PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 179, 517–521 (1985)

Somatostatin Mediates the Effect of Growth Hormone Surges on Splanchnic Biogenic Amines (42132)

M. N. HUSSAIN, A. SIREK, E. CUKERMAN, AND O. V. SIREK

Department of Physiology, University of Toronto, Toronto, Ontario M5S 1A8, Canada

Abstract. Experiments were conducted in trained, conscious dogs fitted with an indwelling portal catheter. Radioenzymatic methods were employed for the quantitative measurement of plasma-free serotonin and catecholamines. An injection of ovine growth hormone (GH, 100 μg/kg) or an equimolar amount of somatostatin (somatotropin release inhibitory factor, SRIF, 7.5 μg/kg) into a saphenous vein led, within the first 15 min, to a transient but significant increase in plasma serotonin and a decrease in the concentrations of dopamine, norepinephrine, and epinephrine. The changes were frequently in excess of 40% of baseline values, and were found only in the portal and not in the peripheral circulation. When the animals were pretreated with an antiserum specifically directed against SRIF, GH surges no longer caused alterations in the portal levels of biogenic amines. Thus, the effects of spike concentrations of GH on plasma serotonin and catecholamines are apparently mediated by SRIF, a novel and unexpected function for a hormone that is known as an inhibitor of GH secretion.

It was shown in our laboratory that a spike concentration of growth hormone (GH), induced experimentally in dogs by a single intravenous injection, produced in the hepatic portal blood a rise in the concentration of plasma-free serotonin and a concomitant reduction in the plasma concentration of dopamine, norepinephrine, and epinephrine (1, 2, 3). These changes were measurable only in the portal (prehepatic) and not in the peripheral (posthepatic) circulation. The portal concentrations of insulin, glucagon, and somatostatin (somatotropin release inhibitory factor, SRIF) also became elevated and the turnover of glucose (Ra) was increased sevenfold (4). The changes occurred in the following sequence: glucagon peaked in 2 min, insulin and SRIF in 4 to 6 min, the Ra in 4 min, and serotonin in 10 to 12 min; the latter coincided with the nadir of the three catecholamines. Because of this time sequence we wondered if insulin, glucagon, or SRIF might participate in the GH effect on the release of splanchnic biogenic amines. We have undertaken a systematic investigation along these lines, and the present report concerns the involvement of SRIF in the GH effects. We found that if we administered SRIF instead of GH, not only were the levels of biogenic amines nearly identical with our GH data, but the administration of a specific SRIF antiserum abolished the response to GH, indicating that the GH effect was mediated by SRIF.

Materials and Methods. Six healthy mongrel dogs of either sex, weighing approximately 20 kg each, were used in this study. An indwelling catheter was placed into the portal vein under aseptic conditions under halothane anesthesia (Ayerst, Montreal) as described earlier (5). Experiments were conducted when the animals had fully recovered from surgery and the leukocyte count was normal. Hormone injections and withdrawal of blood samples for serotonin and catecholamine determinations were always done in fasting, unrestrained, and fully conscious dogs, which were conditioned to handling and withdrawal of blood samples.

Ovine GH (Lot No. NIH-GH-S9, a gift of the NIAMDD, Hormone and Pituitary Program, Baltimore, Md.) was dissolved in 1 ml alkaline saline, pH 9, and was administered iv in the dose of 100 μg/kg, a "superphysiologic" dose, which is the standard amount used in our laboratory (2, 4). Somatostatin (Lot No. 004635, Peninsula Laboratories Inc., Belmont, Calif.) was dissolved in 1 ml saline and administered iv in a dose of 7.5 μg/kg, which is equimolar to the "superphysiologic" dose of GH. Somatostatin antiserum (Lot No. R101, Laboratories for Molecular Endocrinology and Diabetes, Herbert Research Center, Los Angeles, Calif., courtesy of Dr.

0037-9727/85 $1.50

A. Arimura), in a dilution of 1/10,000, was administered iv in a dose of 0.5 cc/kg, 5 min before administration of either GH or SRIF. Based on a slightly excessive plasma volume of 1 liter, it was calculated that this amount of antiserum was sufficient to neutralize some 2.5 ng/ml, i.e., approximately 10 times the peak plasma concentration of SRIF. This was considered adequate, since the peak portal SRIF concentration after a standard dose of 100 μg GH is in the order of 226 pg/ml (4). The neutralizing potency of 1 ml undiluted antiserum equaled 2.5 mg SRIF. Controls were given 1 ml of saline in place of GH or SRIF.

