Fasting differentially regulates plasma corticosterone-binding globulin, glucocorticoid receptor, and cell cycle in the gastric mucosa of pups and adult rats

Daniela Ogiás,1 Eunice Ribeiro de Andrade Sá,1 Ariane Kasai,1 Marie-Pierre Moisan,2 Eliana Parisi Alvares,1 and Patrícia Gama1

1Department of Cell and Developmental Biology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil; 2Laboratory PsyNuGen, Université Bordeaux 2, Bordeaux, France

Submitted 24 June 2009; accepted in final form 10 October 2009

Ogiás D, de Andrade Sá ER, Kasai A, Moisan M, Alvares EP, Gama P. Fasting differentially regulates plasma corticosterone-binding globulin, glucocorticoid receptor, and cell cycle in the gastric mucosa of pups and adult rats. Am J Physiol Gastrointest Liver Physiol 298: G117–G125, 2010. First published October 15, 2009; doi:10.1152/ajpgi.00245.2009.—The nutritional status influences gastric growth, and interestingly, whereas cell proliferation is stimulated by fasting in suckling rats, it is inhibited in adult animals. Corticosterone takes part in the mechanisms that govern development, and its effects are regulated in particular by corticosterone-binding globulin (CBG) and glucocorticoid receptor (GR). To investigate whether corticosterone activity responds to fasting and how possible changes might affect gastric epithelial cell cycle, we evaluated different parameters during the progression of fasting in 18- and 40-day-old rats. Food restriction induced higher corticosterone plasma concentration at both ages, but only in pups did CBG binding increase after short- and long-term treatments. Fasting also increased gastric GR at transcriptional and protein levels, but the effect was more pronounced in 40-day-old animals. Moreover, in pups, GR was observed in the cytoplasm, whereas, in adults, it accumulated in the nucleus after the onset of fasting. Heat shock protein (HSP) 70 and HSP 90 were differentially regulated and might contribute to the stability of GR and to the high cytoplasmic levels in pups and elevated shuttling in adult rats. As for gastric epithelial cell cycle, whereas cyclin D1 and p21 increased during fasting in pups, in adults, cyclin E slowly decreased, concomitant with higher p27. In summary, we demonstrated that corticosterone function is differentially regulated by fasting in 18- and 40-day-old rats, and such variation might attenuate any possible suppressive effects during postnatal development. We suggest that this mechanism could ultimately increase cell proliferation and allow regular gastric growth during adverse nutritional conditions.

food restriction; p21; p27; stomach

THE POSTNATAL DEVELOPMENT and maintenance of gastrointestinal tract is orchestrated by the intricate balance of cell proliferation, migration, differentiation, and death, which, in turn, are controlled by an interaction among milk molecules, hormones, luminal microbes, and genetic program (13, 32, 37). Glucocorticoids also take part in this mechanism, as plasma corticosterone levels increase during the third postnatal week, preceding functional maturation in rodents (1, 24). In addition, hydrocortisone administration promotes the differentiation of gastric and intestinal cells and consequently induces many enzymatic functions in the stomach and small intestine (30, 38, 56).

Corticosterone is the main glucocorticoid hormone in the rat, and its concentration and activity are regulated by different factors, which include adrenocorticotropic hormone, corticosterone-binding globulin (CBG) (17, 33, 42), and glucocorticoid receptor (GR) (23). Furthermore, there is a close relationship between the nutritional status and the response of the hypothalamic-pituitary-adrenal axis. Accordingly, when pups and adult animals are submitted to food restriction, total corticosterone levels increase (21, 39, 42).

CBG is the major transport protein for corticosterone and is mostly produced in the liver. In the rat, binding capacity rises from postnatal day 12 onward, preceding corticosterone upsurge by two days (24, 51) and directly affecting hormonal action on target tissues (10, 17). Nutritional status also influences CBG and consequently corticosterone availability to cells and plasticity to stress responses (42, 55).

