The acidic glycosaminoglycans in gastric mucosa of athyroid rats: The effects of L-tri-iodothyronine[1]

J.A. Kofoed[2] and Ana C. Barceló

Cátedra de Fisiología, Facultad de Odontología, Universidad de Buenos Aires, M.T. de Alvear 2142, Buenos Aires (Argentina), 14 November 1977

Summary. Hyaluronic acid is increased about 12%, whereas dermatan sulfate is decreased about 34%, in gastric mucosa of athyroid rats when compared with controls. Administration of 2 doses of L-tri-iodothyronine reversed the effects of thyroidectomy.

Since the work of Warson and Pearce[3] it has been known that subnormal thyroid activity affects the glycosaminoglycans of skin. It has been reported also that an increase in hyaluronic acid and a decreased dermatan sulfate content of the skin and gingiva[4,5] occurs. These results seem to be independent of thyrotrophin, because the same changes are noted after hypophysectomy as in hypothyroidism[4]. We now present data on the glycosaminoglycans content in gastric mucosa of normal, athyroid and athyroid-treated rats with tri-iodothyronine ($L\text{-}T_3$) using cellulose microcolumn techniques for separation of glycosaminoglycans in order to get further insight into the relationship between the thyroid gland and the skin and mucosas.

Material and methods. 32 Wistar male rats weighing between 210 and 215 g were used throughout these experiments. The animals were maintained on a standard laboratory diet (Forramwz Lab., Argentina) in pellet form with water ad libitum and kept in a constant temperature room. The animals were divided into 4 groups; thyroid destruction was accomplished in 3 of them by administration of 700 µCi of I^{131}. They were used after 3 months of administration. Group 1 acted as untreated normal controls; group 2 was considered as athyroid rats and groups 3 and 4 were athyroid rats injected daily with 0.1 and 1 µg of $L\text{-}T_3$ (Sigma Chemical Co., USA) for 20 days. Completeness of thyroid destruction was checked at autopsy on each animal.

Gastric mucosa was obtained by deep mucosal scrapings of all the inner surface of the stomach. Histological controls were made in some animals in order to ensure reproducibility of samples. In all cases scrappings were made excluding the muscularis mucosae, and their wet weight varied between 890 and 1125 mg. Samples were then defatted and dehydrated for 36 h in ether-acetone (1:1 v/v) and then dried at 60 °C for 5 h until constant weight. 50 mg samples of dry defatted tissue were suspended in 2 ml of phosphate buffer pH 7.2 and heated at 100 °C for 5 min. 15 mg of papain (Sigma Chemical Co. USA) were activated in 20 ml of the same buffer with the addition of 0.005 M EDTA and 0.005 M cysteine HCl at 58 °C for 30 min. The activated papain was then added to the cooled samples (1 ml/sample) and the mixture was incubated for 24 h at 58 °C after 12 h another aliquot of the enzyme was added. Following digestion, 10% TCA (trichloroacetic acid) was added to reach a final concentration of 5% to precipitate any residual protein.

The precipitate was then washed with another volume of 10% TCA; 3 vol. of 5% potassium acetate in ethanol were then added to the combined TCA solutions. After standing for 3 h at 3 °C, the precipitated crude total glycosaminoglycans were centrifuged off and dissolved in 1 ml of 3.5% saline solution. Uronic acids were determined on an aliquot of the above by the carbazole method of Bitter and Muir[6] using Na glucuronate as standard. All the determinations were made in duplicate. The crude glycosaminoglycans were then fractionated on cellulose microcolumns by the technique of Svejcar and Robertson[7]. Uronic acid concentration was also determined in each glycosaminoglycan fraction. Recoveries of 50–1000 µg of chondroitin-4-sulfate alone, or when added to a given sample of dry gastric mucosa, varied between 84 and 95%.