Blood samples were withdrawn at stated intervals into plastic syringes, simultaneously from both the indwelling catheter and from a catheter inserted temporarily into a cephalic or saphenous vein, other than the one used for hormone and antiserum injections. Free serotonin in platelet-poor plasma was determined by modifications (2) of the original method of Hussain and Sole (6) and catecholamines by modifications (2) of the method of Sole and Hussain (7). Plasma glucose was determined as "true" sugar by the Beckman glucose analyzer (Beckman Instruments Inc., Fullerton, Calif.).

To compensate adequately for unavoidable differences in baselines, data for each graph were calculated as follows: absolute values in the legend correspond to 0 time, i.e., immediately prior to the injection of SRIF or GH. All subsequent numbers are percentage changes from the 0 time value that were calculated for each dog separately and are expressed as means ± SEM. Differences between hormone- and saline-injected controls were considered as statistically significant if P was less than 0.05.

Results. Figure 1 gives the results of our experiments with SRIF. It will be noted that a single injection of the hormone produced a statistically significant rise in the hepatic portal concentration of free serotonin and a concomitant statistically significant reduction in the hepatic portal concentrations of dopamine, norepinephrine, and epinephrine. The changes were transient, reaching their respective maxima or minima in about 12 min and returning to basal levels in about 30 min from the time of injection of the hormone.

The fact that the response to SRIF was practically identical to that obtained with GH (Fig. 2) led us to suspect that SRIF might be the mediator of the GH effect on biogenic amines. In order to test this possibility, we injected the same dogs with SRIF antiserum 5 min before the injection of GH. It can be seen in Fig. 2 that the customary response to GH was completely abolished by pretreating our animals with the SRIF-specific antiserum. In spite of the technical complexity of this animal preparation, which necessitated the use of a relatively small number of dogs, the total suppression of the GH effect was a consistent observation and, therefore, the data left little doubt that the effect of GH was mediated by SRIF. Administration of SRIF antiserum, either alone or in combination with SRIF to the same animals, produced data that were indistinguishable from saline-injected controls (data not shown). It is our experience that the difference in baselines can, at times, be quite striking. The particularly low basal levels seen in the set of experiments with SRIF antiserum (Fig. 2) are coincidental and are not related to the administration of the antiserum. Values obtained prior to antiserum were virtually identical to those obtained at 0 time, i.e., at the time of GH injection.

Biogenic amine levels in the peripheral (posthepatic) circulation were not appreciably changed. Similarly, plasma glucose levels in both portal and peripheral blood samples were not significantly altered; presentation of data is therefore omitted.

Discussion. The results of our present experiments are unexpected and we have little theoretical reasoning to offer in trying to explain why SRIF would mediate the GH effect on the monoamine-producing structures of the gut. The curious reciprocal relationship between serotonin and catecholamines has been reported to exist in the brain (8), in the heart (9), and we have also found it in the gut (3). Moreover, unpublished experiments from this laboratory have shown that when L-tryptophan, a specific serotonin precursor, was injected under similar conditions to those of GH or SRIF experiments, the expected rise of the hepatic portal serotonin concentration was accompanied by a reduction in portal catecholamine levels.