Corticosterone triggers its effects by binding to cytoplasmic GR, which belongs to a family of steroid/thyroid/retinoic acid receptors and functions as a ligand-dependent transcriptional factor. GR is ubiquitously expressed in most tissues in the rat (34), and in the gastric mucosa it was identified mainly in parietal cells (28). In the absence of glucocorticoids, GR is found in cytoplasm as part of a large multiprotein complex, which contains heat shock proteins (HSPs), specifically HSP 70 and HSP 90 that allow the proper folding of the receptor and control entrance to the nucleus (23). After glucocorticoids bind GR, a conformational change releases the receptor from the complex, and it translocates into the nucleus to bind glucocorticoid-responsive elements at regulatory regions of target genes, to which repression is the most common response (23).

GR arrests cell cycle through induction of antimitogenic factors and inhibition of proliferative molecules (35, 45, 50). Progression through cell cycle is accomplished by the formation of complexes between cyclins and cyclin-dependent kinases (CDKs), which are regulated by CDK-inhibitory proteins. Among CDK-cyclin complexes, CDK4-cyclin D is induced during G1 phase, whereas CDK2-cyclin E appears lately at G1-S transition (48). Together with cyclin D and CDKs, p21^{waf1} and p27^{kip1} (hereafter referred to as p21 and p27) constitute targets of GR (5, 45, 50). Conversely, the activity of this receptor is also influenced by the phosphorylation promoted by cell cycle proteins (25, 53).

Throughout development, the administration of glucocorticoids exerts early inhibitory effects on gastric epithelial cell proliferation either in vivo or in vitro (20, 21, 35), besides inducing apoptosis (21). The nutritional status also influences gastric growth, and interestingly, whereas cell proliferation is

Address for reprint requests and other correspondence: P. Gama, Dept. of Cell and Developmental Biology, Inst. of Biomedical Sciences, Av Prof Lineu Prestes 1524 ICB I, Univ. of São Paulo, São Paulo, SP, 05508-900, Brazil (e-mail: patgama@usp.br).

http://www.ajpgi.org 0193-1857/10 $8.00 Copyright © 2010 the American Physiological Society

G117
stimulated by fasting in suckling pups, it is inhibited in adult animals (4). As mentioned above, the nutritional status regulates glucocorticoids and CBG levels in the plasma, but paradoxically the high concentrations detected after fasting sucking pups (21) are concomitant with increased gastric cell proliferation, whereas, in adults, proliferative indices are low. Therefore, the cell proliferation in the gastric mucosa seems to be differentially affected by corticosterone in pups and adult rats when they are submitted to fasting condition.

Because the growth of the gastric mucosa depends on a plethora of factors that include the action of glucocorticoids and nutritional status, we investigated more closely how corticosterone responds to fasting and how the possible changes might interfere in cell cycle control. To that end, we evaluated total corticosterone plasma levels, CBG binding capacity after short- and long-term fasting, GR expression and localization, HSP 70, HSP 90, and cell cycle protein concentration after fasting progression in rats at different stages of development.

MATERIALS AND METHODS

Animals. Eighteen- and forty-day-old male Wistar rats from the Department of Cell and Developmental Biology Animal Colony (ICB USP) were used according to the protocol reviewed and approved by the Ethical Committee on Animal Experimentation (CEEA protocol number 124/2006). All animals were kept at 22°C under a 12-h:12-h light/dark cycle with lights on at 0600. Water was offered ad libitum. Rats were anesthetized with isoflurane (Cristália, São Paulo, Brazil) by 30 s, and blood was collected by puncture from abdominal aorta into heparinized tubes. This procedure was conducted in 30 s to avoid any disturbance of glucocorticoids levels because of manipulation. Plasma was collected after centrifugation (12,000 revolution/min, 30 min) and kept at −20°C.