Results and discussion. The table demonstrates the concentration of all glycosaminoglycan fractions in gastric mucosa of normal, athyroid and athyroid-treated rats, expressed as µg of uronic acids/g dry defatted mucosa. It is shown that the concentration of hyaluronic acid was significantly increased (about 12% $p < 0.01$) and that of dermatan sulfate was significantly decreased (about 34% $p < 0.001$) in the athyroid rats when compared with controls, whereas the other fractions did not show any significant differences. From similar data obtained on skin of rats[9], it should be noted that gastric mucosa has about 36% less of total uronic acids. This is due mainly to the absence of chondroitin-6-sulfate and decreased amounts of hyaluronic acid and dermatan sulfate. The administration of both doses of $L\text{-}T_3$ reversed the effects of thyroidectomy. The findings of increased hyaluronic acid and decreased dermatan sulfate with no significant differences among the other sulfated fractions in gastric mucosa are similar to those found by us in cartilage and skin of rats[8,9]. Apparently, thyroid hormone deficiency affects these tissues in a similar way. Treatment of thyroidectomized rats with the same doses of $L\text{-}T_3$, increased heat production by 30% in excess of normal, whereas administration of $D\text{-}T_3$ produced a lesser increase which was maintained throughout the injection period and then returned to the thyroidectomized level[10]. Wheter $L\text{-}T_3$ exerts a direct effect on cartilage, skin and gastric mucosa glycosaminoglycans, or whether the results

Effects of thyroidectomy and $L\text{-}T_3$ on glycosaminoglycans concentration in gastric mucosa of rats

	Normal rats	Athyroid rats	Athyroid 0.1 µg $L\text{-}T_3$	Athyroid 1 µg $L\text{-}T_3$
Total uronic acids	705.3	698.6	689.9	697.4
Hyaluronic acid	396.4 ± 15.8	448.7 ± 17.3	419.7 ± 13.6	391.4 ± 8.9
Chondroitin-4-sulfate	86.3 ± 3.4	83.2 ± 4.7	87.9 ± 5.6	82.9 ± 4.3
Chondroitin-6-sulfate	-	Traces	-	-
Heparitin sulfate	68.3 ± 5.3	63.7 ± 4.9	67.1 ± 2.9	69.7 ± 3.5
Dermatan sulfate	128.6 ± 9.0	84.7 ± 7.8	98.9 ± 5.3	134.6 ± 4.6
Heparin	16.3 ± 2.9	18.4 ± 3.1	15.7 ± 2.7	16.6 ± 2.8

Concentration of glycosaminoglycans expressed as µg total uronic acids/g dry tissue. Results are expressed as mean ± SE. Hyaluronic acid: Normal vs athyroid $p < 0.01$. Dermatan sulfate: Normal vs athyroid $p < 0.001$.

noted are secondary to the caloric effect of the hormone, remains as an open question.

1 This work was partially supported by the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.
2 Member of the Research Career Investigator of CONICET, Argentina.
3 E.M. Warson and R.D. Pearse, Am. J. clin. Path. *19*, 442 (1949).
4 S. Schiller and A. Dorfman, Biochim. biophys. Acta *58*, 27 (1962).
5 J.A. Kofoed and C.E. Bozzini, Acta physiol. latioam. *17*, 323 (1967).
6 T. Bitter and H.M. Miur, Analyt. Biochem. *4*, 330 (1962).
7 J. Svejcar and W. van B. Robertson, Analyt. Biochem. *18*, 333 (1967).
8 J.A. Kofoed and C.E. Bozzini, J. Endocr. *45*, 609 (1969).
9 J.A. Kofoed, Experientia *27*, 702 (1971).
10 C.E. Bozzini, H.F. Niotti and M.E. Barrio Rendo, Acta physiol. latinoam. *26*, 423 (1969).

Plasma motilin levels in duodenal ulcer and effect of a truncal vagotomy and hypoglycaemia

N.D. Christofides and S.R. Bloom[1]

Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 OHS (England), 3 November 1977

Summary. The identical response of plasma motilin levels, in duodenal ulcer patients and healthy controls, to a test meal and insulin induced hypoglycaemia, fail to demonstrate any significant abnormalities in motilin release. The close correlation between blood glucose and motilin suggest a possible role of this new hormone in carbohydrate metabolism.

The mechanisms that control the levels of plasma motilin have not been elucidated. Recently it has been demonstrated in man that instillation of acid into the duodenum results in a sharp rise of plasma motilin[2]. Infusions of exogenous motilin at concentrations within the physiological range have been reported to produce a significant delay in gastric emptying in heathly volunteers[3]. The enhanced rate of gastric emptying and increased gastric acid production in patients with duodenal ulcer led us to study the effects of a test meal and also of insulin hypoglycaemia on plasma motilin in such subjects.

