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# Effect of Hypoxia on Parathyroid Hormone in Lactating and Neonatal Rats

## *Interaction with Halothane*

Hershel Raff

*Endocrine Research Laboratory, St. Luke's Medical Center, Medical College of Wisconsin, Milwaukee, WI*

**Low oxygen in the blood (hypoxemia) may occur in the neonate or women in the postpartum period. Administration of inhalation anesthetic may be required in this period. The purpose of this study was to evaluate the effect of 7 d of hypoxia on the neonatal rat pup and lactating dam without or with acute halothane anesthesia on serum calcium and calciotropic hormones. Ionized calcium was not altered by hypoxia or halothane administration. Hypoxia from birth had no effect on serum parathyroid hormone (PTH) in 7-d-old rat pups (48 ± 4 pg/mL). Halothane increased PTH in rat pups (74 ± 8 pg/mL). The effect of halothane was not augmented in hypoxic pups. Hypoxia for 7 d had no effect on serum PTH in lactating dams (23 ± 3 pg/mL). Halothane resulted in an increase in PTH (106 ± 17 pg/mL). When halothane was administered to hypoxic lactating dams, a striking increase in serum PTH was observed (401 ± 50 pg/mL). We hypothesize that halothane and hypoxia alter parathyroid gland function by a direct effect on cellular calcium dynamics. This interaction may have clinical significance in hypoxic patients requiring general anesthesia.**

**Key Words:** Parathyroid hormone; hypoxia; halothane; calcium; osteocalcin; neonate; lactation.

## Introduction

We have extensively evaluated the effect of hypoxia from birth on a wide variety of metabolic and endocrine functions in neonatal and juvenile rats *(1–5)*. In the process of analyzing body composition and gastrointestinal function, we recently discovered that rat pups exposed to hypoxia from birth have decreased bone mineral density *(3,5)*. We are unaware of studies that have systematically evaluated the response of calciotropic hormones to hypoxia in lactating dams and neonatal rats.

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Author to whom all correspondence and reprint requests should be addressed: Hershel Raff, Ph.D., St. Luke's Phys Office Building, 2801 W KK River Pky, Suite 245, Milwaukee, WI 53215. E-mail: hraff@mcw.edu

Hypoxia is a common complication in the neonatal period *(6–9)*. Congenital cardiopulmonary abnormalities causing neonatal hypoxia often require emergency corrective surgery *(8)*. Furthermore, maternal hypoxia can occur postpartum as a result of a variety of cardiac and respiratory emergencies. It has been previously reported that inhalation anesthetics in rats increase serum intact parathyroid hormone (PTH) independently of changes in ionized calcium *(10)*. Furthermore, the regulation of PTH is altered in lactating rats *(11)*. The effect of inhalation anesthesia on the regulation of parathyroid gland function has not been evaluated in the neonate, nor has the interaction of inhalation anesthetics and hypoxia been evaluated in neonatal or lactating rats.

The purpose of this study was to evaluate the effect of 7 d of hypoxia from parturition, without or with acute halothane anesthesia, on ionized calcium and serum intact PTH. We used our well-established model of maternal–neonatal hypoxia in which lactating dams and their litters are exposed to hypoxia from parturition *(1–5)*.

## Results

There was no effect of 7 d of hypoxia from birth on intact PTH and ionized calcium in rat pups (Fig. 1). Halothane resulted in a significant increase in intact PTH in rat pups without any change in ionized calcium. There was no interaction between halothane anesthesia and hypoxia from birth on either intact PTH or ionized calcium in 7-d-old pups.

The responses in the lactating dams exposed to 7 d of hypoxia from parturition were quite different than the neonate (Fig. 2). Hypoxia without halothane anesthesia had no significant effect on intact PTH or ionized calcium, although ionized calcium tended to be lower during hypoxia. Halothane anesthesia led to a significant increase in intact PTH in the normoxic controls despite no change in ionized calcium. When lactating dams exposed to hypoxia for 7 d from parturition were anesthetized with halothane, there was a dramatic augmentation of serum intact PTH levels despite no change in ionized calcium.

