Tissue and Serum Somatostatin-like Immunoreactivity in Fed, 15-h-fasted, and 72-h-fasted Rats

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SUMMARY

Somatostatin-like immunoreactivity (SLI) was measured in extracts of gastric antrum, colon, pancreas, and central nervous system, as well as in unextracted portal and inferior vena caval serum from fed, 15-h-fasted, and 72-h-fasted rats. No differences were found in SLI in the central nervous system of the three groups. However, striking variations were found in the gastrointestinal tract and pancreas; the antrum, colon, and pancreas of 15-h-fasted rats contained the least SLI, the content being significantly elevated in these three areas after feeding and after a 72-h fast. Portal serum levels were highest after feeding but lowest in 72-h-fasted rats, in spite of high intestinal and pancreatic SLI content in both. These tissue and serum differences suggest a physiologic role for SLI in nutrient homeostasis not only at tissue level, but also putatively as a hormone in the portal system.

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Somatostatin, although originally isolated as a hypothalamic inhibitory hormone for growth hormone, has since been shown as having a widespread tissue localization, especially in the intestine and pancreas.[1–3] Pharmacologic studies have shown that somatostatin has numerous actions (mainly inhibitory) on intestinal[4–6] and pancreatic hormone secretion,[7,8] intestinal[4] and pancreatic exocrine secretion,[9] intestinal motility,[6] and absorption.[10] If somatostatin has a physiologic role in the regulation of intestinal or pancreatic endocrine or exocrine function, then changes in tissue or blood levels may occur in various nutritional states. In vitro evidence for such a role is the release of somatostatin-like immunoreactivity (SLI) from isolated islets in response to glucose[11] and the release of insulin[12] and glucagon[13] from pancreatic islets incubated with anti-somatostatin antiserum. An in vivo increase in portal vein SLI after oral glucose load has also been recently demonstrated.[14,15] We report here our investigation into the changes found in the tissue distribution and serum levels of SLI in fed, 15-h-, and 72-h-fasted rats.

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MATERIALS AND METHODS

Male Long-Evans rats weighing 240–270 g, housed under constant conditions, were used. All animals were fed a standard rat cube diet (Epol Feeds, Cape Town) containing 20% protein, 70% carbohydrate, 2.5% fat, 6% fiber, and adequate vitamins and minerals, and had free access to tap water. Fed animals had free access to food up to the time of sacrifice while the two other groups of animals had food withdrawn 15 and 72 h before sacrifice respectively. Animals were weighed before and after the period of food withdrawal.

All animals were killed under light ether anesthesia after simultaneous blood samples had been rapidly taken from the portal vein and inferior vena cava into Trasylol (500 KIU/ml), placed on ice, and then the serum was separated by centrifugation at 4°C. The pancreas, gastric antrum, and distal colon were dissected free of omental fat and removed. The brain was rapidly excised, transferred to an ice tray, and dissected into regions (septum and preoptic area, striatum, thalamus, cortex, and hypothalamus) as previously described by Brownstein et al.[1] The cervical and thoracic spinal cord were dissected out. All tissues were placed in capped vials, snap frozen in liquid nitrogen, and stored in a container of dry ice; after this they were wiped free of ice and weighed. The frozen tissues were then thoroughly homogenized in 2 N acetic acid, boiled, sampled for protein estimation, centrifuged, and the supernatant lyophilized before assay for somatostatin.

Tissue extracts diluted in assay buffer and serum samples were assayed for immunoreactive somatostatin content by a well validated radioimmunoassay[2] and the serum immunoreactivity carefully characterized as described elsewhere.[16] Protein was measured on sodium deoxycholate solubilized tissue homogenates by the method of Lowry et al.[17]

TABLE 1
Tissue somatostatin-like immunoreactivity (SLI) in 15-h-fasted, 72-h-fasted, and fed rats

	ng SLI/g wet wt		
	Fed	15-h fasted	72-h fasted
Septum and preoptic	528.4 ± 73.7 (10)	551.2 ± 77.6 (12)	955.2 ± 251.1 (5)
Striatum	379.0 ± 48.5 (10)	379.0 ± 57.3 (10)	318.2 ± 87.9 (5)
Hypothalamus	1068.5 ± 162.4 (10)	1502.8 ± 271.6 (16)	2039.2 ± 872.3 (5)
Thalamus	382.7 ± 49.2 (10)	360.6 ± 57.2 (12)	549.6 ± 141.9 (5)
Cortex	509.6 ± 96.4 (10)	306.8 ± 61.7 (12)	531.0 ± 229.7 (5)
Spinal cord	538.0 ± 103.4 (10)	642.3 ± 72.2 (12)	767.6 ± 194.1 (5)
Pancreas	188.1 ± 26.1 (10)	70.6 ± 9.3 (11)*,††	422.0 ± 75.8 (5)
Antrum	676.8 ± 120.3 (15)	166.7 ± 29.0 (12)†,††	462.6 ± 142.5 (5)
Colon	106.7 ± 9.9 (9)	55.2 ± 6.0 (11)*,††	171.0 ± 15.5 (5)

Results are expressed as mean ± SEM, with numbers in parentheses. In pancreas, antrum, and colon, levels in 15-h-fasted rats are lower than in 72-h-fasted rats (*,†) and fed rats (††). In pancreas, levels in fed rats are lower than in 72-h-starved rats ($P = 0.013$).

* $P < 0.001$.

† $P = 0.064$.

†† $P < 0.001$.

The three groups were compared with each other using Student's *t* test for unpaired groups.

