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Inhibition of Insulin and Glucagon Secretion by Somatostatin: are there Indirect Effects?[1] (40113)

PHILLIP H. SMITH[2], MEI CHEN, STEPHEN C. WOODS, JOHN W. ENSINCK, AND DANIEL PORTE, JR.

Division of Metabolism, Seattle Veterans Administration Hospital; and Department of Medicine, University of Washington School of Medicine, Seattle, Washington 98195

Somatostatin, a peptide isolated originally from the hypothalamus, is known to inhibit the secretion of insulin and glucagon (1). Since this effect can be demonstrated *in vitro,* it has been concluded that somatostatin acts directly upon the pancreatic A- and B-cells (2, 3). Further, immunocytochemical studies have shown the presence of somatostatin-like material within islet D-cells suggesting that it plays a role in the local regulation of endocrine pancreatic function (4, 5). Recent investigations by this laboratory showed that alpha-adrenergic blockade with phentolamine markedly reduced the inhibitory action of somatostatin upon insulin and glucagon release from the pancreas of dogs (6–8). Those observations raise the possibility that somatostatin also affects islet function indirectly by interacting with neural signals to the endocrine pancreas. In the present study, somatostatin was infused at various doses and at different anatomic sites into anesthetized dogs to determine whether an inhibitory action of this peptide upon insulin and glucagon secretion could be found via specific routes using a dose that was not effective when given directly into the pancreas. Our results indicate that somatostatin may inhibit the release of insulin and glucagon by indirect as well as direct mechanisms.

Material and methods. The surgical procedure used in these experiments has been described previously (9). Briefly, mongrel dogs (25–35 kg) were anesthetized with morphine sulfate and sodium pentobarbital following an overnight fast. A midline laparotomy was performed, and the blood flow of the superior pancreaticoduodenal vein (PDV) was shunted through an extracorporeal flow-meter circuit before reaching the portal vein. Blood samples were collected from this circuit for the determination of plasma glucose, immunoreactive insulin (IRI), and immunoreactive glucagon (IRG) using methods reported previously (9, 10). Measurement of PDV blood flow and hematocrit in addition to plasma hormone concentrations in the PDV enabled the calculation of IRI and IRG output from the pancreas (9). Throughout these experiments, rectal temperature was maintained (38.5 ± 0.5°) by using an autoregulated thermal blanket. Tracheal intubation and artificial respiration permitted maintenance of arterial blood gases within the normal range (pO_2 80–90; pCO_2 30–40; pH 7.37–7.0).

Dihydrosomatastatin (a gift from Dr. Roger Guillemin, Salk Institute) was infused into the superior pancreatic artery at rates of 0.17, 1.7, or 17 μg for 3 min. In other experiments, somatostatin was administered at a rate of 1.7 μg/min for 30 min into either the hepatic portal vein or a femoral vein. Blood samples were collected at −30, −15, −5, and 0 min prior to the infusions, at +5, +10, +20, and +30 min during somatostatin infusion, and thereafter at +35, +40, +50, and +60 min.

Statistical evaluation of the data was performed by correlation analysis and Student's paired *t* test for IRI and IRG outputs, and analysis of variance for plasma glucose and PDV blood flow.

Results. Regardless of the site of infusion, somatostatin produced a decline of IRI (range, 217–21,874 μU/min) and IRG (range,

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[2] Send reprint requests to: Dr. Phillip H. Smith, Division of Metabolism (151), Veterans Administration Hospital, 4435 Beacon Avenue South, Seattle, Washington 98108.

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1509–24,577 pg/min) output that was correlated linearly with the basal secretory rates of these hormones ($r = 0.88$ and 0.79, respectively, both $P < 0.001$, $n = 20$). Because of this consistent relationship, IRI and IRG output during all somatostatin infusions were calculated as the percent change (%Δ) of the basal rates of secretion. Figure 1 shows the temporal responses of plasma glucose levels and the outputs of IRI and IRG during an infusion of somatostatin (1.7 μg/min for 30 min) via the pancreatic artery.

