Original article

Submandibular glands and the regulation of gastric proliferation and TGFα distribution during rat postnatal development

Lestingi, JFP., Cabral, RS., Gama, P.* and Alvares, EP.

Department of Cell and Developmental Biology, Institute of Biomedical Sciences,
University of São Paulo – USP, CEP 05508-000, São Paulo, SP, Brazil
*E-mail: patgama@usp.br

Abstract

Rodent gastric mucosa grows and differentiates during suckling-weaning transition. Among the molecules in rat milk, EGF and TGFβ are important peptides in the control of cell proliferation, and together with TGFα, they are also produced by submandibular glands. We aimed to determine the effect of saliva and milk on epithelial cell proliferation in the stomach of rat pups. We also examined the distribution of TGFα in the gastric mucosa after sialoadenectomy (SIALO) and fasting in order to determine whether this growth factor is affected by the deprivation of molecules derived from saliva and milk. SIALO was performed at 14 days and fasting was induced 3 days later. Cell proliferation was evaluated through metaphasic index and TGFα was detected by immunohistochemistry. We observed that whereas SIALO did not alter cell division, since the metaphasic index (MI) was unchanged, fasting stimulated cell proliferation ($P < 0.05$). After SIALO and fasting, MI was reduced when compared to the fasted group ($P < 0.05$). We found that TGFα is distributed along gastric gland and SIALO did not interfere in the localization and number of immunolabeled cells, but fasting increased their density when compared to the control ($P < 0.05$). The association of SIALO and fasting reduced TGFα immunostaining ($P < 0.05$). Therefore, during fasting, high MI was parallel to increased TGFα in gastric epithelium, but interestingly, this effect was found only in the presence of submandibular glands. We suggest that during suckling, peptides derived from saliva and milk are important to regulate gastric growth.

Keywords: TGF alpha, sialoadenectomy, gastric growth, milk, fasting.

1 Introduction

The rodent gastric mucosa undergoes differentiation during the first month of life, but more importantly, maturation is complete after the transition from suckling into weaning (HENNING, 1981; KEELEY and SAMUELSON, 2010; OSAKI, CURI, ALVARES et al., 2010). Milk-born peptides are essential to maintain growth (KOLDOVSKÝ, ILLNEROVÁ, MACHO et al., 1995; PENTTILA, SPRIEL, ZHANG et al., 1998; AGARWAL, KARMAUS, DAVIS et al., 2011) and their role in the differentiation of gastric cells is being studied mainly through the use of animal models. Epidermal growth factor (EGF) (DVORÁK and KOLDOVSKÝ, 1994) and transforming growth factor β (TGFβ) are among the active molecules present in rat milk (KOLDOVSKÝ, ILLNEROVÁ, MACHO et al., 1995; PENTTILA, SPRIEL, ZHANG et al., 1998) that might regulate gastric growth during postnatal development (DE ANDRADE SÁ, BITENCOURT, ALVARES et al., 2008).

EGF was isolated from the mouse submandibular gland by Cohen (1962) and it is a potent stimulator of cell proliferation in epithelial tissues, including those in gastrointestinal (GI) tract (AL-NAFUSSI and WRIGHT, 1982). The submandibular glands also secrete TGFα, while TGFβ1 is distributed in the granular and striated ducts (AMANO, TSUJI, NAKAMURA et al., 1991) and the other isoforms and receptors appear during development in mice (JASKOLL and MELNIK, 1999). At birth, TGFα is already expressed and found in saliva, whereas EGF will be secreted only during the third postnatal week, when ducts differentiate (MOGI, MATSUURA, SUZUKI et al., 1995).

Transforming growth factor (TGFα) is structurally related to EGF, shares the same receptor and triggers similar mitogenic and maturational responses (HORMI and LEHY, 1996). It is synthesized in fetal gastric and intestinal epithelia, and tissue concentration progressively increases during postnatal growth (HORMI, ONOLFO, GRES et al., 1995). However, TGFα was not characterized in the rat milk, but its production in GI epithelia can be modulated by milk-born EGF (DVORÁK, WILLIAMS, McWILLIAM et al., 2000).