Fasting treatment for plasma collection. Plasma was collected to evaluate the effects of fasting progression on corticosterone levels and CBG binding capacity, and, therefore, we defined different periods of treatment for each age. Briefly, we first took the schedule and periods of fasting that have been previously determined to influence gastric cell proliferation (4, 20) and considered them as the maximum intervals for 18- and 40-day-old rats. Then, we established a short- and long-term period of treatment for each age. For 18-day-old suckling animals, half of pups were fasted and the other half remained with the dam, as control. For them fasting began at 1500, and they were euthanized after 6 h (short-term fasting) or 18 h (long-term fasting). For 40-day-old rats, half of the group was fasted while the other half remained fed ad libitum until euthanization. To them, fasting started at 2100, and they were euthanized after 12 h (short-term fasting) or 35 h (long-term fasting). All fasted animals were placed in aluminum cages to avoid coprophagy. Blood was collected at mentioned above.

Fasting treatment for stomach collection. The stomach collection was conducted to verify GR localization and expression, the levels of GR, HSP 70, and HSP 90, and cell cycle control proteins in gastric mucosa after fasting progression. For 18-day-old pups, fasting began at 1500, and they were euthanized after 0 h of immunohistochemistry, 2) 0, 12, 18, and 24 h for Western blot analyses or 3) 0, 1 h and 30 min, and 3 h for RT-PCR analyses (0 h was always considered as control). After anesthesia, the stomachs were excised, rinsed in saline 0.9%, and submitted either to mucosal scraping or fixation in 10% formaldehyde.

Total corticosterone assay. Serum levels of total corticosterone were measured by RIA using Rat 125I Corticosterone Kit according to manufacturer’s instruction (Biorad Assay System Kit with Amerlex-M Magnetic Separation; GE Healthcare, Freiburg, Germany). The amount of radioactivity was determined for at least 1 min in a γ-scintillation counter (Wizard Automatic Gamma Counter, PerkinElmer, Waltham, MA). The intra-assay coefficient variation was 4.3%, and interassay coefficient variation was 4.1%.

CBG binding assay. CBG was measured using a saturation binding assay following a procedure previously described (22). Briefly, plasma samples were diluted 1:50 and incubated 30 min at room temperature with dextran-coated charcoal suspension (DCC; Merck, Darmstadt, Germany) to remove endogenous steroids. DCC was then precipitated by centrifugation, and an aliquot of the samples was added into duplicate tubes containing different concentrations (16 to 0.5 nM) of [1,2-3H]corticosterone (specific activity: 79 Ci/mmol; GE Healthcare) and into one tube containing 32 μM of cold corticosterone for evaluation of nonspecific binding. After the incubation at 40°C followed by ice-water bath, cold DCC was added to remove unbound steroids, and the reaction was incubated and terminated by centrifugation at 3,000 revolution/min at 4°C for 15 min. Supernatants were transferred into scintillation vials, and simultaneous triplicates (Tri-Carb 1600TR Liquid Scintillation Counter, PerkinElmer). Specific binding was determined for each sample and converted into nM of serum.

Immunohistochemistry. For immunohistochemistry, the stomachs were embedded in paraffin wax. Nonserial 6-μm sections were placed on slides coated with 3-aminoethyltriethoxy-silane (Sigma Chemical, St Louis, MO). Sections were deparaffinized and rehydrated, and peroxidase activity was blocked with 0.3% H2O2 (Sigma Chemical) in methanol. Nonspecific binding was blocked with 10% goat serum (Jackson ImmunoResearch Laboratories, West Grove, PA), and antigen retrieval was performed with 10 mM citric acid pH 6.0 in microwave. Sections were incubated overnight at 4°C with rabbit antibody for GR (M-20) (2 μg/ml, Santa Cruz Biotechnology, Santa Cruz, CA). After adding goat anti-rabbit antibody (Jackson Laboratories) and streptavidin-biotin complex, the peroxidase activity was developed by 0.05% 3,3-diaminobenzidine (Dako, Carpinteria, CA) in 50 mM Tris containing 0.15% H2O2. Slides were counterstained with Mayer’s hematoxylin. Negative controls were incubated with normal serum to replace the primary antibody. Labeling index was estimated by counting 2,500 epithelial cells as labeled or nonlabeled along the whole extension of the gastric gland. Only longitudinal sections were considered. The index was determined for each animal as labeled cells/total epithelial cells × 100.

