In Vitro and *In Vivo* Effect of Acylated and Unacylated Ghrelin on Neonatal Glucose Homeostasis

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ABSTRACT: Maintenance of normal glucose homeostasis is crucial for survival during the perinatal period. Acylated ghrelin (AG) but not unacylated ghrelin (UAG) inhibits insulin release from pancreatic islets in adult rats. Circulating AG concentrations are low in the fetus and progressively increase in the postnatal period. We tested the hypothesis that AG has insulinostatic effects *in vitro* and *in vivo* during the perinatal period. *In vitro*, AG (10^{-10} – 10^{-8} M) caused a 25–53% decrease in insulin secretion by islets from 5-d-old rat pups under normo- and hyperglycemic conditions, an effect that was mediated through the growth hormone secretagogue receptor (GHSR-1a). Ghrelin (1–5) amide, [Dap3]-octanoyl, a pentapeptide that is resistant to deacylation and binds the GHSR-1a, had similar effects at 10^{-8} M. *In vivo*, AG, UAG, or GHRP-6 [D-Lys3], a GHSR-1a antagonist, did not affect insulin or glucagon concentrations during the first 3 h of life. In 6-d-old pups, AG, UAG, or ghrelin (1–5) amide, [Dap3]-octanoyl did not affect glucose-induced insulin or C-peptide concentrations. In summary, AG has insulinostatic effects *in vitro* as early as during the perinatal period. These effects could not be confirmed *in vivo*, possibly due to the short half-life of AG in rat neonates. (*Pediatr Res* 67: 609–613, 2010)

Ghrelin is a peptide hormone mainly secreted by the stomach in adult rodents and humans. It circulates as acylated ghrelin (AG) and unacylated ghrelin (UAG). Acylation with octanoate is required for binding of ghrelin to the growth hormone (GH) secretagogue receptor (GHSR-1a) that mediates many of the actions ascribed to ghrelin. Originally, AG was considered to be the active form of the hormone and its main roles were felt to be limited to GH secretion (1) and stimulation of appetite at the hypothalamus level (2). It is now clear that AG has many metabolic effects, that UAG is not inactive, and that both AG and UAG potentially play a physiologic role in energy balance (reviewed in Ref. 3).

The source of ghrelin and its concentrations in tissues and plasma differ according to age. The rat embryo contains elevated plasma concentrations of UAG but little AG (4), and the circulating AG to UAG ratio increases markedly between fetal day 20 and postnatal day (PN) 1 (5). Concentrations of ghrelin are barely detectable in the fetal stomach and increase progressively to reach adult values by 3–4 wk. In contrast, the

fetal pancreas contains elevated concentrations of ghrelin that progressively decrease after birth (5). In pancreas, ghrelin originates from glucagon producing alpha (α) cells but also from a novel cell type, called epsilon (ϵ). Insulin-producing beta (β) cells do not contain ghrelin (6). In adult pancreas, GHSR-1a is present on the α -cell as well as on the β -cell (7), raising the possibility of a direct, paracrine action of ghrelin on insulin and glucagon secretion.

Indeed, AG has been shown *in vitro* to inhibit insulin and stimulate glucagon secretion by cultured pancreas islets from adult rodents (rev in Ref. 8). The *in vivo* role of ghrelin on glucose metabolism remains unclear but endogenous ghrelin secreted by islets was suggested to modulate insulin secretion by the β -cell in adult rodents (9).

We hypothesized that AG could contribute to neonatal normoglycemia by inhibiting insulin production by the β -cell. Maintenance of normoglycemia is a requirement for early PN survival in rodents like in most mammals. This is achieved through a marked decrease in insulin and increase in glucagon production and by the development of hepatic glucose production in a matter of minutes following birth (10).

In this study, we investigated the effects of ghrelin (1) *in vitro* on insulin production by pancreas islets from 5-d-old rats; (2) *in vivo* on glucose homeostasis in rat neonates born by Cesarean section on the expected day of delivery and in 6-d-old rat pups.

METHODS

Animals. Sprague Dawley rats obtained from Charles River Laboratories (St. Constant, Quebec, Canada) were time-mated in the animal care center at the Child and Family Research Institute (day of conception was day 0). They had free access to water and to a standard chow diet. Except if otherwise noted, all chemical reagents were obtained from Sigma Chemical Co.-Aldrich Canada (Oakville, ON). All experiments were approved by the University of British Columbia Animal Care Committee.

