypoxia.

Adenosine A_{2A}-receptor blockade abolished the roll-off respiratory response to hypoxia, with the magnitude of the initial rise in ventilation maintained throughout the entire 15 min of hypoxia. These results differ from prior studies (10, 28, 35) in which nonselective adenosine receptor antagonists (e.g., theophylline, aminophylline, caffeine) have generally only attenuated the roll-off. Although peripheral effects of ZM-241385 cannot be excluded, the maintenance of ventilation for the entire 15 min of hypoxia appears to result from antagonism of adenosine A_{2A} receptors in the brain, because *1*) the inhibitory influences to respiratory output in the roll-off response to hypoxia have a central origin, *2*) the maximum *phase 1* stimulatory response was not significantly altered by ZM-241385, and *3*) hypoxic inhibition of breathing in the fetus is abolished by intravascular administration of adenosine receptor antagonists that cross the blood-brain barrier but not by those that poorly diffuse into the brain (21, 25). This highly selective and potent adenosine A_{2A}-receptor antagonist may have eliminated the depressant phase by modulating intracellular cAMP concentration, protein kinase activity, and calcium signaling pathways (33).

Hypoxia increases brain adenosine concentrations, which activate adenosine A_{2A} receptors that modulate a number of functions, including sleep state and motor activity (24). Thus the findings of this study are consistent with the hypothesis that the depressant effects of hypoxia on breathing in developing lambs, as in the fetus (25), involve central adenosinergic pathways regulating behavioral adaptations to acute falls in Pa_{O_2} and possibly to other noxious stimuli, such as pain (16, 22).

Hypoxia induces a prolonged hyperventilation in conscious adult rats, which is augmented by intracerebroventricular injection of aminophylline (2). Thus central adenosine A_{2A} receptors likely modulate hypoxic respiratory drive, even in mammals that do not display the biphasic response.

The respiratory effects of ZM-241385 do not appear to be elicited by secondary effects of adenosine A_{2A}-receptor blockade on brain blood flow or body temperature. First, ZM-241385 would likely blunt hypoxia-induced vasodilatation (and reduce brain O_2 delivery) by antagonizing adenosine A_{2A} receptors involved in hypoxic cerebral vasodilatation. Thus increased brain Po_2 is not a likely cause of the persistence of

peak ventilation in lambs treated with ZM-241385. Second, the similarity in the rate of rectal temperature decline for lambs infused with ZM-241385 and vehicle suggests that an increase in O_2 consumption (and CO_2 production) was not a significant factor in sustaining hypoxic hyperventilation in lambs with adenosine A_{2A}-receptor blockade.

Clinical Significance

Apnea and hypoventilation, which occur frequently in premature infants, can be associated with significant morbidity if prolonged and associated with hypoxemia. Methylxanthines have long been used to treat respiratory depression involving central apnea, mixed (central and obstructive) apnea, periodic breathing, and idiopathic apneas of prematurity. In the latter, aminophylline has been effective in only about half of the cases, which may be related to variable maturation in the expression of brain stem receptors and/or intracellular transduction pathways involved in respiratory control (41). The fall in arterial O_2 saturation during an apneic episode may be another variable relevant to therapeutic success. When significant declines in arterial O_2 saturation accompany apnea, the present study indicates that a potent, highly selective antagonist of adenosine A_{2A} receptors might provide better defense against hypoxia-induced respiratory depression compared with aminophylline or caffeine, which are comparatively weak, nonselective adenosine receptor antagonists. DPCPX was not effective in blunting hypoxic respiratory depression, and it enhanced the vulnerability of the lambs to hypoxia. These results suggest that potent highly selective antagonists of adenosine A_1 receptors should not be used in this clinical setting until more is known about their metabolic and cardiorespiratory effects in acute hypoxia.

In the conscious state, the wakefulness stimulus generally preserves minute ventilation when accompanied by reduced respiratory stimuli, such as hypocapnea (12). Sleep lacks this stabilizing influence on respiratory rhythm. Besides the absence of the wakefulness drive to breathing, respiration in sleep can be critically compromised by hypoxic inhibition and impaired arousal to acute falls in Pa_{O_2}, which increases the vulnerability of infants to central, obstructive, or mixed apnea (34).

The depressant effects of hypoxia on the ventilatory roll-off are substantially greater up to at least 5 mo of age in infants identified as high-risk for sudden infant death syndrome (SIDS) (17). SIDS victims, who commonly display evidence of chronic hypoxemia, likely have elevated adenosine concentrations, as described in some adults with sleep apnea disorders (15). Thus the use of selective adenosine A_{2A}-receptor antagonists has the potential to reduce the risk of SIDS in infants with prolonged (>15 s) sleep apneic episodes.

In summary, blockade of adenosine A_{2A} receptor, which did not affect ventilation in normoxia, abolished the depressant effects of hypoxia on ventilation in developing lambs. Antagonism of adenosine A_1 receptors increased ventilation in normoxia but did not blunt the roll-off in hypoxia. These observations indicate that hypoxia triggers, via activation of adenosine A_{2A} receptors, a crucial neuronal pathway that depresses respiratory motoneurons.

Perspectives

Fetal breathing movements, which are episodic and inhibited by hypoxia, would appear at first glance to have little in common with respiration after birth. However, respiratory stimuli and modulators generally have similar qualitative effects on breathing in both the fetus and newborn (3, 11). In the case of adenosine receptors, the A_{2A} (but not A_1) subtype mediates two hypoxic respiratory responses: *1*) inhibition in the fetus and *2*) the *phase 2* decline in the neonate. Thus fetal breathing adaptations to hypoxia appear to persist postnatally in the roll-off response to hypoxia. Differences in respiratory control in the fetus (e.g., episodic apnea, hypoxic inhibition), which generally subserve a survival role and/or a lack of functional requirement, likely persist to a variable extent in the newborn and contribute not only to the regulated decrease in metabolic rate in hypoxia (30) but also to pathological disorders, such as sleep apnea, hypoxic ventilatory depression, and SIDS.

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