In the dog, the adrenal medulla does not

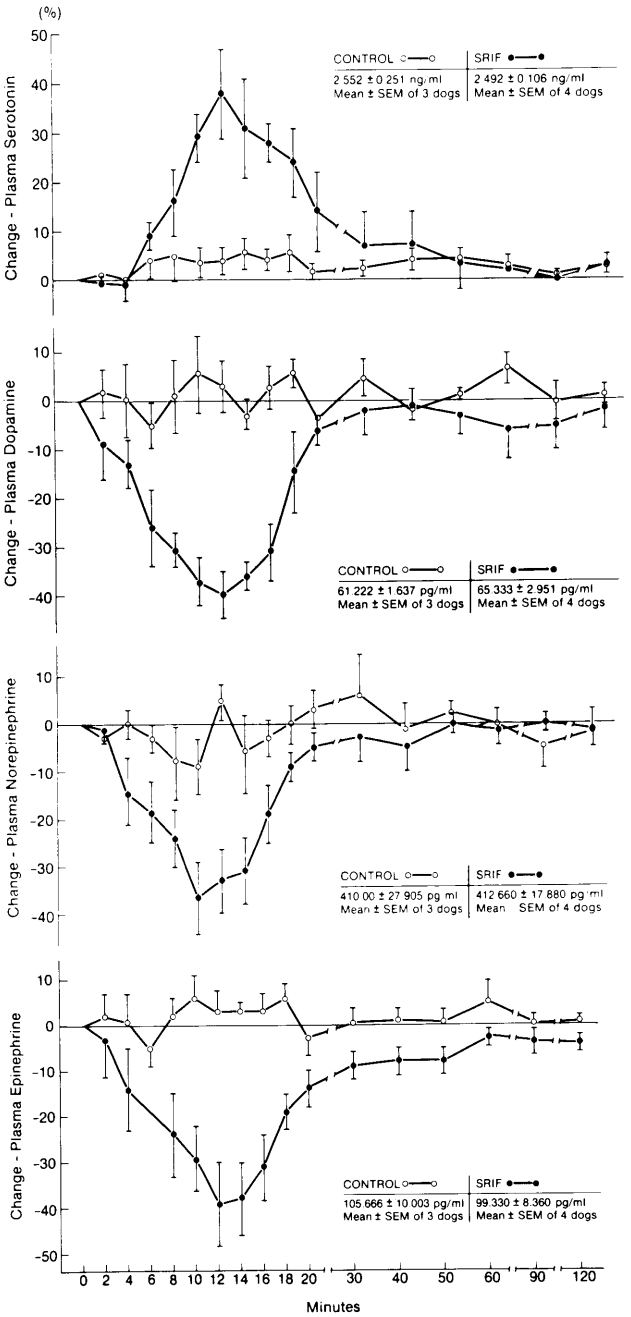

FIG. 1. Hepatic portal plasma-free serotonin, dopamine, norepinephrine, and epinephrine concentrations following a single iv injection of somatostatin (SRIF) at 0 time. Differences from saline injected controls were statistically significant between 10 and 16 min for serotonin, between 8 and 16 min for dopamine, between 10 and 14 min for norepinephrine, and between 8 and 18 min for epinephrine.

deliver its secretions into the splanchnic area (10), and in cats, the enterochromaffin cells of the gut seem to release serotonin into the intestinal lumen and not into the splanchnic circulation (11). Thus, serotonergic and adrenergic gut neurons would appear to be the major source of monoamines in the portal circulation. At least three possibilities may