***In vitro* studies.** Pancreases from 5-d-old rat pups killed by decapitation were removed. Islets were isolated by collagenase XI digestion (1000 U/mL in Hanks' solution incubated at 37°C for 17 min with gentle shaking). The suspension was washed in Hank's solution, centrifuged at 1000 rpm for 30 s at 4°C and the supernatant aspirated. This step was performed three times. Islets were then resuspended in RPMI 1640 medium (Invitrogen, Carlsbad, CA) and poured on a prewet 70 μ m nylon mesh cell strainer (BD Biosciences, Mississauga, ON). Islets are hand-picked under a microscope, and incubated overnight in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin G, 100 μ g/mL streptomycin (Invitrogen). After prein-

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Abbreviations: AG, Acylated ghrelin; CV, Coefficient of variation; GHSR-1a, Growth hormone secretagogue receptor 1a; PN, Postnatal; UAG, Unacylated ghrelin

cubation for 2 h at 37°C with 5% CO₂ in Krebs-Ringer-Bicarbonate-HEPES (KRBH) buffer with 1.67 mM glucose, islets were incubated for 1 h at 37°C in 500 μ L of the same KRBH buffer in 48-well plates (five islets per well) under hypoglycaemic (1.67 mM), euglycemic (8.5 mM), or hyperglycaemic conditions (16.7 mM glucose). Half of the cultures were performed in male and half in female pups. The effect of the following chemicals (Phoenix Pharmaceuticals, Burlingame, CA) on insulin secretion by the pancreas islet were tested: rat AG (031-31); rat UAG (031-33); ghrelin (1–5) amide [Dap3]-octanoyl (032-14, a pentapeptide that binds to the GHSR-1a (11)); GHRP-6 [D-Lys3] (031-22, a GHSR-1a antagonist (12)) or [D-Arg1, D-Phe5, D-Trp7,9, Leu11]-substance P (061-21, a GHSR-1a inverse agonist (13)). The supernatant was collected and stored at –80°C until analysis.

In vivo effect of AG, UAG, and GHRP-6 [D-Lys3] administration on glucose homeostasis at birth. Twenty-one dams were randomly assigned to a baseline, 45 min and 180 min group. All pups were delivered by Cesarean section immediately after decapitation of the dam on the morning of pregnancy day 21.5, a few hours before the expected time of spontaneous delivery. In the baseline group ($n = 6$), all pups in one litter were killed immediately after delivery. In the 45 min group ($n = 8$), 3–4 pups in each litter were randomly assigned to receive at birth an i.p. injection (50 μ L) of either saline, 1 μ g AG (= 0.3 nmol), 1 μ g UAG (= 0.3 nmol), or a mixture of 1 μ g AG and 25 μ g (= 27 nmol) GHRP-6 [D-Lys3] and were killed 45 min after the injection. In the 180 min group ($n = 7$), 3–4 pups in each litter were randomly assigned to receive 2 consecutive injections (at birth and after 120 min) as described above and were killed at 180 min of life. Blood from pups receiving the same treatment at the same time point in the same litter was pooled as one sample. Thus, each litter from the six dams in the baseline group contributed one baseline blood sample. Each litter from the eight dams in the 45 min group and from the seven dams in the 180 min group each contributed one blood sample to the control, one to the AG, one to the UAG and one to the AG/GHRP-6 [D-Lys3] groups. Pups were kept in a warm (33°C) and humid (60%) environment (Ohio Servo-Controlled Isolation Incubator, Aireo, Madison, WI) and were killed by decapitation. Trunk blood was collected in a tube containing EDTA and 250 U aprotinin (Trasyol, Bayer, Toronto, ON) per ml blood. All samples were kept on ice until centrifugation and plasma was frozen at –80°C until hormonal determinations were performed. Previous data from our group have shown that i.p. administration of 1 μ g AG or UAG in rat neonates results in a rapid and sustained (at least for 90 min) absorption of the peptide into the circulation (14). Intraperitoneal administration of GHRP-6 [D-Lys3] was previously shown to be an effective way of decreasing appetite in rodents, suggesting that it is also readily absorbed (12).

