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Total and Active Ghrelin in Developing Rats During Hypoxia

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Hypoxia is well known to decrease appetite and weight gain in growing rats, and to induce weight loss in humans. It has been hypothesized that this is mediated by a change in ghrelin, an orexigenic peptide synthesized and released primarily from the stomach. Rats were exposed to hypoxia for 7 d as neonates (birth–7 d of age), weanlings (28–35 d of age), and juveniles (49–56 d of age). Hypoxia had no effect on total or active plasma ghrelin. There was a significant decrease in active ghrelin in weaned rats (0.8 ± 0.1 ng/mL) compared to nursing pups at 7 d of age (2.3 ± 0.2 ng/mL). The proportion of total ghrelin that was active decreased significantly between 7 and 35 d of age. We conclude that the anorexia and weight loss associated with hypoxia is probably not mediated by ghrelin. There appear to be changes in active ghrelin levels in plasma during early development in the rat.

Key Words: Ghrelin; des-acyl ghrelin; hypoxia; development; neonate; weaning.

Introduction

Hypoxia is well know to induce a decrease in appetite, body weight, and weight gain in growing animals *(1)*. We have performed several studies evaluating possible hormonal mediators of this phenomenon and so far have not found a role for growth hormone, growth hormone therapy, IGF-I, or leptin *(2–4)*. Furthermore, although developing rats exposed to hypoxia are smaller, their body composition is essentially normal *(3,4)*.

It has recently been suggested that ghrelin, an orexigenic peptide that is increased during fasting *(5,6)*, may be involved in the decrease in food intake and body weight during hypoxia *(1)*. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized primarily in the stomach and appears in the blood in two forms: so-called "active" ghrelin, is a 28 amino acid peptide in which the Ser^3 residue is n-octanoylated, and "total" ghrelin *(7,8)*.

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In the current study, we used new, commercially available radioimmunoassays (RIA), one specific for the octanoyl-modified portion of ghrelin at the N-terminal (active ghrelin) and the other that recognizes the C-terminal portion of ghrelin (total ghrelin). We measured both forms in plasma from neonatal, weaned, and juvenile rats exposed to hypoxia for 7 d but allowed free access to water and food.

Results

Table 1 shows active and total plasma ghrelin levels in rats exposed to 7 d of hypoxia vs normoxic controls. There were no significant effects of hypoxia at 7, 35, or 56 d of age. Therefore, these data were combined to increase statistical power and are presented in Fig. 1. Seven-day-old rats had plasma ghrelin levels approx 15% of total plasma ghrelin. Active plasma ghrelin in 35- and 56-d-old rats was significantly lower than 7-d-old rats. There was a small, but significant decrease in total plasma ghrelin in 35-d-old compared to 7-d-old rats, but these levels were no longer different at 56 d of age.

Discussion

This brief study failed to find any effect of 7 d of hypoxia on active or total plasma ghrelin in neonatal, weaned, and juvenile rats. Therefore, it seems unlikely that ghrelin is a major factor in the anorexia and decreased weight-gain that we have documented during hypoxia in developing rats *(2–4)*. It is important to note that the rats in this and our previous studies had free access to their lactating dams (0–7 d old) or food and water (28–35 and 49–56 d of age). This was necessary because hypoxic pups do not tolerate food restriction and because fasting increases ghrelin in the neonate *(9)*.

The most striking finding was that the percentage of circulating ghrelin that was active was higher in the nursing (7 d old) vs the weaned (35 d old) rat. This is in contrast to the expression of ghrelin mRNA in the stomach, which increases with age *(10)*. It is also interesting that a previous report demonstrated no detectible ghrelin in 5-d-old rat pups suggesting a very rapid activation of ghrelin gene expression thereafter *(11)*. The higher active ghrelin may be involved in the stimulation of growth hormone release in the neonate, but is also likely to have many other wide-ranging effects *(5,6)*. The mechanism of this change in ghrelin processing is unknown at this time.

Table 1
Effect of 7 d of Hypoxia on Plasma Active and Total Ghrelin in Suckling Rats (7 d old), Postweaned (35 d old), and Juvenile (56 d old)

	Days of Age		
Ghrelin	7	35	56
Active (ng/mL)			
Normoxia	2.4 ± 0.2	0.8 ± 0.1	0.9 ± 0.1
Hypoxia	2.4 ± 0.1	0.9 ± 0.1	0.7 ± 0.1
Total (ng/mL)			
Normoxia	13.9 ± 0.7	13.4 ± 0.4	13.7 ± 0.4
Hypoxia	14.7 ± 0.2	12.0 ± 0.8	13.5 ± 1.0

Rats were exposed to hypoxia (12% 02) vs. normoxic (21% O2) controls (n = 4–8 per mean ± standard error) for 7 d. There were no significant effects of hypoxia.