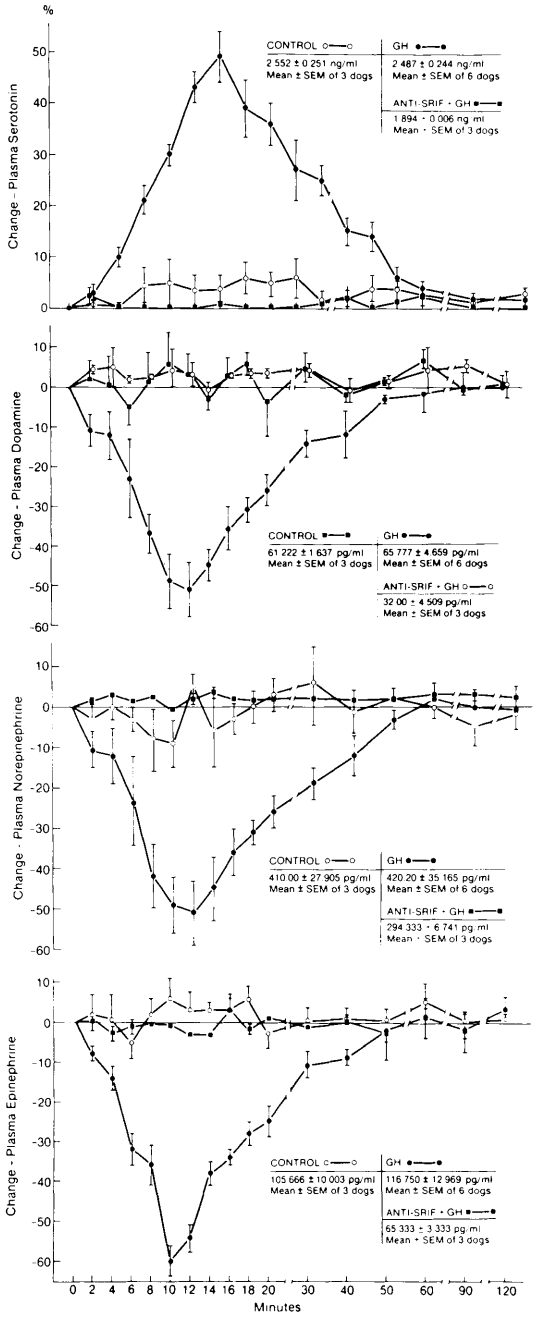

FIG. 2. Hepatic portal plasma-free serotonin, dopamine, norepinephrine, and epinephrine concentrations following a single iv injection of growth hormone (GH) at 0 time, either given alone or preceded by SRIF antiserum. Note that the antiserum caused a complete suppression of response. In comparison to saline controls, the changes in the concentration of biogenic amines after GH alone were statistically significant between 8 and 30 min for serotonin, between 8 and 18 min for dopamine, between 8 and 20 min for norepinephrine, and between 6 and 20 min for epinephrine.

be considered as hypothetically reasonable mechanisms of hormonal and neuronal interaction in this area.

First, the secretion of serotonin may be stimulated by the injected hormone, resulting in elevated plasma levels. The fall of catecholamine levels would then be the consequence of a reciprocal response. Since a similar effect is produced by both GH and SRIF and is abolished by a specific SRIF antiserum, we questioned whether SRIF, a known inhibitory hormone (12, 13) could, under certain conditions or in certain tissues, have other than inhibitory effects. A literature search revealed that a stimulatory effect of SRIF has been reported in rat mast cells, where it leads to an enhanced release of histamine (14–18) and serotonin (19, 20). The process seems to be calcium and energy dependent. A similar effect on histamine release has also been shown in human leukocytes (21). However, it remains to be determined how generalized this stimulatory effect of SRIF is and whether it could apply to serotonin-producing cells of the gut of the dog.

A second possibility is the reverse sequence of events. SRIF could suppress the release of catecholamines, leading to a fall in their portal plasma levels. A rise in serotonin would then take place as a reciprocal response. Such a primary inhibitory action of SRIF on catecholamines is more in line with the known actions of the hormone. It has been demonstrated in many types of cells and is based, in part, on the lowering of intracellular Ca^{2+} (22, 23).

The third possibility is a dual effect of SRIF on the monoaminergic neurons, namely, a concurrent stimulation of serotonin-producing and suppression of catecholamine-producing structures of the gut. The fact that in our experiments the rise in portal serotonin concentrations was consistently accompanied by a simultaneous reduction in catecholamine levels, without appreciable lag time argues in favor of this possibility.

However, since our experiments were primarily designed to study the hepatic portal levels of agents potentially involved in carbohydrate metabolism of the liver, our data are hardly suitable for dealing specifically with the ill-understood mechanisms of gut serotonin-catecholamine interrelationships. It is, therefore, impossible for us to favor any

one of the three proposed theories at this time. Indeed, in this report the main emphasis is placed on the fact that the effects of spike concentrations of GH on portal plasma serotonin and catecholamines are apparently mediated by SRIF, a novel and unexpected function for a hormone that is primarily known as an inhibitor of GH secretion.

Many of the data presented in this paper are part of a thesis submitted (by M. N. Hussain) to the School of Graduate Studies in conformity with the requirements for the degree of Doctor of Philosophy in the University of Toronto. This work was supported by grants from the Medical Research Council of Canada (No. MA-7778) and Denison Mines Limited of Toronto. The expert secretarial assistance of Mrs. S. Meredith is gratefully acknowledged.