In vivo effect of AG, UAG, ghrelin (1–5) amide [Dap3]-octanoyl, and GHRP-6 [D-Lys3] administration on glucose-induced hyperinsulinemia and C-peptidemia in 6-d-old pups. Eight dams were allowed to deliver normally and the litter culled to 10 pups on PN1 (five male and five female pups). On PN6, each male and female pup in each litter was fasted for 30 min and randomly assigned to one of the following five groups: glucose only (control group, i.p. injection of 1.4 g glucose/kg body weight in 100 μ L) or glucose plus either AG (25 nmol/kg body weight), UAG (25 nmol/kg), ghrelin (1–5) amide [Dap3]-octanoyl (25 nmol/kg), or a mixture of ghrelin (1–5) amide, [Dap3]-octanoyl (25 nmol/kg), and GHRP-6 [D-Lys3] (1 μ mol/kg). The dose of AG and time of sacrifice were chosen following an experiment previously performed in adult male rats. Eight adult animals were given an i.p. administration of glucose (1.4 gr/kg) with or without AG (25 nmol/kg) in random order at 1 week interval. When glucose was administered with ghrelin, blood glucose concentrations 15 and 30 min after the injection were, respectively, 50% (13.2 versus 9.2 mM) and 48% (13.6 versus 9.2 mM) higher than when glucose was injected alone. The pups were kept in a warm (33°C) and humid (60%) environment and fasted for 30 min before i.p. injection. The pups were killed 20 min after the injection and trunk EDTA blood was collected. Blood glucose was measured in duplicate by glucometer. All samples were kept on ice until centrifugation and plasma was frozen at –80°C until determinations of insulin and C-peptide were performed.

Assays. Blood glucose was measured using the One-Touch Ultra glucose meter (Life Scan Canada Ltd, Burnaby, BC, Canada). In the *in vivo* experiment at birth, insulin (RI-13 K, inter-assay coefficient of variation [CV] <9.4%, intra-assay CV <4.6%) and glucagon (GL-32 K, intra-assay CV 4.3%) were determined by RIA (Millipore, St-Charles, MO). In the other experiments, insulin was determined by rat specific ELISA (80-INSRT-E01, or 80-INSRTH-E01 [high range], intra-assay CV <6.4%, inter-assay CV <9.9%, Alpco, Salem, NH). C-peptide was determined by ELISA (rat specific, recognizes C-peptide I and II, 48-CPERT-E01, intra- and inter-assay CVs <8.8%, Alpco).

Statistics. Results are expressed as mean (standard error of the mean). The differences between the groups were assessed using ANOVA with posthoc analysis for comparison with the control group using *t* test for unpaired values. Data were log transformed for the *in vitro* experiments. A *p* value

Figure 1. Effect of acylated ghrelin (AG) on insulin secretion by cultured pancreas islets from 5-d-old rats under hypoglycemic (1.67 mM), normoglycemic (8.3 mM), and hyperglycemic (16.7 mM) conditions. ■, control; □, AG 0.1 nM; ▒, AG 1.0 nM; ▨, AG 10 nM. Two-way ANOVA: $p < 0.001$ for glucose; $p < 0.05$ for AG concentration; $p < 0.05$ for glucose $\times$ AG concentration interaction. * $p < 0.05$ compared with all other groups. Values are given in mean (SE).

<0.05 was considered significant. Data were analyzed with SPSS version 17.0 (2008, Chicago, IL).

RESULTS

In vitro studies. As expected, the concentration of insulin in the culture medium after incubation of the pancreas islets increased with the concentration of glucose. Addition of AG caused a marked decrease in insulin secretion (ANOVA: $p < 0.05$) that was similar for AG concentrations of 0.1, 1, and 10 nM and was greater under hyperglycemic (–39 to –53%) compared with euglycemic conditions (–25 to –29%) (Fig. 1). In contrast, addition of UAG or of a mixture of AG and UAG did not affect insulin secretion (Fig. 2A). The effect of AG on insulin secretion under hyperglycemic conditions was prevented by co-incubation with 1 μ M of an inverse agonist ([D-Arg1, D-Phe5, D-Trp7, 9, Leu11]-substance P) or an antagonist (GHRP-6 [D-Lys3]) of GHSR-1a (Fig. 2B). Incubation of pancreas islets with 10 nM ghrelin (1–5) Amide [Dap3]-octanoyl, a pentapeptide that binds to GHSR-1a, caused a 57% decrease in insulin secretion that was similar to the effect of 0.1 nM AG (Fig. 2C). There was no significant difference between islets from male and from female pups in any of the experiments.