Fasting represents the deprivation of milk for pups and food for adult animals, but interestingly, though used as a routine in clinics, its effects on gastric epithelium growth are opposite when the two developmental periods are considered. Accordingly, fasting affects tissue kinetics and induces the cell proliferation in the gastric gland only during suckling period (ALVARES and GAMA, 1993; OGIAS, DE ANDRADE SÁ, KASAI et al., 2010). When EGF concentration is considered, fasting induces the secretion by submandibular gland (GRAU, RODRÍGUEZ, SOLEY et al., 1994), whereas in the small intestine, levels are reduced in suckling pups or not altered in adults (SHAUDIES, GRIMES, DAVIS et al., 1989). As for TGFα, it seems to be less influenced in the intestinal mucosa, suggesting that though both growth factors trigger similar functions, they are under different regulatory pathways (DVORÁK and KOLDOVSKÝ, 1994).

Although the role of salivary glands in the control of digestive epithelia development has been studied, it is still not completely understood. Sialoadenectomy is the surgical excision of submandibular glands, and in adult animals, it

results in higher susceptibility of gastric mucosa to injury (TEPPERMAN and SOPER, 1990), while affecting liver structure in mice (BUIRA, POCH, SÁNCHEZ et al., 2004). Yang, Tyler, Donoff et al. (1996) showed that in hamsters salivary EGF regulates eosinophil-derived TGFα and suggested that these growth factors may take part in oral ulcer healing.

In the current study, we aimed to evaluate the effect of saliva and milk on epithelial cell proliferation in the stomach of rat pups. In addition, we examined the distribution of TGFα in the gastric mucosa after sialoadenectomy and fasting in order to determine whether this growth factor is affected by the deprivation of proteins derived from saliva and milk, and if so, how TGFα correlates to proliferative control in the gastric epithelium.

2 Material and methods

2.1 Animals and tissue collection

Wistar rats from Cell and Developmental Biology Department Animal Colony were used according to the procedures of Ethics Committee on Animal Use (CEUA ICB protocols 68/2001 and 27/2004). Rats were mated and pregnant females were kept in isolated cages until the time of birth. Animals were maintained at 22 °C and under 12/12 hours light dark schedule. Water was offered *ad libitum* and supplemented with a 0.9% multivitamin complex (Vitagold, Tortuga, Brazil). Delivery was set as day 0 and litters were culled to 8 pups around the 3rd day. Sex and weight of animals were recorded throughout experimental period.

In other to evaluate the effect of saliva on gastric growth, each litter was divided into groups that were either submitted to sialoadenectomy (SIALO) or sham operation on the 14th postnatal day. Three days later (17th day) animals were handled again and randomly separated into fed and fasted groups, so that milk was deprived and its effects could be observed. Of note, at 17 days milk is still the main source of nutrition (ALVARES and GAMA, 1993). For fasting, pups were placed in aluminum cages to avoid coprophagy and were food- deprived for 16 h before euthanasia. Fed rats (SIALO or not) remained with dams until euthanasia. Sialoadenectomy was performed under anesthesia with xilasine (Bayer, Brazil) and ketamine chloridrates (Agener, Brazil) that were i.p. injected at 1:1 (0.15 mL/100 g body weight). Submandibular and sublingual glands were excised bilaterally and fixed in Bouin's fluid for surgery control. Sham operated rats were also incised cervically, and underwent a divulsion around the glands. Suture was carried out with Superbonder glue (Loctite, Brazil) to avoid licking and biting from the mother. All animals were kept on warm plates at 37 °C to allow body temperature maintenance after surgery. Soon after recovery, water was offered to pups in Pasteur pipettes, so that we could check for suckling and swallowing conditions. Whenever incapacity for these functions was detected, animals did not proceed in experimentation.

In order to study gastric cell proliferation after sialoadenectomy and fasting through metaphasic index, 18-day-old pups were i.p. injected with vincristine (0.5 mg/kg, Oncovin, Eli-Lilly, Brazil) at 8:00 AM and euthanized 2 hours later with excessive dose of same anesthetics used for surgery (0.5 mL/ 100 g). Stomachs were collected, opened along the lesser curvature, stretched on a cork, and fixed in Bouin's fluid for morphological analyses, or in 4% paraformaldehyde solution in 0.1 M phosphate buffer (pH 7.4) for immunohistochemistry.

