Somatostatin concentration in gastric and colonic mucosa of normal and streptozotocin-diabetic pregnant rats

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Radioinmunoassayable somatostatin content was measured in stomach antrum and fundus, and in colon mucosa from virgin and 20-day pregnant rats made diabetic with streptozotocin treatment before gestation, and which either did or did not receive daily insulin substitution therapy. They were compared with normal untreated rats. Somatostatin content in both antrum and fundus was lower in normal pregnant animals than in virgin animals. Diabetes produced an increase in somatostatin content, and insulin therapy caused a reduction in antrum, fundus and colon somatostatin content in the virgin animals, only in the fundus and colon in pregnant rats. It is proposed that these findings may be related to changes in gastric acid secretion and digestive cell proliferation known to occur during diabetes and pregnancy although their precise physiological significance remains to be established.

Key words: Somatostatin. Insulin. Pregnancy. Streptozotocin. Diabetes. Rat. Stomach. Colon.

CONCENTRACIÓN DE SOMATOSTATINA EN LA MUCOSA DEL ESTÓMAGO Y DEL COLON EN RATAS NORMALES Y RATAS PREÑADAS DIABÉTICAS POR ESTREPTOZOTOCINA

Se determinó el contenido de somatostatina por radioinmunoensayo en el antrum y el fundus del estómago y en la mucosa del colon de ratas vírgenes y preñadas de 20 días, que fueron hechas diabéticas mediante tratamiento con estreptozotocina antes de la gestación. Una parte de los animales fue tratada diariamente con terapia de sustitución con insulina. En las ratas preñadas normales, el contenido de somatostatina en antrum y fundus apareció disminuido con relación al de las vírgenes. En las ratas vírgenes, la diabetes produjo un incremento en el contenido de somatostatina y el tratamiento con insulina originó una reducción de este parámetro en antrum, fundus y colon, mientras que en las preñadas se observó el efecto de la diabetes sólo en fundus y colon y la insulina produjo un efecto inferior que en las vírgenes. Se sugiere que estos resultados pueden relacionarse con los cambios en la secreción ácida gástrica y en la proliferación de las células del tracto digestivo que tiene lugar con la diabetes y la gestación, aunque queda por determinarse su papel funcional.

Palabras clave: Somatostatina. Insulina. Gestación. Estreptozotocina. Diabetes. Rata Estómago. Colon.

Different authors have found that basal gastric hypersecretion in pregnant rats was associated to an increase in histamine synthesis and release in gastric mucosa as well as mucous cell hyperplasia and hypertrophia[1-5]. These changes have been related to vagal innervation and serum histamine level increases occurring at late gestation in the rat[2]. Gastrin increases the gastric acid secretion[6] whereas both gastric inhibitory polypeptide[7] and somatostatin[8,9] have inhibitory effects. It is known that pregnancy affects neither serum and antral gastrin[2] nor serum inhibitory polypeptide levels[10], but to our knowledge no study has been reported on the potential changes in the somatostatin gastrointestinal mucosa content. We therefore performed these determi-

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Recibido el 25-5-1989; aceptado para su publicación el 6-7-1989.

nations in both gastric and colonic mucosa in late pregnant rats. As somatostatin content is known to increase in the gastric and colonic mucosa of diabetic rats[11,12], the present work was extended to determine the effects of streptozotocin diabetes and insulin therapy on these parametes in both pregnant and virgin rats.

MATERIAL AND METHODS

Wistar female rats weighing 160-180 g were made diabetic with a single i.v. injection of streptozotocin (45 mg/kg body weight) freshly dissolved in 50 mM citrate buffer pH 4.5, after 24 hours of fasting. In the streptozotocin rats glucosuria was checked (Dextrostise, Ames) one day after the treatment, and those with a negative response were discadted. These animals were treated with a daily s.c. injection of 1.5 IU of porcine insulin (Insulin Novo Ultralente, from Novo Industri A/S, Denmark)/100 g body weight for 8 days at which time half of them were mated with untreated normal males and the others were kept virgin. Rats receiving just the citrate buffer i.v. and handled in the same manner as above were always run in parallel, and were considered Controls. At day 0 of gestation (timed by the appearance of spermatozoids in vaginal smears) half of the streptozotocin treated rats were kept under the daily insulin treatment whereas this treatment was suppressed in the other half. At day 20 of gestation all the animals were decapitated without anesthesia.

Blood samples

Plasma was separated from blood samples collected from the neck wound into heparinized receptacles, and some aliquots were deproteinized[13] for glucose analysis[14] whereas other aliquots were frozen and kept at -20 °C until RIA-insulin determination. This was done using a radioimmunoassay kit specific for rat[15] generously provided by Novo Industri A/S (Copenhagen, Denmark).