Effect of AG, UAG, and GHRP-6 [D-Lys3] administration on glucose homeostasis at birth. Maternal glucose, insulin, and glucagon concentrations in dams at delivery were 92 [2] mg/dL, 1.1 [0.2] ng/mL, and 109 [6] pg/mL, respectively. In control neonates, glucose concentrations decreased significantly between birth and 45 min and went up again by 180 min (ANOVA: $p < 0.001$) while insulin concentrations were lower at 45 and 180 min compared with baseline (ANOVA: $p = 0.001$). Glucagon concentrations remained stable during the first 180 min of life (Fig. 3). Administration of AG, UAG, or GHRP-6 [D-Lys3], a GHSR-1a antagonist, did not affect glucose (Fig. 3A), insulin (Fig. 3B) or glucagon (Fig. 3C) concentrations at 45 min or 180 min of life (Fig. 3).

Effect of AG, UAG, ghrelin (1–5) amide [Dap3]-octanoyl or co-administration of ghrelin (1–5) amide, [Dap3]-

Figure 2. Effects of (A): unacylated ghrelin (UAG, $n = 26-30/\text{gr}$); (B): GHSR-1a antagonists (Lys GHRP-6) or inverse agonists (Subst P) ($n = 14-16/\text{gr}$); C: [DAP-3]-ghrelin (1-5) ($n = 14-16/\text{gr}$) on insulin secretion by cultured pancreas islets from 5-d-old rat neonates under hyperglycemic (16.7 mM) conditions. ■, control; □, AG only; ▤, specific experimental conditions. One-way ANOVA: A, $p < 0.001$; B, $p = 0.044$; (C), $p = 0.001$. * $p < 0.05$ compared with control group. Values are given in mean (SE). Subst P: [D-Arg1, D-Phe5, D-Trp7,9, Leu11]-substance P. [Dap3]-ghrelin (1-5): Ghrelin (1-5) Amide, [Dap3]-octanoyl.

octanoyl and GHRP-6 [D-Lys3] on glucose homeostasis in 6-d-old pups. Blood glucose and plasma insulin and C-peptide concentrations were similar in 6-d-old rat pups 20 min after the i.p. administration of 1.4 g/kg of glucose or of glucose together with AG, UAG, ghrelin (1-5) amide [Dap3]-octanoyl or a mixture of ghrelin (1-5) amide [Dap3]-octanoyl and GHRP-6 [D-Lys3] (Table 1). The results were not influenced by gender.

DISCUSSION

This is the first *in vitro* demonstration in rat neonates that AG causes a marked decrease in insulin secretion from cultured pancreas islets. We also show that the ghrelin analogue ghrelin (1-5) amide [Dap3]-octanoyl has similar effects, although at concentrations that are 1-2 orders of magnitude greater than for AG. In contrast, *in vivo*, exogenous administration of AG in rat neonates at birth or at 6 d of age did not cause any detectable change in glucose homeostasis.

In vitro inhibition of insulin secretion by neonatal pancreas islets was observed for AG but not for UAG. Furthermore, the effect of AG was inhibited by an antagonist or an inverse

Figure 3. Effect of acylated ghrelin (AG), unacylated ghrelin (UAG), and GHRP-6 [D-Lys3] on (A) glucose, (B) insulin, and (C) glucagon concentrations in rat neonates at birth $n = 6-8/\text{group}$ and time point. ■, baseline; □, control; ▤, AG; ▥, UAG; ▦, GHRP-6 [D-Lys3]. Glucose—2-way ANOVA: time $p < 0.001$; group: not significant. Insulin—2-way ANOVA: time $p < 0.001$; group: not significant. Glucagon: not significant. Values are given in mean (SE).

agonist of GHSR-1a. These data suggest that, similar to adult animals, the effect of ghrelin on insulin secretion in rat neonates is mediated by GHSR-1a and that octanoylation of ghrelin is required (9). The insulinostatic effect of AG was abolished by co-incubation with UAG at a 1 to 10 (AG to UAG) molar ratio. Several explanations have been proposed for this observation: competition of UAG with AG for GHSR-1a (15) (which seems unlikely as UAG does not bind GHSR-1a even at micromolar concentrations (11)) and stimulation of insulin release by UAG through nonGHSR-1a mechanisms (16). Furthermore, we show that *in vitro*, ghrelin (1-5) amide, [Dap3]-octanoyl, an octanoylated pentapeptide that includes the first 5 aminoacids of ghrelin (the minimal sequence required for binding to GHSR-1a) (11), also inhibits insulin secretion but at concentrations that are 1-2 orders of magnitude greater than for AG. This is consistent with *in vitro* data showing that the concentration of a similar pentapeptide required for 50% specific binding to the GHSR-1a is 200 times greater than for natural ghrelin (11).