2.2 Cell proliferation through metaphasic index (MI) investigation

Non-serial 5 µm paraffin sections were stained with hematoxylin-eosin and studied under light microscopy at ×800 magnification (Nikon, Japan) using an integrative eyepiece with ocular grid (Kpl X8, Zeiss, Germany). Only areas that were perpendicular to the gastric lumen were considered and interphasic and metaphasic epithelial cells were counted inside the proliferative compartment that comprises the whole gland in 18-day- old rats (ALVARES and GAMA, 1993). At least 2,000 cells were recorded and the MI was obtained as the number of metaphasic cells/total epithelial cells × 100. No anaphases were registered, but prophases were consistently seen. At least 5 pups were used for each condition.

2.3 Immunohistochemistry and TGF alpha detection

Non-serial 6 µm sections were cleared of paraffin one day before immunostaining procedure. Reactions were performed as described before (OSAKI, CURI, ALVARES et al., 2010). Briefly, sections were rehydrated with 0.05M phosphate buffered saline, the endogenous peroxidase was blocked with 0.3% hydrogen peroxide (H_2O_2) in methanol, and the non-specific binding of antibodies was blocked with 10% goat serum. Tissue sections were incubated with the monoclonal antibody for TGFα (6.7 µg/mL, overnight at 4 °C, Oncogene-Calbiochem, USA). After washing, samples were incubated with the biotinylated secondary antibody (13 µg/mL, Jackson ImmunoResearch Laboratories, USA), followed by the streptavidin-peroxidase complex (10 µg/mL, Jackson ImmunoResearch Labs). Reaction was developed by 0.05% 3,3'- diaminobenzidine (DAB) (DAKO, Germany) in H_2O_2 and slides were then counterstained in Mayer's Hematoxylin. Negative control was performed by omission of primary antibody and incubation with normal serum.

Sections were analyzed as mentioned above for MI. The presence and localization of TGFα in the corpus region of the stomach were determined and labeled epithelial cells were counted in microscopic fields. Five to ten fields were evaluated per animal and at least three rats were used for each condition. Because differences between the foveolar and glandular compartments have been detected (OSAKI, CURI, ALVARES et al., 2010), we registered the immunostaining in surface mucous cells and gland cells independently. Results were obtained as the number of labeled cells/field per animal and images were captured under light microscopy (Olympus system, using Image Pro Plus Software, Media Cybernetics, USA).

2.4 Statistical analyses

The results obtained for each of the four experimental conditions were grouped, represented as the means ± SD and submitted to ANOVA followed by Tukey's test (GraphPad

Prism 5.01, GraphPad Software, Inc., USA). Significance level was set at $P < 0.05$.

3 Results

In the current study, we aimed to evaluate the effects of saliva and milk on the growth of gastric mucosa and the distribution of TGFα in the epithelium, and we used sialoadenectomy followed by fasting as experimental model. The excision of submandibular glands was carefully conducted and we did not find morphological disruption while checking for the integrity of tissues. The condition of pups after surgery was also controlled and most of them were able to suckle regularly after recovery, being gently nursed by the mother. Body mass was not significantly changed by sialoadenectomy and fasting (data not shown). We should mention that pups that were not suckling or did not gain weight did not proceed in experimentation.

The gastric mucosa of rats submitted to sialoadenectomy, sham-operation and fasting was not injured, and we did not detect morphological differences among the groups (Figure 1a-d).

When we evaluated gastric epithelial cell proliferation, we observed that sialoadenectomy did not alter the metaphasic index (MI) in suckling pups, whereas fasting significantly stimulated cell division ($P < 0.05$) when compared to the group fed normally (Figure 1e). Interestingly, after sialoadenectomy and fasting, i.e., in the absence of salivary and milk-born peptides, cell proliferation was significantly reduced ($P < 0.05$) when compared to fasted group.