1. Hussain MN, Sirek A, Dvorak M, Cukerman E, Sirek OV. Growth hormone stimulates serotonin secretion into the hepatic portal circulation. Canad J Physiol Pharmacol **61**:95–97, 1983.
2. Hussain MN. Functional Interrelationships between Biogenic Amines and Other Glucoregulatory Hormones. Ph.D. thesis, University of Toronto, 1984.
3. Hussain MN, Sirek A, Cukerman E, Sirek OV. Effect of growth hormone on biogenic amines in the hepatic portal circulation of the dog. Canad J Physiol Pharmacol 1985, in press.
4. Vaitkus P, Sirek A, Norwich KH, Sirek OV, Unger RH, Harris V. Rapid changes in hepatic glucose output following a pulse of growth hormone. Amer J Physiol **246**:E14–E19, 1984.
5. Sirek A, Sirek OV. A new technique for hepatic portal sampling in the conscious dog. Proc Soc Exp Biol Med **172**:397–399, 1983.
6. Hussain MN, Sole MJ. A simple, specific radioenzymatic assay for picogram quantities of serotonin or acetylserotonin in biological fluids and tissues. Anal Biochem **111**:105–110, 1981.
7. Sole MJ, Hussain MN. A simple, specific radioenzymatic assay for the simultaneous measurement of picogram quantities of norepinephrine, epinephrine and dopamine in plasma and tissues. Biochem Med **18**:301–307, 1977.
8. Roccatagliata G, Albano C, Cocito L, Maffini M. Interactions between central monoaminergic systems: Dopamine–serotonin. J Neurol Neurosurg Psychiatry **42**:1159–1162, 1979.
9. Martinez AA, Linkhandwala MF. Evidence for a presynaptic inhibitory action of 5-hydroxytryptamine on sympathetic neurotransmission to the myocardium. Eur J Pharmacol **63**:303–311, 1980.
10. Miller ME, Christensen GC, Evans HE. Anatomy of the Dog. Philadelphia/London, Saunders, 1964.
11. Ahlman H, DeMagistris L, Zinner M, Jaffe BM. Release of immunoreactive serotonin into the lumen of the feline gut in response to vagal nerve stimulation. Science (Washington, DC) **213**:1254–1255, 1981.
12. Efendic S, Luft R. Somatostatin, a classical hormone, a locally active polypeptide and a neurotransmitter. Ann Clin Res **12**:87–94, 1980.
13. Pace CS, Tarvin JT. Somatostatin: Mechanism of action in pancreatic islet B-cells. Diabetes **30**:836–842, 1981.
14. Theoharides TC, Douglas WW. Somatostatin induces histamine secretion from rat peritoneal mast cells. Endocrinology **102**:1637–1640, 1978.
15. Baxter JH, Adamik R. Differences in requirements and actions of various histamine-releasing agents. Biochem Pharmacol **27**:497–503, 1978.
16. Theoharides TC, Betchaku T, Douglas WW. Somatostatin-induced histamine secretion in mast cells. Characterization of the effect. Eur J Pharmacol **69**: 127–137, 1981.
17. Theoharides TC, Douglas WW. Mast cell histamine secretion in response to somatostatin analogues: Structural considerations. Eur J Pharmacol **73**:131–136, 1981.
18. Theoharides TC, Sieghart W, Greengard P, Douglas WW. Somatostatin-induced phosphorylation of mast cell proteins. Biochem Pharmacol **19**:2735–2736, 1981.
19. Theoharides TC, Bondy PK, Taskalas ND, Askenase PW. Differential release of serotonin and histamine from mast cells. Nature (London) **297**:229–231, 1982.
20. Carraway RE, Cochrane DE, Granier C, Kitabgi P, Leeman E, Singer EA. Parallel secretion of endogenous 5-hydroxy-tryptamine and histamine from mast cells stimulated by vasoactive peptides and compound 48/80. Brit J Pharmacol **81**:227–229, 1984.
21. Diel F, Bethge N, Oprée W. Histamine secretion in leukocyte incubates of patients with allergic hyperactivity induced by somatostatin-14 and somatostatin-28. Agents Actions **13**:216–218, 1983.
22. Kraicer J, Spence JW. Release of growth hormone from purified somatotrophs: Use of high K^+ and the ionophore A23187 to elucidate interrelations among Ca^{++}, adenosine 3′,5′-monophosphate and somatostatin. Endocrinology **108**:651–657, 1981.
23. Kracier J, Chow AEH. Release of growth hormone from purified somatotrophs: Use of perifusion system to elucidate interrelations among Ca^{++}, adenosine 3′,5′-monophosphate and somatostatin. Endocrinology **111**:1173–1180, 1982.

Received December 4, 1984. P.S.E.B.M. 1985, Vol. 179.
Accepted April 2, 1985.