Western blot. The gastric mucosa of the corpus region was scraped and stored in 10 mM PMSF (Merck) in 0.02 M Tris-buffered saline (TBS) at −80°C. For total protein extraction, samples were homogenized in ice-cold NP40 lysis buffer (20 mM Tris–HCl pH 8.0, 135 mM NaCl, 1% NP-40, and 10% glycerol) (19) containing a cocktail of proteases inhibitors (Sigma Chemical). Total protein concentration was estimated by the Bradford method (9). Cytoplasmic and nuclear proteins were separated by cellular fractioning using NE-PER Kit (Pierce, Rockford, IL), and concentration was determined by BCA method (BCA Kit, Pierce). All samples were maintained at −80°C.

Thirty micrograms of total protein extracts and 20 μg of cellular fractioning were separated by 12% SDS-PAGE and transferred onto nitrocellulose membranes (Hybond, GE Healthcare), which were washed in TBS containing 0.1% Tween 20 (TBS-T) and blocked overnight in TBS-T with 5% nonfat dry milk. Membranes were incubated with rabbit polyclonal antibodies to GR (M20) (20 μg/ml, Santa Cruz Biotechnology), HSP90, and the cell cycle proteins cyclin D1, CDK 4, p27, cyclin E, CDK 2 (20 μg/ml, Santa Cruz Biotechnology), and p21 (10 μg/ml; Abcam, Cambridge, MA). Monoclonal antibodies were used to HSP 70, α-tubulin (20 μg/ml, Santa Cruz Biotechnology), and β-actin (1 μg/ml, Sigma Chemical), and these structural proteins were taken respectively as fractioning and loading controls. In addition, the equal concentration of nuclear samples was checked by immunoblotting against rabbit anti-lamin B1 (20 μg/ml,
Abcam). Bands were detected using ECL system (GE Healthcare). Densitometry was performed using public domain program (Image J, 1.37 v. software, NIH public domain).

**RT-PCR amplification.** The gastric mucosa of the corpus region was scraped, immediately frozen in liquid nitrogen, and kept at –80°C. RNA from tissues was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA). PCR for GR was performed using the forward primer 5’-TTCTGTACAC-3’ and the reverse primer 5’-AGCAGGGTGATCACCAGCAG-3’. After preheating for 10 min at 94°C, amplification was performed using 30 cycles at 94°C for 30 s, at 55°C for 30 s, and at 72°C for 30 s, followed by a final extension of 5 min at 72°C. PCR reaction mixture contained 1 μl of sample cDNA, 2 μl PCR buffer (pH 8.4), 0.5 μl dNTP at 10 mM, 0.5 μl of each GR-specific primers, 0.3 μl Taq DNA polymerase (5 U/μl, Invitrogen), and water. Under the same conditions, β-actin was taken as an internal control, using the forward primer 5’-CTGTTGGAAGACGTCATTACCCACAG-3’ and the reverse primer 5’-GACTCATCGTACTCTGCTCTC-3’. Reaction products were submitted to electrophoresis in 1% agarose gels containing ethidium bromide. Fluorescence measurements were made using G:Box (Syngene, Frederick, MD). Results were expressed as relative densitometric units, as a ratio of β-actin mRNA.

**Statistical analyses.** All data were expressed as means ± SD and were compared by ANOVA followed by Tukey’s test. Statistical differences were considered when P < 0.05. Analyses were performed on Graphpad Prism 5.1 software (Graphpad, La Jolla, CA).