Because TGFα distribution might be altered in the gastric mucosa after treatments, we evaluated the presence and localization of this peptide in the epithelium (Figure 1a-d). We observed that sialoadenectomy did not change the number of surface mucous cells immunolabed for TGFα in suckling pups, whereas fasting increased this population, especially those cells immunostained in the apical cytoplasm ($P < 0.05$) (Figure 1f). However, when fasting was used after sialoadenectomy, this augment was not observed (Figure 1f), and we recorded a significant decrease of immunolabeling when compared to fasted rats submitted to sham operation ($P < 0.05$). As mentioned before, we analyzed the apical and basal cytoplasm of surface mucous cells, but we did not identify any change in the basal region.

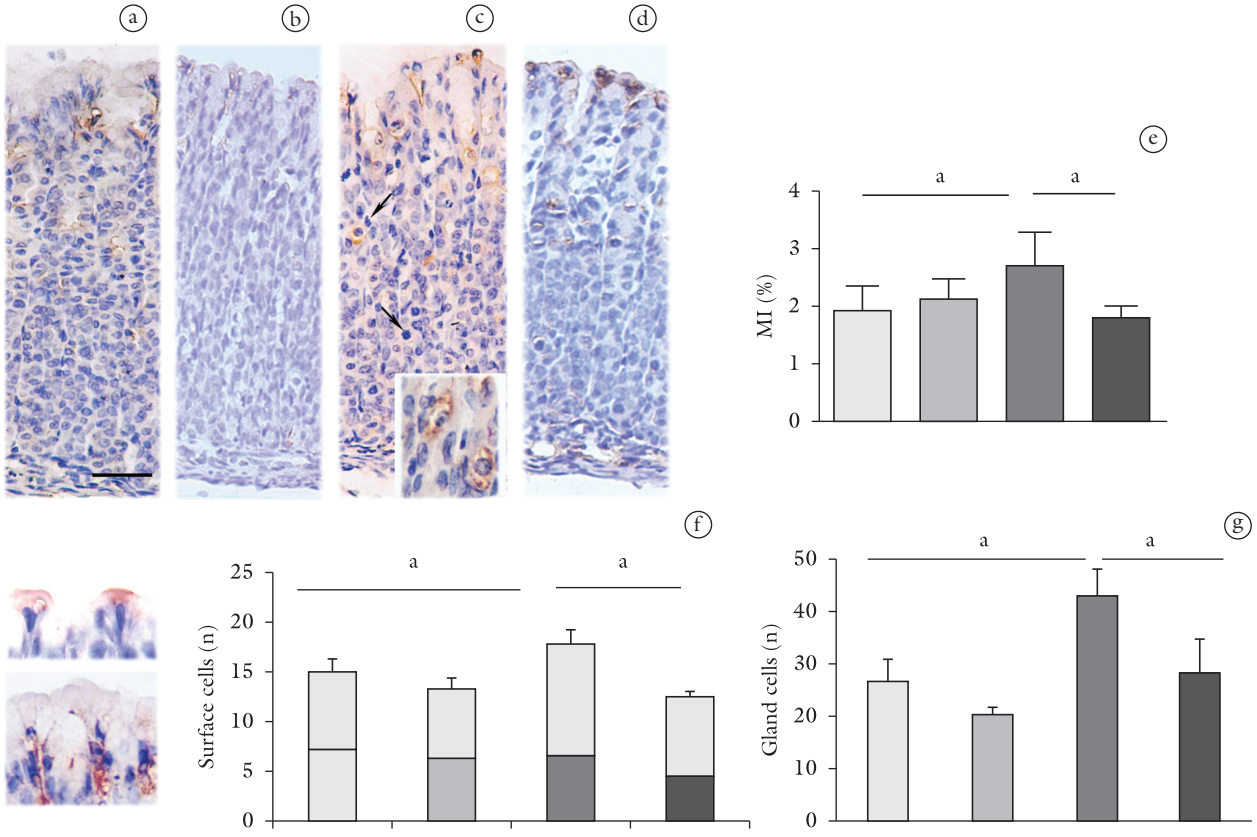

Figure 1. Sialoadnectomy (SIALO) and fasting (FA) effects on cell division and TGFα distribution in the gastric mucosa of 18-day-old rats. Immunostaining for TGFα in the following groups: a) sham-operated fed, b) SIALO- fed, c) FA and d) SIALO -FA. Treatment did not change morphology and TGFα was detected mainly on superficial mucous cells (in detail in panel f) and parietal cells (inset in panel c). Scale bar:a-d: 50μm; insets at (c) and (f): 25μm. (↙) indicates metaphasic cells. e) Metaphasic index (MI) shown as means ± SD for sham-operated –fed (light gray), SIALO –fed (gray) FA (dark gray), and SIALO + FA (black). f-g):The number of cells immunostained for TGFα was determined in the foveola (panel f) and gland (panel g), separately. Values are shown as means ±SD and the same graph attributes were used for each treatment. In (f) immunostaining was identified in the basal and apical compartments separately, which are represented at the bottom and top of bars, respectively. (*n*) = 5-6 pups at each condition. (*a*) $P < 0.05$ when FA compared to sham- operated –fed pups or SIALO+FA.

In the gastric gland, we found that TGFα is distributed equally along the extension of the gland (Figure 1a-d). Sialoadenectomy did not interfere in the localization and number of immunolabeled cells in suckling pups. Fasting, however, increased significantly the density of TGFα expressing cells when compared to the control group ($P < 0.05$). The association of sialoadenectomy and fasting reduced immunolabeling when sham-operated pups submitted to fasting were considered ($P < 0.05$) (Figure 1f).

4 Discussion