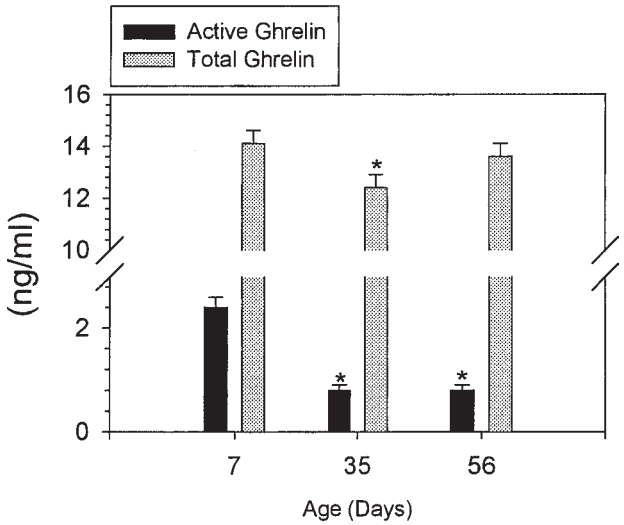

Fig. 1. Plasma active and total ghrelin in rats at 7, 35, and 56 d of age (n = 6–10 per mean ± standard error). *indicates different from 7 d of age ($p < 0.05$).

One may speculate about why active ghrelin may be higher in the 7 d old (nursing rat). It is possible that ghrelin may be present in rat milk, although I am not aware of a study demonstrating this. Because leptin, which is present in milk, does have dramatic effects in the neonate and subsequently in the adult *(12,13)*, it is possible that higher active ghrelin is necessary in the neonate to maintain the drive to feed. Obviously, the discovery of ghrelin is relatively recent and many of these questions have yet to be addressed.

Materials and Methods

Timed pregnant Sprague-Dawley rats (Harlan, Indianapolis, IN, n = 12) were obtained at 14 d gestation and maintained on a standard sodium diet and water *ad libitum* in a controlled environment (0600–1800 lights on). Parturition occurred spontaneously on the afternoon of gestational d 21 during which rats were kept under observation. As soon as a litter was completely delivered, the pups were weighed and cross-fostered (8–10 pups/dam). Four dams with their litters were moved to an environment chamber and exposed to normobaric normoxia (21% O_2) or hypoxia (12% O_2) as described in detail previously *(14)*. We have previously shown that this exposure leads to arterial pO_2 levels in adults of about 50–55 torr with sustained respiratory alkalosis with metabolic compensation *(15)*. The remaining litters were weaned at 21 d of age. At 28 or 49 d of age, rats (either gender) were moved to the chamber and exposed to normoxia or hypoxia for 7 d. At 8 AM of the seventh day in the chamber, rats were quickly removed and decapitated with trunk blood collected in EDTA. Blood was immediately centrifuged and plasma frozen for measurement of ghrelin. Rats were not weighed to minimize handling before obtaining blood samples. However, we have published extensive analyses of body weights in normoxic vs hypoxic rats of the same age *(3,4)*.

Ghrelin radioimmunoassays were obtained from Linco Research (St. Charles, MO). The active ghrelin RIA (cat. no. HGRA-88HK) used a guinea pig anti-ghrelin serum specific for the N-terminal containing the octanoyl group on serine 3. The antiserum had 100% crossreactivity with rat ghrelin and <0.1% crossreactivity with des-octanoylghrelin. The total ghrelin RIA (cat. no. GHRT-89HK) used a rabbit anti-ghrelin serum directed toward the C-terminal and does not require the presence of the octanoyl group in serine 3. The antiserum has 100% crossreactivity with rat ghrelin, ghrelin 14–28, and des-octanoylghrelin, with no detectible crossreactivity with ghrelin 1–10, motilin-related peptide, glucagon, or Glp-1 (7-36). Plasma was diluted 1:20 in assay buffer to give concentrations between ED_{20} and ED_{80} of the binding curve.

Data were anlyzed by one- and two-way analysis of variance and Duncan's multiple range test ($p < 0.05$). Data are presented as mean ± standard error.

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