RESULTS

Fasting for 72 h resulted in a loss of body wt of 46.2 ± 3.8 g (mean ± SEM, N = 5), which represented 18.8 ± 1.8% of original body wt. An overnight fast of 15 h resulted in a loss of original body wt of only 4.3 ± 1.7% (mean ± SEM, N = 12). It was noted that fed animals had food in their stomachs and lost no weight.

The tissue distribution of SLI is represented in Table 1. Tissue SLI was higher in the pancreas, antrum, and colon of the fed and 72-h fasted animals compared with the 15-h fasted group; pancreatic SLI in 72 h fasted animals was also significantly higher than in fed animals. Recoveries of added somatostatin to tissue samples from all areas before homogenization were comparable (55–65%) in all three nutritional states. There was no significant difference in the central nervous system SLI content among any of the three groups.

Serum SLI of portal venous and inferior vena caval blood behaved identically with synthetic cyclic somatostatin on serial dilution, and showed parallelism with the standard curve and a similar elution profile on Sephadex G25(f) chromatography, as has been described for somatostatin in human and rat serum.[15,16] More than 90% of synthetic somatostatin added to rat serum was recovered; incubation damage to the assay tracer was no greater in rat serum than in assay buffer as assessed by paper chromatoelectrophoresis.

TABLE 2
Portal and inferior vena caval (IVC) somatostatin-like immunoreactivity (SLI) in fed, 15-h-fasted, and 72-h-fasted rats

	SLI ng/ml	
	Portal vein	IVC
Fed	0.840 ± 0.100 (16)	0.130 ± 0.020 (11)
15-h fasted	0.579 ± 0.048 (18)	0.130 ± 0.021 (11)
72-h fasted	0.301 ± 0.082 (10)	0.055 ± 0.007 (10)

Results are expressed as mean ± SEM, with numbers in parentheses. Portal levels are higher than those of IVC in all three nutritional states (in fed and 15-h fasted, $P < 0.001$, in 72-h fasted, $P < 0.008$). Portal levels in fed rats are higher than in 15-h fasted ($P = 0.025$) or 72-h-fasted ($P < 0.001$), while in 15-h-fasted animals SLI is also higher than in 72-h-fasted rats ($P = 0.007$). There is no significant difference in IVC levels between fed and 15-h-fasted animals, but values in 72-h-fasted rats are lower than in both other groups ($P = 0.001$).

In all three groups (Table 2) the SLI in portal venous serum was significantly greater than in inferior vena caval serum. The portal venous SLI was significantly higher in fed than in 15-h fasted animals while that from 72-h fasted animals was significantly lower than either. There was no significant difference in the inferior vena caval serum SLI in fed and in 15-h fasted animals but that of 72-h fasted animals was significantly lower than either.

DISCUSSION

We report alterations in SLI in the intestine and pancreas in different nutritional states. When comparing the fed and 15-h fasted animals, tissue SLI was found to be elevated in pancreas, colon, and most markedly in gastric antrum in the fed group. These changes may reflect a response to gastric distension or play a role in postabsorptive nutrient homeostasis, in that exogenous somatostatin reduces intestinal motility[6] and nutrient absorption,[10] gastrin and gastric acid secretion,[4] and pancreatic exocrine[9] and endocrine secretion.[7,8] There was no significant difference in central nervous system SLI under these nutritional conditions.

Fasting for 72 h also significantly elevated tissue SLI compared with that found in the 15-h fasted state. The reasons for this secondary rise are not clear, but the high levels may be linked to certain physiologic mechanisms operative in starvation, such as reduced intestinal motility and secretion, and hypoinsulinemia,[18] which are observed effects of somatostatin.

Although both feeding and 72-h fasting result in an increase in antral, colonic, and pancreatic SLI, in the case of feeding this is associated with high portal venous SLI while in 72-h fasting the level is very low. Since the tissue content of hormone reflects a balance among synthesis, storage, and release, the last reflected by the serum level, it is conceivable that in fed animals the high portal SLI may represent release into the portal vein with ongoing

synthesis maintaining a high tissue level, while after 72-h fasting accumulation of tissue stores in the absence of release might account for high tissue and low portal SLI. The failure of portal venous levels to reflect tissue somatostatin content in the different states points to the possibility of selective release into the portal system, not merely overflow. This raises the question of whether somatostatin could act as a hormone within the closed portal system. The evidence for this at present is of necessity indirect. Portal SLI levels in intact rats closely follow those of insulin after intragastric glucose while IVC SLI remains unaltered.[14,15] Furthermore, a direct inhibitory effect of somatostatin on glucagon-induced hepatic glucose output in the perfused rat liver has been shown,[19] providing evidence for a target effect for this peptide within the portal system. Finally, the antiserum used in the assay of SLI is directed against the biologically active core of somatostatin.[15] A hormonal role for somatostatin in a closed portal circulation is not without precedent; it is widely regarded as such in the hypophyseal-portal system. A role for portal SLI in nutrient homeostasis is also indirectly supported by increased release from pancreatic islets[11] and perfused pancreas[20] in response to glucose and other nutrients.

In all three nutritional groups the portal SLI levels are greater than those of the inferior vena cava, which is consistent with previously reported observations.[15] Only after 72 h fasting is the change in portal venous SLI reflected in the inferior vena caval levels, confirming the importance of regional blood sampling in the elucidation of the physiology of so widely distributed a substance as somatostatin.[14,15] The present study suggests that tissue as well as regional blood measurement may be required.

The nutritional components of the mixed diet responsible for the above findings and the role of intestinal distension is at present under investigation.

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