There were no major effects of hypoxia on plasma 25-hydroxyvitamin D [25(OH)D], osteocalcin, total calcium, or total phosphate, although there was a small statistically significant decrease in 25(OH)D (Table 1). Hypoxia resulted

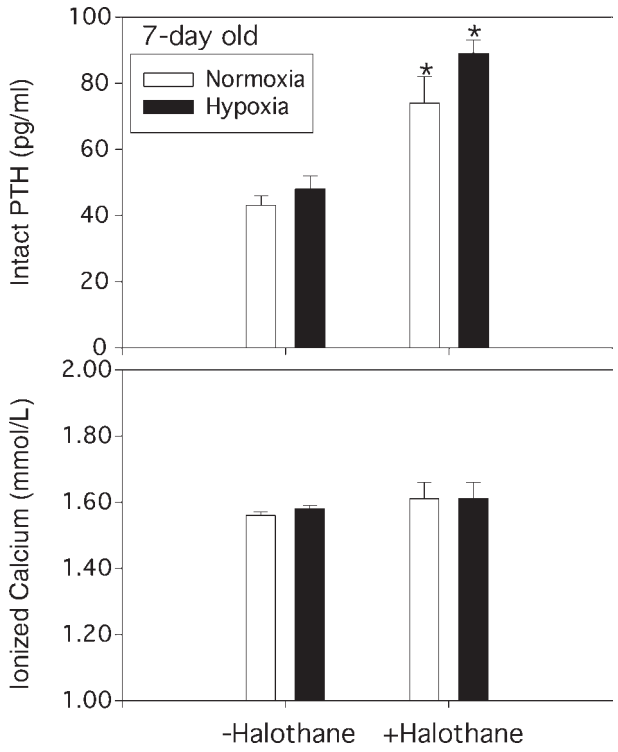


**Fig. 1.** Serum parathyroid hormone (PTH) and ionized calcium in 7-d-old rats exposed to normoxia (control) or hypoxia from birth. Pups were decapitated without (–halthone) or with (+halothane) acute general anesthesia with inhaled halothane. Data are presented as mean ± standard error ($n$ = 8–14 samples/mean). The asterisk indicates + halothane different from –halothane ($p < 0.05$).

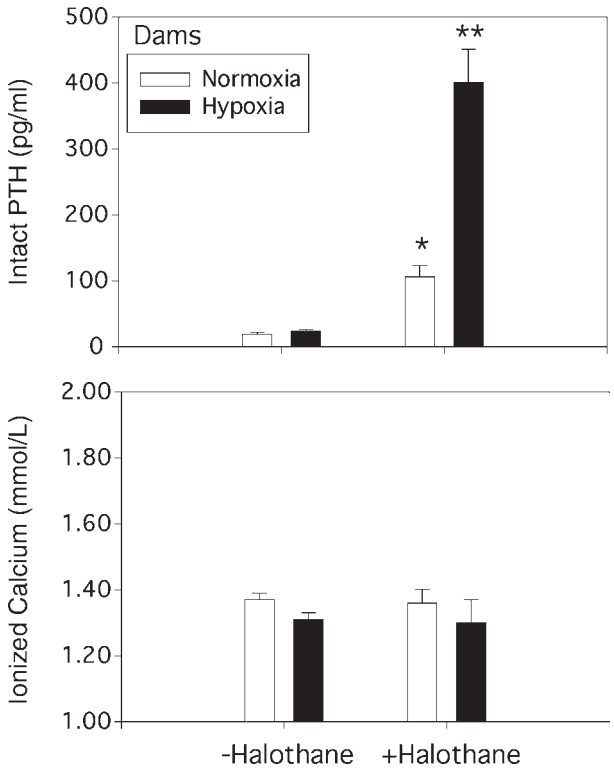


**Fig. 2.** Serum parathyroid hormone (PTH) and ionized calcium in lactating dams exposed to normoxia (control) or hypoxia for 7 d from parturition. Rats were decapitated without (–halothane) or with (+halothane) acute general anesthesia with inhaled halothane. Data are presented as mean ± standard error ($n$ = 6–8 samples/mean). The asterisk indicates + halothane different from –halothane ($p < 0.05$). The double asterisk indicates hypoxia different from normoxia, and +halothane different from –halothane ($p < 0.05$).