Patients and methods. A previously described sensitive radioimmunoassay specific for motilin was used[4] which was able to detect changes in plasma motilin of 3 pmoles/l with 95% confidence. Antibodies were raised to pure porcine motilin and used at a final dilution of 1 in 240,000 bound approximately 50% of the added I^{125} motilin. No cross reaction was observed on addition of 1 µg/tube of the other available intestinal hormones or with 100 ng/tube of the synthetic fragments of motilin (1-6, 7-22, 12-22). Gel chromatography of human gut extracts showed only a single major peak of motilin immunoreactivity eluting in the identical position to pure porcine motilin[4]. Blood samples were taken with 10 units of heparin, rapidly separated and the plasma frozen within 15 min of collection. Plasma motilin immunoreactivity was measured after an overnight fast in controls and patients in response to 2 types of stimuli. Blood glucose was measured by glucose oxidase[5] method.

1. Test meal: A standard breakfast of eggs and toast composed of 66 g of carbohydrate, 18 g of protein and 22 g of fat was given after an overnight fast to 16 healthy controls aged 27-64 (mean 41) and 10 patients with an active duodenal ulcer proven by endoscopy or barium meal aged 29-63 (mean 39).

2. Insulin induced hypoglycaemia: A 2nd group of 6 controls, 16 duodenal ulcer patients and 8 patients following complete truncal vagotomy (Holanda -ve) received an i.v. injection of insulin (0.2 units/kg). Prior to the test a Ryles tube was inserted and gastric juice was drained throughout and discarded.

Calculations: Motilin concentrations are expressed both in pmoles/l and, in order to overcome the considerable variation in basal levels from individual, as percentage increments. Differences were examined parametrically (e.g. t-test) for percentage change and nonparametrically (e.g. Wilcoxon sum of ranks tests) for absolute values.

Results. Figure 1 shows the basal plasma motilin in the 3 groups. A considerable individual variation is seen. The median concentration in the controls is 33 pmoles/l (range 10-110), all duodenal ulcer patients 42 pmoles/l (range 8-258) and patients following complete truncal vagotomy 48 pmoles/l (range 15-316). The differences are not statistically significant.

Figure 2 shows the effect of a standard meal on plasma motilin levels in the group of 16 control subjects and 10 duodenal ulcer patients. In the control group a 15 pmoles/l (range 3-50) incremental rise of plasma motilin was observed 15 min following ingestion of the meal, representing a mean individual percentage increment of $32.5 + 12\%$ ($p < 0.02$). An identical rise was also observed in the duodenal ulcer group. In controls motilin levels then fell below basal reaching statistical significance at 90 and 135 min ($p < 0.02$), whereas in DU patients they only fell slightly after the initial peak and stayed thereafter at approximately fasting values. At no point during the experiment did the motilin concentrations in the DU patients differ significantly from the controls.

Following insulin-induced hypoglycaemia motilin levels fell sharply (figure 3) reaching a nadir at 35 min (a fall of $58 \pm 8\%$ in controls ($p < 0.005$) and $42 \pm 3\%$ in DU ($p < 0.001$), which coincided with the lowest blood glucose

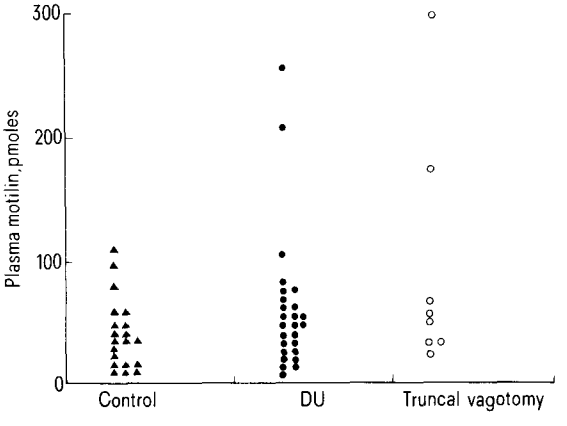

Fig. 1. Basal plasma motilin in 26 active duodenal ulcer patients, 8 post-vagotomy patients and 20 normal volunteers.