The gastric mucosa is constantly exposed to peptides that come from amniotic fluid, colostrum, milk and saliva throughout pre and postnatal development. In the current study, we aimed to investigate how the molecules produced by submandibular glands together with those present in milk might influence the renewal of gastric epithelium and the expression of TGFα in suckling rats.

The transition from suckling into weaning characterizes a period in which the gastric mucosa is highly responsive to dietary changes and tissues are able to modify growth parameters in order to allow maturation and functionality (ALVARES and GAMA, 1993; DE ANDRADE SÁ, BITENCOURT, ALVARES et al., 2008; OGIAS, DE ANDRADE SÁ, KASAI et al., 2010; OSAKI, CURI, ALVARES et al., 2010).

Our results showed that the cell division in the gastric epithelium of 18-day-old pups was not changed by the excision of submandibular glands, but it was increased when rats were submitted to fasting, which corroborates previous reports (ALVARES and GAMA, 1993; OGIAS, DE ANDRADE SÁ, KASAI et al., 2010) and confirms the role of milk-born molecules on growth. However, we further demonstrated that when fasting was induced after sialoadenectomy, the proliferative stimulus was reversed. Such result indicated that peptides from saliva might also take part in the regulation of response detected during food deprivation, and consequently control gastric growth.

The effects of fasting have been studied in our laboratory, and we demonstrated that although it characterizes a stressful condition, corticosterone action is diminished in pups (OGIAS, DE ANDRADE SÁ, KASAI et al., 2010), and TGFβ levels are reduced in the gastric mucosa (ALVARES, JORDÃO and GAMA, 2007). So, because these inhibitory elements are not fully working to balance epithelium renewal and other regulatory molecules from milk are absent (GAMA and ALVARES, 1996), during fasting pro-proliferative peptides might be more active. As mentioned before, we showed that after sialoadenectomy, the stimulatory effect of food deprivation was reversed, and the metaphasic index was found to be similar to sham-operated control.

Luminal factors derived from saliva and milk can modulate gastric cell renewal through pathways that might depend or not on their interaction. We know that saliva is a secretion rich in EGF (KONTUREK, BIELANSKI, KONTUREK et al., 1989; MOGI, MATSUURA, SUZUKI et al., 1995), TGFα, KGF, VEGF, and cytokines (GRÖESCHL, 2009), which altogether induce wound-healing. Similarly milk is also a secretion that contains a wide range of hormones and growth factors (KOLDOVSKÝ, ILLNEROVÁ, MACHO et al., 1995) that affect different gastric functions (GAMA and ALVARES, 1996) and TGFα synthesis (OSAKI, CURI, ALVARES et al., 2010).