We then investigated whether our *in vitro* findings could apply to *in vivo* situations and investigated the effect of exogenous administration of ghrelin in rat pups at birth and on the sixth day of life. The physiology of mammalian glucose homeostasis during the perinatal period is unique and markedly differs from adults. Birth is associated with instant dis-

Table 1. *In vivo* effect of acylated ghrelin, unacylated ghrelin, [DAP-3]-ghrelin (1–5) and co-administration of [DAP-3]-ghrelin (1–5) and GHRP-6 [D-Lys3] on glucose, insulin, and C-peptide concentrations in 6-day-old rat neonates

	Glucose	AG	UAG	[Dap3]-ghrelin (1–5)	[Dap3]-ghrelin (1–5) + GHRP-6 [D-Lys3]
Glucose (mg/dl)	261 (9)	268 (6)	261 (4)	254 (7)	268 (7)
Insulin (ng/ml)	15.8 (2.4)	15.1 (2.5)	13.4 (3.1)	14.9 (3.5)	11.8 (2.3)
C-peptide (ng/ml)	22.2 (1.1)	21.4 (1.1)	20.2 (1.5)	21.1 (1.8)	19.8 (1.6)

Values are given in mean (SE).

n = 16–20/group.

AG, acylated ghrelin; UAG, unacylated ghrelin; [DAP-3]-ghrelin (1–5), Ghrelin (1–5) Amide, [Dap3]-Octanoyl.

continuation of maternal glucose as a source of carbohydrates. Maternal milk in rats contains little lactose and 60% of the calories originate from fat. Thus, conversion of fat into glucose is a key step in the maintenance of the glycemia in the neonate. This is achieved by the rapid development of hepatic neoglucogenesis and is associated with a decrease in insulin secretion and an increase in glucagon secretion (rev in Ref. 10). Consistent with these data, we observed a transient decrease in neonatal glycemia and a marked decrease in circulating insulin during the first 3 h of life, while glucagon concentrations were readily detectable (Fig. 3). However, in contrast to our hypothesis, AG administration did not appear to cause an earlier or higher increase in blood glucose in the first 3 h of life and did not affect insulin or glucagon concentrations. A potential explanation for our finding is that we did not investigate appropriate time points. However, in our experiment, blood glucose and insulin were measured first 45 min after the first injection, at a time when blood glucose was found to be at a nadir, and second at 180 min of life, following two consecutive AG injections, when blood glucose was found to have recovered. Taken together, our data do not support a major role for AG in the maintenance of neonatal glycemia at birth.

In contrast to our own unpublished findings in adult rats and to published data in adult mice (17), AG administration in 6-d-old pups did not affect glucose or insulin concentrations under hyperglycemic conditions. We chose to measure C-peptide in addition to insulin to ensure that the lack of a measurable effect on systemic insulin concentrations was not masked by the degradation of portal insulin when crossing the liver (16). Indeed, the half-life of insulin is short (4–5 min in the adult rat (18)), and the liver takes up and degrades 50% of portal insulin at the first pass (19). In contrast, the concentrations of C-peptide, which is secreted in equimolar concentrations as insulin, are minimally affected by the liver and its half life is 5–10 times longer than insulin (20). Nevertheless, C-peptide concentrations were not affected by ghrelin.