**RESULTS**

**Total corticosterone and CBG binding capacity.** Because changes of nutritional status interfere in the growth control of the gastric mucosa and glucocorticoids are part of this mechanism, we first evaluated total corticosterone levels in the plasma combined with CBG binding capacity. To test whether the progression of fasting might alter these parameters, we established short- and long-term periods of food restriction.

Short-term fasting (6 h for pups or 12 h for adults) increased corticosterone levels in both suckling and adult animals (P < 0.05) (Fig. 1, A and C). After the progression to long-term fasting, we observed that, whereas corticosterone remained constant in pups (18 h), there was a significant increase in adults (35 h) (P < 0.05) (Fig. 1, A and C).

CBG binding capacity behaved differently in the ages studied. We found that short-term fasting significantly enhanced CBG binding capacity in sucking pups (P < 0.05) (Fig. 1B) whereas, in adult animals, it did not alter the parameter (Fig. 1D). After the progression of fasting, CBG binding remained high in pups (18 h) (P < 0.05) and significantly increased in adult animals (35 h) (P < 0.05) (Fig. 1D). By comparing CBG binding capacity between the two ages, we observed that it is much higher in developing rats (Fig. 1, B and D).

**GRs and HSPs.** After the detection of high total corticosterone levels induced by short-term fasting in both suckling and adult rats, we studied the distribution, concentration, and expression of GR, as well as protein levels of HSP 70 and HSP 90 in the gastric mucosa.

By using immunohistochemistry, we observed that, in pups, GR is spread along the gastric gland (Fig. 2, A to D). Fasting increased the number of cells stained for the receptor as detected by a higher labeling index (P < 0.05) (Fig. 2, A, B, and J). In the gastric mucosa of adult animals, GR is distributed as a gradient from the isthmus to the basal region of the gland (Fig. 2, F–I). Fasting increased the intensity of labeling and the number of cell immunostained for GR compared with control group (P < 0.05) (Fig. 2, F–I and K). We observed that labeling is distributed in the cytoplasm and nucleus of epithelial cells (Fig. 2, H and I). By comparing GR presence in pups and adults, we found that it was much higher in adult rats submitted to fasting.

The concentration of GR in the gastric mucosa was also investigated, and samples were collected after different periods of fasting so that we used 0, 3, and 6 h for pups and 0, 12, 18, and 24 h for adult rats. Through the proposed schedule, we verified that, in pups, GR level was higher in the gastric mucosa after 3 h of fasting (P < 0.05) (Fig. 3, A and B) compared with the beginning of treatment (0 h), and the concentration remained constant after 6 h. In adult animals, GR level rose in the gastric mucosa after 12 h of treatment (P < 0.05) (Fig. 4, A and B), returned to initial values after 18 h, and then increased again (P < 0.05).
HSP 70 levels significantly decreased \((P < 0.05)\) during the progression of fasting in 18-day-old rats, whereas HSP 90 was highly expressed throughout treatment (Fig. 3, \(A\) and \(B\)). In 40-day-old animals, HSP 70 remained constant in the gastric mucosa and HSP 90 was significantly reduced only at 18 h of fasting \((P < 0.05)\) (Fig. 4, \(A\) and \(B\)).

GR shuttling from cytoplasm to nucleus was evaluated through cellular fractioning. In suckling rats, we detected that receptor was more concentrated in the cytoplasm \((P < 0.05)\) and the proportion between the compartments did not change with treatment (Fig. 3, \(C\) and \(D\)). In contrast, in 40-day-old animals, the concentration of GR decreased in the cytoplasm concomitant with an increase in the nucleus \((P < 0.05)\) (Fig. 4, \(C\) and \(D\)).