In the stomach, TGFα is expressed by surface mucous and parietal cells (HORMI, ONOLFO, GRES et al., 1995; OSAKI, CURI, ALVARES et al., 2010) and binds EGFR (ALISON and SARRAF, 1994), which directly controls gastric epithelial cell proliferation and differentiation (OSAKI, CURI, ALVARES et al., 2010; OSAKI, FIGUEIREDO, ALVARES et al., 2011). EGFR activity has been described at basolateral membranes for endogenous ligands and at apical surface for luminal factors (CHEN, SOLOMON, KUI et al., 2002). Importantly, during postnatal development, salivary-and milk-born peptides can activate different signaling pathways through surface receptors, since proteolysis is not fully operating (KOLDOVSKÝ, 1989; RAMÍREZ and SOLEY, 2011). Among salivary molecules, EGF would be an important element, as the association of EGF and TGFα has been reported for wound-healing in oral cavity (YANG, TYLER, DONOFF et al., 1996). We observed the increased distribution of TGFα either in the apical compartment of surface cells or in the gastric gland during fasting, and such effect was reversed in SIALO pups. We should mention that SIALO rats that suckled regularly did not present changes in cell proliferation and TGFα immunostaining, and this response might be related to the age studied (RAMÍREZ and SOLEY, 2011). Finally, because TGFβ might be also modified by surgery and feeding, we also studied TGFβ1 and TβRII in our samples, but we did not detect any alteration on their distribution (data not shown).

5 Conclusion

Taken together, our results indicated that saliva upregulated TGFα in the absence of milk, but also that in SIALO rats, milk maintained gastric growth. Therefore, we can suggest that during fasting, pro-proliferative molecules from submandibular glands might act in the gastric epithelium either directly and indirectly through the increase of TGFα, and in parallel to lower corticosterone and TGFβ activities, they might be involved in the control of stomach growth during postnatal development.

Acknowledgements: the authors would like to thank Cruz Alberto Mendoza Rigonati and Luciana Renata Jordão for technical assistance. J F P Lestingi and R S Cabral were recipients of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) fellowships for undergrad students.

References

AGARWAL, S., KARMAUS, W., DAVIS, S. and GANGUR, V. Immune markers in breast milk and fetal and maternal body fluids: a systematic review of perinatal concentrations. *Journal of Human Lactation*, 2011, vol. 27, p. 171-186. PMid:21678611. http://dx.doi.org/10.1177/0890334410395761

ALISON, MR. and SARRAF, CE. The role of growth factors in gastrointestinal cell proliferation. *Cell Biology International*, 1994, vol. 18, p. 1-10. PMid:8186766. http://dx.doi.org/10.1006/cbir.1994.1001

AL-NAFUSSI, AL. and WRIGHT, NA. The effect of epidermal growth factor (EGF) on cell proliferation of the gastrointestinal mucosa in rodents. *Virchows Archiv - B*: Cell Pathology, 1982, vol. 40, p. 63-69. PMid:6126955. http://dx.doi.org/10.1007/BF02932851

ALVARES, EP. and GAMA, P. Fasting enhances cell proliferation of gastric epithelium during the suckling period in rats. *Brazilian Journal of Medical and Biological Research*, 1993, vol. 26, p. 869-873. PMid:8298523.

ALVARES, EP., JORDÃO, LR. and GAMA, P. Differential distribution of transforming growth factor beta and receptors in the hyper or hypoproliferative gastric mucosa of developing and adult rats. *Histology and Histopathology*, 2007, vol. 22, p. 147-157. PMid:17149687.

AMANO, O., TSUJI, T., NAKAMURA, T. and ISEKI, S. Expression of growth factor b1 in the submandibular gland of the rat. *Journal of Histochemistry & Cytochemistry*, 1991, vol. 39, p. 1707-1711. PMid:1940322. http://dx.doi.org/10.1177/39.12.1940322

BUIRA, I., POCH, E., SÁNCHEZ, O., FERNÁNDEZ-VARO, G., GRAU, M., TEBAR, F., RAMIRÉZ, I. and SOLEY, M. Sialoadenectomy alters liver cell turn-over and function in mice. *Journal of Cellular Physiology*, 2004, vol. 198, p. 12-21. PMid:14584039. http://dx.doi.org/10.1002/jcp.10402

CHEN, MC., SOLOMON, TE., KUI, R. and SOLL, AH. Apical EGF receptors regulate epithelial barrier to gastric acid: endogenous TGF-alpha is an essential facilitator. *AJP - Gastrointestinal and Liver Physiology,* 2002, vol. 283, p. G1098-1106. PMid:12381523.