Because of the rapid deacylation of AG in the neonate (14), which could explain the absence of demonstrable effect of AG on glucose homeostasis *in vivo*, we also administered ghrelin (1–5) amide, [Dap3]-octanoyl. This pentapeptide retains the capability of binding and activating the GHSR-1a (11) and has an amide bond between Ser3 of ghrelin and the octanoyl chain that makes this peptide more resistant to deacylation. Similar to ghrelin, ghrelin (1–5) amide, [Dap3]-octanoyl was shown to inhibit exocrine pancreas secretion *in vivo* (21). In contrast, it did not affect stomach emptying (22). In our *in vitro* experi-

ments, ghrelin (1–5) amide, [Dap3]-octanoyl inhibited insulin secretion by the neonatal pancreas islet at a concentration of 10 nM (1–2 orders of magnitude greater than AG). *In vivo*, we chose a dose of ghrelin (1–5) amide, [Dap3]-octanoyl that was similar to the dose of AG. The rationale for this design was that in contrast to ghrelin (1–5) amide, [Dap3]-octanoyl, AG is degraded very quickly in young rats as recently shown by us (14), resulting in circulating AG concentrations that would be 1–2 orders of magnitude lower compared with the concentrations of ghrelin (1–5) amide, [Dap3]-octanoyl. However, we did not observe an effect of ghrelin (1–5) amide, [Dap3]-octanoyl on glucose-stimulated insulin or C-peptide secretion *in vivo*.

The physiologic role of ghrelin in overall glucose homeostasis remains incompletely understood. Our *in vitro* demonstration that AG under normo or hyperglycemic conditions decreases insulin secretion in rat pups is consistent with data in adult rodents reported in most (15,17,23,24) but not in all (25) studies. However, *in vivo*, the situation is more complex and administration of AG was shown to affect glucose homeostasis not only by decreasing glucose-stimulated insulin secretion (17) but also by decreasing insulin sensitivity (26). In addition, while the *in vivo* effects of AG on glucose homeostasis are usually attributed to a direct effect of the acylated form of ghrelin, it should be remembered that, *in vivo*, AG is rapidly deacylated into UAG (14), which has effects opposite to those of AG on glucose homeostasis. UAG may stimulate insulin secretion under hyperglycemic conditions (16) and increases insulin sensitivity (27). Studies performed in several animal models that overexpress ghrelin have attempted to clarify this situation. By using a transgenic mouse model that leads to a $\approx 50\%$ increase in circulating AG and a $\approx 25\%$ increase in UAG, Bewick *et al.* (28) observed a decrease in glucose-induced insulin secretion without changes in insulin sensitivity. In contrast, Zhang *et al.* (29), using a model that increases mainly UAG concentrations, observed an increase in insulin sensitivity. In the study by Iwakura *et al.* (30), in which both AG and UAG concentrations were markedly increased, glucose intolerance was found to be due to decreased insulin response to a glucose load, while insulin sensitivity was unaffected. Taken together, these data suggest that the ratio of AG to UAG, more than the absolute concentrations of AG, may regulate glucose homeostasis *in vivo*.

This hypothesis is relevant to perinatal rat physiology and may explain the absence of *in vivo* effects of AG in our experiments. We recently demonstrated *in vitro* that AG degradation was much faster in rat neonates compared with adult

rats (14). Although only 59% of AG was transformed into UAG and non-UAG products over 30 min in adult animals, this percentage was 92% in rat neonates. As a consequence, the AG to UAG ratio at the end of the incubation was six times lower in rat neonates compared with adult rat. We speculate that the discrepancy between the *in vitro* effects of AG (similar in adult rats and in rat neonates) and the *in vivo* effects of AG (detectable in adult rats but not, in this study, during the perinatal period) may be due to a smaller AG to UAG ratio in the rat neonate that would counteract the effects of AG on glucose homeostasis.

The AG to UAG ratio may also be relevant to perinatal physiology and to the treatment of Type 2 diabetes in humans. Insulin is an anabolic hormone and is necessary for growth and development, in particular during the perinatal period (31). We speculate that a faster degradation of AG and a lower AG to UAG ratio could prevent an inappropriate inhibition of insulin secretion by the pancreas islet and favor anabolism. From a clinical point of view, in subjects with Type 2 diabetes, a lower AG to UAG ratio may be of benefit by preventing inhibition of insulin release and increasing peripheral insulin sensitivity. This could be achieved by the development of inhibitors of the ghrelin O-acyl transferase, the enzyme that was recently shown to acylate proghrelin in tissues (32,33).

In conclusion, our *in vitro* data show that perinatal islets, similar to adult islets, are sensitive to the insulinostatic effects of AG. However, in contrast to adult rodents, our data do not support our hypothesis that AG regulates insulin secretion *in vivo* during the perinatal period. This may be due to a faster degradation of AG compared with the adult animal and may represent a physiologic mechanism aiming at preserving the secretion of insulin, an anabolic hormone important for optimal growth and development.

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