To study GR mRNA expression, we used a different schedule to collect the samples from the gastric mucosa because, if protein was already altered after 3 h in pups and 12 h in adults, we should explore transcription earlier. Thus we used fasting periods of 0, 30, and 90 min for pups and 0, 1 h and 30 min, and 3 h for adult rats. We verified that GR expression increased in the gastric mucosa of pups soon after 30 min of fasting \((P < 0.05)\) (Fig. 3, \(E\) and \(F\)) compared with the beginning of treatment \((0 \text{ h})\), and levels were maintained after 90 min \((P < 0.05)\). In adult animals, GR mRNA was higher in the gastric mucosa at 18 and 40 days.
mucosa after 1 h and 30 min of treatment ($P < 0.05$) (Fig. 4, E and F) and remained elevated at 3 h.

**Cell cycle control proteins.** To investigate the effects of fasting on cell cycle control in the gastric mucosa of pups and adult rats, we evaluated the protein concentration of cyclin D1, CDK 4, p21, cyclin E, CDK 2, and p27. We observed that, in suckling rats, cyclin D1 was higher after 3 h of food restriction ($P < 0.05$) (Fig. 5, A and C) and then returned to initial levels. Whereas CDK 4 did not change, p21 increased after 3 h ($P < 0.05$) and remained high until 6 h of fasting ($P < 0.05$) (Fig. 5, A and D).

![Figure 3. GR, heat shock protein (HSP) 70, and HSP 90 levels in the gastric mucosa of pups. A: representative immunoblots are shown for each molecule. B: densitometry is represented as fold of control group (0 h) and shown as means ± SD. *$P < 0.05$ vs. 0 h. #$P < 0.05$ vs. 3 h of fasting. C: representative immunoblots of GR after cellular fractioning to detect cytoplasmic (C) and nuclear (N) proteins. Total and nuclear loadings were verified respectively by β-actin and lamin B1. Cellular fractioning was confirmed by α-tubulin. D: integrative optical densitometry (IOD) is represented by means ± SD in arbitrary units (a.u.). *$P < 0.05$ vs. cytoplasmic fraction. E: representative RT-PCR for GR in gastric mucosa. F: relative densitometry as ratio of β-actin represented as means ± SD. *$P < 0.05$ vs. 0 h; $n = 3$ for each condition.]

![Figure 4. GR, HSP 70, and HSP 90 levels in the gastric mucosa of adult rats. A: representative immunoblots are shown for each molecule. B: densitometry is represented as fold of control group (0 h) and shown as means ± SD. *$P < 0.05$ vs. 0 h. #$P < 0.05$ vs. 12 h of fasting. C: representative immunoblots of GR after cellular fractioning to detect cytoplasmic and nuclear proteins. Total and nuclear loadings were verified respectively by β-actin and lamin B1. Cellular fractioning was confirmed by α-tubulin. D: integrative optical densitometry is represented means ± SD in arbitrary units. E: representative RT-PCR for GR in gastric mucosa. F: relative densitometry as ratio of β-actin represented as means ± SD. *$P < 0.05$ vs. 0 h; $n = 3$ for each condition.]

AJP-Gastrointest Liver Physiol • VOL 298 • JANUARY 2010 • www.ajpgi.org
Cyclin E, CDK 2, and p27 were not altered by food restriction in the gastric mucosa of suckling rats (Fig. 5, A and D). 

In adults, we found a different pattern of regulation as cyclin D1, CDK 4 and p21 only slightly varied (Fig. 5, B and E). Concomitantly, cyclin E slowly decreased, whereas p27 significantly increased (*P < 0.05) (Fig. 5, B and F).

**DISCUSSION**

Food restriction is a stressful condition that induces high glucocorticoid concentration in blood (20, 55). In the present study, we investigated corticosterone plasma levels and CBG binding capacity, the expression of GRs and their distribution, as well as the concentration of cell cycle control proteins in the rat gastric mucosa to elucidate whether fasting influences stomach growth and development through corticosterone action.