**Table 1**
Other Serum Measurements and Body Weight

| | 25(OH)D (ng/mL) | Osteocalcin (ng/mL) | $TotCa^{2+}$ (mg/dL) | $TotPO4^{-}$ (mg/dL) | BW (g) |
|---|---|---|---|---|---|
| 7-d-old pups | | | | | |
| Normoxia | 16.5 ± 1.0 | 46 ± 9 | 9.3 ± 0.1 | 10.1 ± 0.1 | 14.0 ± 0.4 |
| Hypoxia | 15.6 ± 2.2 | 48 ± 10 | 9.5 ± 0.1 | 10.6 ± 0.1 | 10.4 ± 0.3* |
| Lactating dams | | | | | |
| Normoxia | 17.5 ± 0.8 | 16 ± 2† | 9.8 ± 0.2 | 5.0 ± 0.5† | 290 ± 4† |
| Hypoxia | 14.0 ± 0.2* | 20 ± 2† | 9.6 ± 0.2 | 6.4 ± 0.4† | 262 ± 3† |

*Note*: Plasma 25-hydroxyvitamin D [25(OH)D], osteocalcin, total calcium ($TotCa^{2+}$), total phosporus ($TotPO4^{-}$), and body weight (BW) in 7-d-old rats and their dams both exposed to normoxia (control) or hypoxia from parturition. The asterisk indicates hypoxia different from normoxia. † indicates that lactating dams are different from 7-d-old pups. Mean ± SE values for pups are $n$ = 8–14 (except BW: mean ± SE for pups, $n$ = 30–47). All mean ± SE for dams are $n$ = 6–8.

in a significant decrease in body weight in both rat pups and lactating dams (Table 1). Intact PTH and ionized calcium concentrations were both significantly greater in 7-d-old pups compared to lactating dams (Fig. 1 vs Fig. 2). Plasma osteocalcin and total phosphate were significantly lower in lactating dams compared to 7-d-old pups (Table 1). Otherwise, there was no difference in 25(OH)D or total calcium between pups and dams.

## Discussion

The main finding of this study was the quite dramatic increase in intact PTH in hypoxic lactating dams anesthetized with halothane. Despite the dramatic effects of chronic hypoxia on growth and body composition *(3,5)*, it was remarkable that hypoxia *per se* had no major effects on PTH, ionized calcium, 25(OH)D, or osteocalcin. A previous study of severe hypoxia for several weeks in humans found a small increase in PTH levels *(12)*, whereas milder hypoxia for about 1 wk had no effect on PTH or $1,25(OH)_2D$ *(13)*.

One possible explanation for a lack of effect of hypoxia is that the rat chow used in this study has a relatively high calcium and phosphorus content compared to other studies of bone metabolism in lactating rats *(10,11)*. Furthermore, the diet is quite replete in vitamin D. Therefore, any decrease in food intake or gastrointestinal (GI) absorptive capacity *(1)* was probably compensated for by the diet. Although hypoxia induces an initial decrease in body weight in rats, we have previously shown that growth velocity returns to normal, although catch-up growth is not exhibited *(5)*. It seems unlikely, then, that a decrease in food intake could account for the changes in PTH in hypoxic dams in response to halothane. It also suggests that hypoxia decreases bone mineral density by an effect independent of major changes in calciotropic hormones, perhaps by a direct effect on bone metabolism *(3,5)*.

Halothane anesthesia increased PTH in neonates and lactating dams. This effect has been observed in adult rats previously and is independent of ionized calcium *(10)*. The mechanism of this effect is unknown *(10)*. However, one can hypothesize that it is likely the result of direct effects of halothane on cellular function. For example, halothane has dramatic effects on calcium channel function and membrane potential, as well as many other parameters of activity *(14–18)*. Because the parathyroid gland is controlled by extracellular ionized calcium and the calcium receptor *(19)*, it would not be surprising if halothane altered the relationship between calcium and PTH secretion. Further experiments examining the effects of halothane on parathyroid cells will be necessary to resolve this question.