Tissue extraction

Immediatly after sacrifice the stomach and colon were removed and washed with 0.9 % NaCl. The stomach was dissected into the cardia, fundus and antrum. The mucosa was then dissected from the underlying muscle layer and immediately boiled for 5 min in 1 M acetic acid to inactivate the proteolytic enzymes before being homogenized for 2 min with a motor-driven teflon pestle. The homogenate was centrifuged at 3000 rpm for 30 min at 4 °C and the resultant supernatant was stored at −70 °C until assay. Just prior to assay extracts were neutralized with 1 N NaOH, and whereas one aliquot was used for protein determination[16] other aliquots were subjected to appropriate dilution in 0.01 M phosphate buffer, pH 7.4, containing 0.15 M NaCl, 0.05 M EDTA, 0.1 % (w/vol) bovine serum albumin and 100 Kalikrein Inhibitor Units (KIU) of aprotinin (Trasylol)/ml, for RIA-somatostatin assay.

Radioimmunoassay of somatostatin

Tyr^{11}-somatostatin was radioiodinated by a chloramine-T method[17], obtaining a specific tracer of arounf 350 Ci/g. Rabbit antibody against somatostatin-14 was purchased from The Radiochemical Center (Amersham, U.K.) and somatostatin was determined by radioimmunoassay[18]. Separation of bound and free hormone was accomplished with dextran-coated charcoal. The observed limit of sensitivity for the assay was 10 pg/ml, dilution curves for tissue extracts were parallel to the standard curve, and percentages of intra- and inter-assay variations were 5.2 and 8.1, respectively. Recovery of somatostatin added to different tissue samples before homogenization was always between 85-87 %.

Statistical analysis

All values are given as mean ± SEM. Statistical comparisons were done by the Student's «t» test for unpaired samples.

RESULTS

As shown in table 1, 20 day pregnant rats showed lower plasma glucose and higher RIA-insulin levels than virgin animals. Treatment with streptozotocin 8 days before the onset of gestation (and comparaible time in the case of virgins) produced a significant increase in plasma glucose and a decrease in plasma RIA-insulin in both pregnant and virgin animals with no difference between these two groups, whereas daily treatment with 1.5 IU of porcine insulin/100 g body weight/day in the streptozotocin treated animals reverted both plasma glucose and RIA-insulin levels to values that did not differ from those of their respective virgin or pregnant non-diabetic controls (table 1).

As shown in figure 1, somatostatin content in both antrum and fundus was significantly lower in pregnant than in virgin normal control animals. In the antrum of virgin rats but not of pregnant rats and in the fundus of both virgin and pregnant animals, streptozotocin diabetes produced significant increases in the somatostatin content as compared to their respective normal control

TABLE 1. Effect of streptozotocin diabetes and insulin therapy on body weight and plasma glucose and RIA-insulin levels in the 20-day pregnant rat

	Conceptus-free body weight (g)	Plasma glucose (mg/dl)	Plasma RIA-insulin (μU/ml)
Normal virgin	228 ± 2	139 ± 3	64 ± 11
Normal pregnant	251 ± 4	92 ± 5	96 ± 8
p	< 0.001	< 0.001	< 0.05
STZ virgin	201 ± 8**	544 ± 34***	16 ± 5***
STZ pregnant	196 ± 10***	451 ± 26***	24 ± 2**
p	NS	NS	NS
STZ + ins virgin	230 ± 6	199 ± 32	59 ± 17
STZ + ins pregnant	251 ± 3	75 ± 25	209 ± 57
p	< 0.05	< 0.05	< 0.05

p: pregnant *vs* virgin; STZ = streptozotocin treated rats; ins = insulin treated rats.
* Statistical comparison between STZ or STZ + ins *vs*. normal rats.
** $p < 0.01$. *** $p < 0.001$. Means ± SEM of 6 rats/group.

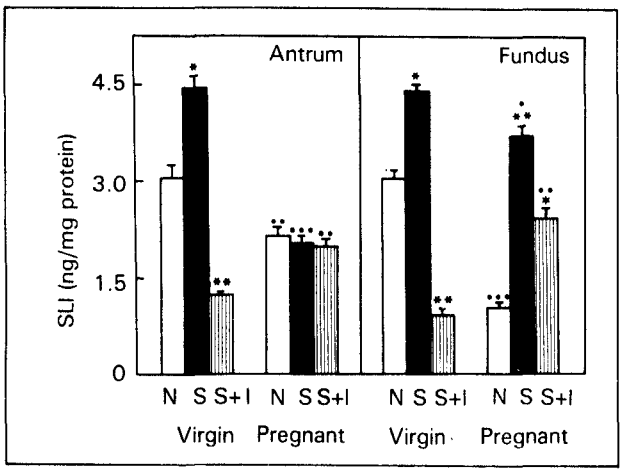

*Fig. 1. RIA-somatostatin (SLI) content in antrum and fundus mucosa of normal (N), streptozotocin-treated (S) or streptozotocin-and insulin-treated (S+I) virgin and 20-day pregnant rats. Means ± SEM of 6 rats/group. Significance of the difference between S or S+I group and N is shown by: * $p < 0.01$, ** $p < 0.001$, and that between pregnant and virgin animals by: ● $p < 0.05$; ●● $p < 0.01$; ●●● $p < 0.001$.*