By using different periods of food restriction and considering that, at 18 days, circadian variations are not fully established in rat pups (44), we evaluated how the progression of nutritional stress might interfere in glucocorticoid activity. We showed that short- and long-term fasting increased corticosterone plasma levels in pups and adult rats. Given that the handling of animals, especially at early stages of development, can also generate stress responses (8, 36), we tried to reduce the manipulation at most to minimize its effects. We confirmed that the high corticosterone concentrations found in fasted rats were attributable to food restriction and not to handling, as the control groups, which were equally manipulated, presented basal hormone levels. In addition, our results are in accordance with studies that reported higher corticosterone when animals are submitted to stressful stimuli as tail shocks (17), burn injury (12), and fasting (21).

CBG is an important element in the coordination of corticosterone activity. Henning (24) showed that plasma CBG significantly rises in the rat from postnatal day 9 to day 24, reaching a plateau from day 24 to day 28. Total corticosterone increases concomitantly (24) so that there is little change in the proportion of hormone bound to CBG. In fact, Henning (24) reported that CBG binding increases from day 9 to day 17, and, from then on, it is kept at high rates. We studied 18- and 40-day-old rats and detected similar CBG binding in control-fed groups at both ages. However, the response of these animals to the stress induced by fasting was completely different. Short-term treatment enhanced CBG binding in suckling pups, and it remained high throughout long-term fasting. In contrast, adult rats presented a slight increase of CBG binding capacity only after long-term fasting, but curiously these values were much lower than in pups. It is known that CBG regulation is diverse and depends on age, stressor stimuli,
and hormone levels. Different analyses indicate that stress-induced corticosterone downregulates CBG expression in the liver (49), whereas glucocorticoid administration leads to opposite responses in suckling and adult animals (58). When CBG binding is examined, it is lower after burn injury in adult mice (12). We suggest that the high CBG binding capacity in pups might be a compensatory mechanism to avoid any inhibitory effects of high corticosterone over gastric growth, mainly because suckling represents a critical stage during the postnatal development of gastrointestinal tract.

Glucocorticoid action still depends on the expression and distribution of receptors in either normal or tumoral cell types (18, 19, 57). We detected GR along the extension of gastric gland in pups, whereas, in adult animals, it was more restricted to the isthmus and basal regions, which agrees with a previous study (28). Adding to that, we demonstrated that, in 18- and 40-day-old rats, fasting increased GR at transcriptional and protein levels. Although food restriction doubled GR labeling index and protein concentration in the gastric mucosa at both ages, the effect was slightly more pronounced in 40-day-old animals. Through a closer investigation using cellular fractioning and identification of immunolabeled cells, we observed that, in pups, GR is mainly cytoplasmic, whereas, in adults, it accumulated in the nucleus after the onset of fasting. These results indicate that, in pups, fasting did not activate receptor translocation to the nucleus, even though, in number, GR labeling index was increased by treatment. In contrast, in adult animals, food restriction induced the shuttling of the receptor into the nucleus, which characterizes the response to corticosterone. Because chaperones (HSP 70 and HSP 90) are essential to maintain GR in a state suitable to bind the hormone and to control GR entrance into the nucleus (14, 23, 57), we also investigated their concentration in the gastric mucosa. HSP 70 and HSP 90 were differentially regulated by fasting in 18- and 40-day-old rats, in a way that their respective increase and decrease might contribute to the stability of GR and to the elevated cytoplasmic levels in pups and higher shuttling in adult rats. Another study also demonstrated that chronic stress increases bound and total cytosolic GR in the liver of adult animals in parallel with high corticosterone plasma levels (2). Taken together, the present results suggest that, during suckling, the high corticosterone and receptor levels induced by fasting might not immediately affect the gastric epithelium because of the higher CBG binding capacity and consequent lower traffic of GR into the nucleus. In contrast, in adult animals, increased corticosterone levels and lower CBG allow the binding to GR and its shuttling into the nucleus to trigger the proper effects. These data support the idea of a stress-hyporesponsive period during postnatal development (46).