The most striking finding of this study is the marked increase in PTH independent of any change in ionized calcium that was found when halothane anesthesia was administered to hypoxic lactating dams. There are many possible mechanisms for this interaction. Hypoxia may alter parathyroid cell calcium flux, intracellular pH, or membrane potential, rendering the cell more sensitive to the action of halothane. Hypoxia can induce respiratory alkalosis, which may also alter the control of parathyroid hormone *(13)*. We have shown that adult rats develop mild hypocapnia when exposed to similar inspired oxygen concentrations, but that there is a significant metabolic compensation (decrease in base excess) *(20)*. Therefore, major alkalosis does not occur in this model of hypoxia, at least in adults, and probably does not account for the effects observed in the present study.

Of interest was that the interaction of halothane and hypoxia from birth was not evident in the 7-d-old rat pup. It is possible that the PTH response to halothane takes longer in pups and that an increase would have been observed if sampling had been performed more than 5 min after induction of anesthesia. The only other difference noted was that the neonatal rats had quite high serum phosphorus levels, consistent with previous studies *(21)*.

These results have several potential clinical implications. It would be interesting if (1) inhalation anesthetics increase parathyroid hormone levels in humans and (2) if patients with cardiopulmonary disease have an exaggerated PTH response to inhalation anesthetics. Because a tight control of extracellular ionized calcium is vital for normal cardiovascular function, wildly fluctuating PTH levels may be detrimental in patients during and after surgery under general anesthesia. This is particularly so because PTH has demonstrable hemodynamic effects *(22)*.

## Methods

Timed pregnant Sprague-Dawley rats (Harlan Sprague Dawley, $n = 32$) were obtained at 14 d gestation and maintained on Test Diet 5001 (Richmond, IN) and water *ad libitum* in a controlled environment (0600–1800 lights on). The diet contained 4.5 IU/g vitamin D, 0.95% calcium, and 0.67% phosphorus. Parturition usually occurred on the afternoon of gestational day 21, during which rats were kept under observation. As soon as a litter was completely delivered, the pups were weighed and then randomly assigned to treatment groups (approx 10 pups/litter). The dam and her pups were moved to an environment chamber and exposed to normobaric normoxia (21% $O_2$) or hypoxia (12% $O_2$) as described in detail previously *(1–5)*. We have previously shown that this hypoxic exposure leads to arterial $PO_2$ levels in adults of about 50–55 torr with respiratory alkalosis with significant metabolic compensation *(20)*. Rat pups with their lactating dams were maintained for 7 d (0–7 d of age) in a normoxic or hypoxic environment.

At 7 d of age, rat pups with their lactating dams were removed from chambers and quickly weighed. Rats were either quickly decapitated or were anesthetized with halothane (2-bromo-2-chloro-1,1,1,-trifluoroethane; Sigma, St. Louis, MO) in an inhalation narcosis chamber (Harvard Apparatus, South Natick, MA). Halothane-exposed rats were carefully observed and decapitated as soon as they became motionless (except for respiratory movements), which normally took about 30–60 s. Trunk blood was collected in serum separator tubes and allowed to clot. Blood from pups was pooled (three to four per sample). Blood was then centrifuged and the serum analyzed for ionized calcium within 2 h, as described previously *(10,11)*. Remaining serum was frozen for future measurements.

Ionized calcium was analyzed by ion-selective electrode (Nova 8, Waltham, MA) and corrected for pH. Total calcium and phosphorus were measured by colorimetric method using a Vitros autoanalyzer (Ortho-Clinical Diagnostics, Rochester, NY). Intact PTH was analyzed using a two-site homologous immunoradiometric assay (IRMA) developed by Immunotopics, Inc. (San Clemente, CA) and distributed by Diagnostic Systems Laboratories (Webster, TX). The details of the rat PTH IRMA have been previously reported *(23)*. Serum 25(OH)D was analyzed by radioimmunoassay with reagents purchased from Diasorin, Inc. (Stillwater, MN) as described previously *(24)*. Serum osteocalcin was measured by a two-site IRMA using reagents specifically generated for measurement of rat osteocalcin and purchased from Immunotopics (distributed by Diagnostic Systems Laboratories).

Data were analyzed by unpaired *t*-test or two-way analysis of variance ($p < 0.05$). Data are presented as mean ± standard error.

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