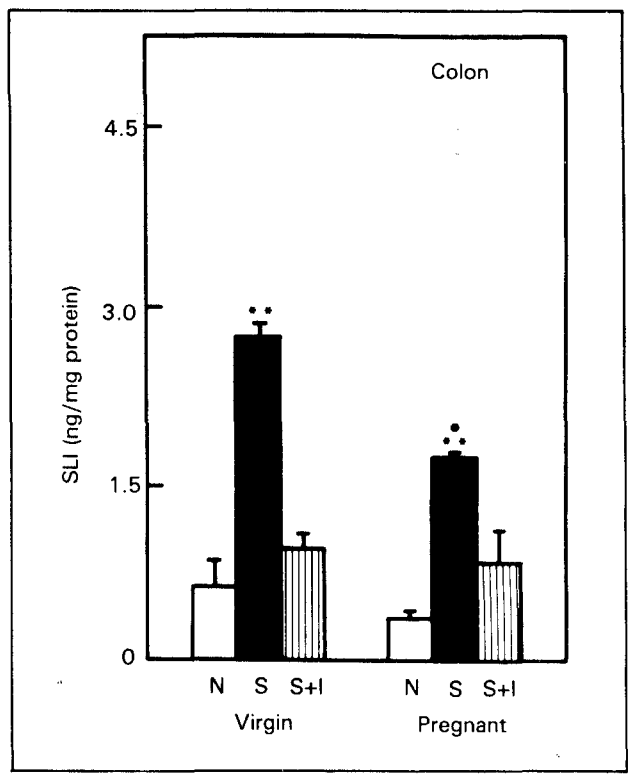

Fig. 2. RIA-somatostatin content in colon mucosa of normal (N), streptozotocin-treated (S) or streptozotocin- and insulin-treated (S+I) virgin and pregnant rats. Other specifications as indicated in figure 1.

groups (fig.1). Insulin treatment caused an intense reduction in the somatostatin content of both antrum and fundus in streptozotocin treated virgin rats and this effect was smaller, but still statistically significant, in streptozotocin treated pregnant animals (fig. 1). No change was found with any of these treatments in the antrum of the pregnant rats (fig. 1). As shown in figure 2, somatostatin content in the colon did not differ between normal pregnant and virgin rats. Streptozotocin diabetes caused an increase in colon somatostatin content in both groups, although the effect was smaller in pregnant than in virgin rats, and insulin treatment in streptozotocin-treated animals reverted this parameter to normal levels in both groups (fig. 2).

DISCUSSION

Present results show that somatostatin content in gastric (fundic and antral) mucosa decreases at late pregnancy in the rat whereas no change was found in the colonic somatostatin content. They also show that streptozotocin diabetes increases somatostatin content in the mucosa of these tissues in the virgin rats, and the effect is completely reverted with insulin therapy, whereas no change was found in the antrum of the streptozotocin pregnant rats. Insulin had a smaller effect on these and the other tissues studied in the streptozotocin pregnant rats than in the virgin ones.

The mechanism responsible for the changes of the gastrointestinal mucosa somatostatin content under different condition remains to be established, but some indirect derivations may help to explain the observed findings. It is known that insulin exerts a suppresive effect on somatostatin containing cells[19,20] whereas glucose has a stimulatory effect[21]. Hyperinsulinemia and reductions in plasma glucose found here in the normal pregnant rats are in agreement with previous reports[22,23] and could therefore be responsible for their reduction in gastric (fundus and antrum) mucosa somatostatin content. The effect of streptozotocin-diabetes enhancing the somatostatin content in gastric mucosa found here in both virgin and pregnant rats confirms reported results in both streptozotocin-treated rats[11,24] and human juvenile diabetes[25] that show increases in somatostatin content in stomach and pancreas. These findings may also be related to the enhanced gastric somatostatin release found in streptozotocin-treated rats[11], suggesting hyperfunction in the gastric D-cells with this condition. Since somatostatin is a potent inhibitor of gastrin release[26], and serum gastrin levels are decreased in streptozotocin diabetes[27], we may also propose that this change may be a consequence of the enhanced gastric mucosa somatostatin content found here.

The role of the observed decrease in somatostatin content in gastric mucosa in late pregnant rats is not clear. Since somatostatin is known to suppress both gastric acid secretion[8,9] and digestive mucosa cell proliferation[28], it may be proposed that the decrease is responsible for both the gastric acid hypersecretion[1,2] and the gastric mucosa hyperplasia[4] present at late pregnancy. Although in an indirect manner, this explanation also agress with the enhanced triglyceride intestinal absorption recently described in the rat during late gestation[29] indicating an active digestive activity.

In conclusion, although more studies are required to understand their physiological significance the present finding show that in both diabetes and pregnancy there are alterations in somatostatin gastric mucosa content which may be responsible for some of the functional changes known to occur in the digestive tract in these conditions.

ACKNOWLEDGEMENTS

The present study was carried out in part with a grant from the Dirección General de Investigación Científica y Técnica (n.º PM88-0050). The authors thank Carol F. Warren, from ICE of Alcalá de Henares University, for her editorial help.

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