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Lung Cyclic AMP: Selective Decrease with Hypoxia (39422)

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Recent evidence suggests that adenosine 3′,5′-monophosphate (cAMP) and guanosine 3′,5′-monophosphate (cGMP) levels are potentially important in the regulation of lipid metabolism, contraction of smooth muscle in blood vessels and airways, and tissue growth (1-3). Since hypoxia is known to affect all of these parameters (4-6), we investigated the alterations in cyclic nucleotide levels in lung following hypoxic exposure. Cyclic nucleotides were also examined in liver tissue to evaluate comparatively with lung, and with fasting, since food intake is substantially reduced in rats during hypoxic exposures (7).

Methods. Male Long Evans Hooded rats weighing 250-300 g were exposed to 24-hr hypobaric hypoxia in an altitude chamber at 7193 m (23,600 ft: p_B = 280 mm Hg). Rate of ascent and descent was 1000 ft·min^{-1}. The level of hypoxia was chosen to simulate clinical and altitude conditions as well as to correlate with previous experiments on the effects of hypoxia, performed in this laboratory (8). All animals received water *ad libitum* and the hypoxic group was food deprived for the 24-hr period. At the appropriate times animals were removed from the altitude chamber and killed within 15 min by decapitation. Tissues were quickly removed, freeze clamped in liquid nitrogen, and stored at −76° until assayed. Forty milligrams of the frozen tissue were homogenized in 2 ml of 6% trichloroacetic acid, centrifuged (2000 *g*), and the supernatant extracted with ethyl ether saturated with water. The aqueous portion was dried under nitrogen on a 60-70° steam bath, resuspended in 2-4 ml of 0.05 *M* sodium acetate (pH 6.2), and assayed for cyclic nucleotides using radioimmunoassay kits (9). Statistical analyses were carried out using Student's *t* tests (10).

Results and discussion. No differences in lung dry:wet ratios were observed between hypoxic exposed animals and controls, indicating that little edema was present following acute hypoxic stress in rat lung. Lung and liver cyclic nucleotide levels are shown in Table I. In contrast to liver (fed *ad libitum* condition), lung contains higher endogenous cAMP and cGMP levels. Acute hypoxia resulted in a significant ($P < 0.025$) decrease in cAMP while liver cAMP was unaltered. Cyclic GMP for both tissues was not affected by hypoxia. These data indicate that acute hypoxic stress has a selective action in decreasing lung cAMP.

Although a 24-hr fast did not markedly alter cyclic nucleotide levels, a 72-hr fast resulted in a threefold increase in lung cAMP while liver cAMP increased eightfold. In both liver and lung, cGMP was unaltered. Although not shown, lung glycogen was unaffected by the 72-hr fast, whereas liver showed a 40-fold decrease in glycogen content, consistent with early studies (11, 8). These data emphasize the importance of the nutritional status of the animal on endogenous cyclic nucleotide levels and indicate that the preferential decrease in cAMP seen with hypoxic exposure cannot be explained as an effect due to anorexia. It is interesting to note that epinephrine will increase cAMP levels and that any stress, including starvation, may increase epinephrine and lead to elevated levels of cAMP. Thus, it is not clear at this time if the stress of food deprivation is the critical factor in stimulating cAMP levels. Nevertheless, nutritional stress appears to have a separate and distinct action on cAMP than seen with hypobaric hypoxic stress.

The association of decreased cAMP levels or decreased cAMP-cGMP ratios with vasoconstriction and tissue growth indicate that alterations of cyclic nucleotide levels may be of functional significance in the hypoxic lung. Constriction of vascular smooth muscle associated with a decrease in cAMP/cGMP ratio has been reported by others (12). Hypoxia is known to cause pulmonary

TABLE I. Cyclic Nucleotide Levels in Lung and Liver.[a]

Cyclic nucleotide	pmoles/mg dry weight			
	Control (Fed *ad libitum*)	Hypoxia (24 hr)	Fasted (24 hr)	Fasted (72 hr)
cAMP				
Lung	22.96 ± 2.63 (n = 6)	8.24 ± 0.93* (n = 7)	26.74 ± 4.93 (n = 10)	77.03 ± 14.00* (n = 4)
Liver	4.70 ± 1.47 (n = 6)	5.98 ± 2.06 (n = 4)	6.43 ± 2.28 (n = 4)	37.68 ± 13.70* (n = 4)
cGMP				
Lung	3.01 ± 0.48 (n = 3)	2.53 ± 0.36 (n = 7)	2.00 ± 0.29 (n = 9)	2.90 ± 1.13 (n = 4)
Liver	0.26 ± 0.06 (n = 3)	0.39 ± 0.16 (n = 4)	0.26 ± 0.06 (n = 4)	0.26 ± 0.06 (n = 4)
$\frac{\text{cAMP}}{\text{cGMP}}$				
Lung	7.62	3.25	13.37	26.56
Liver	18.07	15.33	24.73	144.92

[a] Values are mean ± SE. Hypoxic animals were deprived of food and exposed to a simulated altitude of 7193 m.
* Statistically significant from controls ($P < 0.025$).

vasoconstriction. This raises the question of whether the decrease in cAMP observed in our investigation may serve as an underlying mechanism for the hypoxic pressor response (increase in mean pulmonary arterial pressure). Somewhat surprising was that cGMP was not elevated with hypoxia since prostaglandin (PG) $F_{2\alpha}$-like substances have been shown to be released with hypoxia (13), and Vaughan and co-workers (14) have shown that cGMP plays an important role in regulating PG synthesis and release. Whether cGMP is linked to PG $F_{2\alpha}$ in lung remains to be determined.

An additional observation is that decreased levels of cAMP have been associated with tissue growth and elevation of cAMP associated with its suppression (15). In lung, where growth is increased with hypoxia, there is a decrease in cAMP, whereas in liver in which no such growth occurs, the cAMP levels are unchanged.

Since the lung is a heterogeneous organ comprised of at least 38 different cell types it should be noted that changes in tissue cyclic nucleotide levels may not totally reflect the response of any particular function or any specific cell type. Although the present study does not delineate the functional role of lung cyclic nucleotides it does provide fundamental information concerning changes in their levels during altered physiologic states.

Summary. Rats, food-deprived and exposed to hypobaric hypoxia (7193 m or 23,600 ft) for 24 hr showed a significant decrease in lung adenosine 3′,5′-monophosphate (cAMP). Lung guanosine 3′,5′-monophosphate (cGMP) was unaltered as well as liver cAMP and cGMP. In contrast, rats fasted for 72 hr showed a significant threefold increase in lung cAMP and eightfold increase in liver cAMP. Endogenous cGMP for both tissues was unchanged by a 72-hr fast. These data indicate that acute hypoxic stress has a selective action in decreasing lung cAMP and that this effect is not related to anorexia.

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