COHEN, S. Isolation of a mouse submandibular gland protein accelerating incisor eruption and eyelid opening in the newborn animal. *Journal of Biological Chemistry*, 1962, vol. 237, p. 1555-1562. PMid:13880319.

DE ANDRADE SÁ, ER., BITENCOURT, B., ALVARES, EP. and GAMA, P. *In vivo* effects of TGFβ1 on the growth of gastric epithelium in suckling rats. *Regulatory Peptides*, 2008, vol. 146, p. 293-302. PMid:18068234. http://dx.doi.org/10.1016/j.regpep.2007.11.001

DVORÁK, B. and KOLDOVSKÝ, O. The presence of transforming growth factor-α in the suckling rat small intestine and pancreas and the absence in rat milk. *Pediatric Research*, 1994, vol. 35, p. 348-353. PMid:8190525. http://dx.doi.org/10.1203/00006450-199403000-00015

DVORÁK, B., WILLIAMS, C., McWILLIAM, DL., SHINOHARA, H., DOMINGUEZ, JA. and MCCUSKEY, RS. Milk-borne epidermal growth factor modulates intestinal transforming growth factor α levels in neonatal rats. *Pediatric Research*, 2000, vol. 47, p. 194-200. PMid:10674346. http://dx.doi.org/10.1203/00006450-200002000-00008

GAMA, P. and ALVARES, EP. LHRH and somatostatin effects on the cell proliferation of the gastric epithelium of suckling and weaning rats. *Regulatory Peptides*, 1996, vol. 63, p. 73-78. http://dx.doi.org/10.1016/0167-0115(96)00013-4

GRAU, M., RODRÍGUEZ, C., SOLEY, M. and RAMÍREZ, I. Relationship between epidermal growth factor in mouse submandibular glands, plasma, and bile: effects of catecholamines and fasting. *Endocrinology*, 1994, vol. 135, p. 1854-1862. PMid:7956907. http://dx.doi.org/10.1210/en.135.5.1854

GRÖESCHL, M. The physiological role of hormones in saliva. *BioEssays*, 2009, vol. 31, p. 843-52. PMid:19554609. http://dx.doi.org/10.1002/bies.200900013

HENNING, SJ. Postnatal development coordination of feeding, digestion and metabolism. *AJP - Gastrointestinal and Liver Physiology*, 1981, vol. 241, p. G199-214. PMid:7025659.

HORMI, K. and LEHY, T. Transforming growth factor-alpha in vivo stimulates epithelial cell proliferation in digestive tissues of suckling rats. *Gut*, 1996, vol. 39, p. 532-538. PMid:8944561 PMCid:1383265. http://dx.doi.org/10.1136/gut.39.4.532

HORMI, K., ONOLFO, JP., GRES, L., LEBRAUD, V. and LEHY, T. Developmental expression of transforming growth factor-alpha in the upper digestive tract and pancreas of the rat. *Regulatory Peptides*, 1995, vol. 55, p. 67-77. http://dx.doi.org/10.1016/0167-0115(94)00093-D

JASKOLL, T. and MELNIK, M. Submandibular gland morphogenesis: stage-specific expression of TGF-alpha/EGF, IGF, TGF-beta, TNF, and IL-6 signal transduction in normal embryonic mice and the phenotypic effects of TGF-beta2, TGF-beta3, and EGF-r null mutations. *Anatomical Record*, 1999, vol. 256, p. 252-268. http://dx.doi.org/10.1002/(SICI)1097-0185(19991101)256:3<252::AID-AR5>3.0.CO;2-6

KEELEY, TM. and SAMUELSON, LC. Cytodifferentiation of the postnatal mouse stomach in normal and Huntingtin-interacting protein 1-related-deficient mice. *AJP - Gastrointestinal and Liver Physiology,* 2010, vol. 299, p. G1241-1251. PMid:20813912 PMCid:3006248.