Two human variants have been described for GR as hGR-α and hGR-β that differ by 35 amino acids in length at the carboxy terminus (34). In humans, GR-β is considered to function as a repressor of GR-α, mediating negative transcriptional control (11, 29). In addition, GR-β does not bind to hormone (7) and can be correlated to glucocorticoid resistance (52). The mechanisms that underlie the development of such resistance are not completely understood but change with disease, treatment, and genetic background. The presence of GR isoforms in rat or mice is not yet established (41). In rats, GR translational variants were described as A, B, C, and D (34). Recently, GR-β was identified in zebrafish (47), and a splice variant was found in rats (rGR-β) (27). Our results indicated that GR shuttling from cytoplasm into the nucleus is much lower in pups, but it remains to be determined whether such effect can be correlated with other receptor forms and or with a mechanism of resistance to glucocorticoids during postnatal development.

After estimating the different parameters discussed above, we tried to determine the effects of fasting on the control of cell cycle. We have previously shown that food restriction inhibits gastric epithelial cell proliferation in young adult rats (4), reproducing the response to exogenous corticosterone (20, 21). Paradoxically, fasting stimulates epithelial cell division in suckling pups (4, 20). Our present results indicated that cyclin D1 and p21 increased during food restriction in pups, and, therefore, they are parallel with the high proliferative indices reported before (4). The function of p21 as a cell cycle promoter has been discussed by different studies (6, 16, 54), and it was described to act as an adaptor protein and to be involved in the assembly and maintenance of cyclin D1-CDK 4 complex in the nucleus (3, 31, 54). In contrast to our findings for pups, in adult animals, we observed that fasting slightly decreased p21 and cyclin E, concomitant with an important increase of p27. Such effect was also described when epithelial cells were treated with corticosterone (26). p27 delays and arrests cell cycle (15, 16, 26, 43), and, besides being responsive to glucocorticoids, it is part of transforming growth factor (TGF)-β inhibitory cascade in the gastric mucosa (13). Therefore, during fasting periods, a complex mechanism that combines corticosterone, growth factors, and cell cycle proteins is differentially activated to control epithelial cell proliferation throughout postnatal development. Adding to it all, we previously showed that hydrocortisone administration increases TGF-β1 and Smad2P in the gastric epithelium of pups (40). It still remains to be determined whether GR function and TGF-β signaling pathway are integrated through a cross talk to take part in the control of stomach growth.

As summarized in Fig. 6, we demonstrated that, at 18 days, fasting increased corticosterone concentration as well as CBG levels.
binding capacity, which, in turn, reduced hormone availability and the consequent traffic of GR into the nucleus. Lower receptor levels allowed the upsurge of proteins involved in cell cycle progression, especially cyclin D1 and p21, and they might cooperate to stimulate epithelial proliferation during fasting (4, 20). At 40 days, we showed that fasting also increased corticosterone concentration, but it did not affect CBG binding capacity, which allowed the higher availability of the hormone and shutting of GR into the nucleus. The elevated receptor levels were parallel with augmented p27 concentration in the gastric mucosa (Fig. 6), which might be part of the inhibition of cell proliferation induced by fasting at this age (4).

We conclude that the stress induced by fasting activates different mechanisms of corticosterone action in pups and adult rats, and such variation might attenuate any possible suppressive effects during postnatal development. We suggest that this regulatory mechanism would ultimately increase cell proliferation and allow gastric growth during adverse nutritional conditions.

ACKNOWLEDGMENTS

We thank Cruz Alberto Mendoza Rigonatti for preparing the histological sections, Carolina Botini Prates for working on Western blot, and Marlene Santos da Rocha for technical assistance with radioimmunoassay.

GRANTS

This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (São Paulo, Brazil) (05/01273-4) and CNPq (474920/2006-2). Daniela Ogias was a recipient of a CAPES fellowship for PhD.

DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES

Sapolsky RM, Meaney MJ. 

Rogatsky I, Trowbridge JM, Garabedian MJ. 

Ramaley JA. 


Nanthakumar NN, Henning SJ. 