KOLDOVSKÝ, O., ILLNEROVÁ, H., MACHO, L., STRBÁK, V. and STEPÁNKOVÁ, R. Milk-borne hormones: possible tools of communication between mother and suckling. *Physiological Research*, 1995, vol. 44, p. 349-351. PMid:8798268.

KOLDOVSKÝ, O. Critical review: search for role of milk-borne biologically active peptides for the suckling. *Journal of Nutrition*, 1989, vol. 119, p. 1543-1551. PMid:2689607.

KONTUREK, JW., BIELANSKI, W., KONTUREK, SJ., BOGDAL, J. and OLEKSY, J. Distribution and realease of epidermal growth factor in man. *Gut*, 1989, vol. 30, p. 1194-1200. PMid:2806986 PMCid:1434230. http://dx.doi.org/10.1136/gut.30.9.1194

MOGI, M., MATSUURA, S., SUZUKI, K., INAGAKI, H., MINAMI, M., KOJIMA, K. and HARADA, M. Differential expression of transforming growth factor-alpha and epidermal growth factor during postnatal development of rat submandibular gland. *Biochemical and Biophysical Research Communications*, 1995, vol. 217, p. 271-277. PMid:8526922. http://dx.doi.org/10.1006/bbrc.1995.2774

OGIAS, D., DE ANDRADE SÁ, ER., KASAI, A., MOISAN, MP., ALVARES, EP. and GAMA, P. Fasting differentially regulates plasma corticosterone-binding globulin, glucocorticoid receptor, and cell cycle in the gastric mucosa of pups and adult rats. *AJP - Gastrointestinal and Liver Physiology*, 2010, vol. 298, p. G117-125.

OSAKI, LH., CURI, MA., ALVARES, EP. and GAMA, P. Early weaning accelerates the differentiation of mucous neck cells in rat gastric mucosa: possible role of TGFalpha/EGFR. *Differentiation*, 2010, vol. 79, p. 48-56. PMid:19783352. http://dx.doi.org/10.1016/j.diff.2009.09.001

OSAKI, LH., FIGUEIREDO, PM., ALVARES, EP. and GAMA, P. EGFR is involved in control of gastric cell proliferation through activation of MAPK and Src signalling pathways in early-weaned rats. *Cell Proliferation*, 2011, vol. 44, p. 174-182. PMid:21401759. http://dx.doi.org/10.1111/j.1365-2184.2011.00733.x

PENTTILA, IA., SPRIEL, ABV., ZHANG, MF., XIAN, CJ., STEEB, CB., CUMMINS, AG., ZOLA, H. and READ, LC. Transforming growth factor-β levels in maternal milk and expression in postnatal rat duodenum and ileum. *Pediatric Research*, 1998, vol. 44, p. 524-531. PMid:9773841. http://dx.doi.org/10.1203/00006450-199810000-00010

RAMÍREZ, I. and SOLEY, M. Submandibular salivary glands: influence on growth rate and life span in mice. *Journal of Physiology and Biochemistry*, 2011, vol. 67, p. 225-231. PMid:21191684. http://dx.doi.org/10.1007/s13105-010-0067-x

SHAUDIES, RP., GRIMES, J., DAVIS, D., RAO, RK. and KOLDOVSKÝ, O. EGF content in the gastrointestinal tract of rats: effect of age and fasting/feeding. *American Journal of Physiology*, 1989, vol. 256, p. G856-861. PMid:2785766.

TEPPERMAN, BL. and SOPER, BD. Effect of sialoadenectomy on gastric mucosal integrity and growth in the rat-A time-course study. *Digestive Diseases and Sciences*, 1990, vol. 35, p. 943-949. PMid:2166652. http://dx.doi.org/10.1007/BF01537241

YANG , J., TYLER, LW., DONOFF, RB., SONG, B., TORIO, AJ., GALLAGHER, GT., TSUJI, T., ELOVIC, A., McBRIDE, J., YUNG, CM., GALLI, SJ., WELLER, PF. and WONG, DT. Salivary EGF regulates eosinophil-derived TGF-alpha expression in hamster oral wounds. *American Journal of Physiology*, 1996, vol. 270, p. G191-202. PMid:8772518.

Received November 7, 2012
Accepted February 17, 2013