The dose-response relationship between log increases of somatostatin dose infused via the pancreatic artery and the %Δ of IRI and IRG outputs are summarized in Fig. 2. At a rate of 0.17 μg/min ($n = 5$) somatostatin did not reliably alter the basal output of either hormone. When the infusion rate was increased to 1.7 μg/min ($n = 4$), IRI output decreased markedly (%Δ = −76 ± 9, mean ± SEM, $P < 0.01$) as did IRG output (%Δ = −53 ± 8, $P < 0.01$). Increase of the somatostatin infusion rate to 17 μg/min ($n = 3$) resulted in an inhibition of the outputs of IRI (%Δ = −85 ± 3, $P < 0.001$) and IRG (%Δ = −57 ± 12, $P < 0.05$) that was not significantly different from that observed during the preceding dose of somatostatin. Thus, it would appear that the inhibition of both IRI and IRG outputs was approaching maximum using an infusion of somatostatin at 1.7 μg/min.

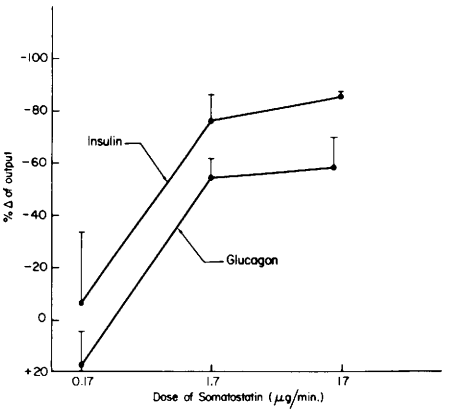

FIG. 2. Relationship between log dose of somatostatin and the %Δ of basal IRI and IRG output. Somatostatin infused via pancreatic artery. Values represent the mean of the 10, 20, and 30 min %Δ of each dog. Mean ± SEM of each data point is given in text.

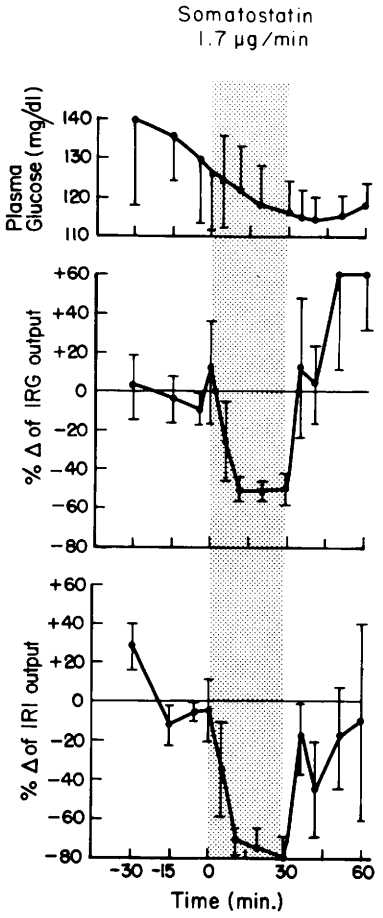

FIG. 1. The effects of somatostatin (1.7 μg/min for 30 min) upon plasma glucose level, IRI, and IRG outputs during an infusion via the pancreatic artery of dogs. Data are shown as mean ± SEM.

To evaluate further its inhibitory action upon the endocrine pancreas, somatostatin was infused into either the pancreatic artery, the hepatic portal vein, or a femoral vein at a rate of 1.7 μg/min for 30 min. Figure 3 summarizes the findings of this study. As indicated above, an infusion of somatostatin at 1.7 μg/min via the pancreatic artery produced a significant inhibition of both IRI and IRG outputs. This infusion also decreased the level of plasma glucose ($P < 0.05$). When infused into the hepatic portal vein, this dose of somatostatin elicited a significant decrease of IRI output (%Δ = −46 ± 12, $n = 4$, $P < 0.05$) while the apparent decline of IRG from basal levels was not significant (%Δ = −20 ± 12). Plasma glucose levels also decreased ($P < 0.05$). An identical infusion into a fem-

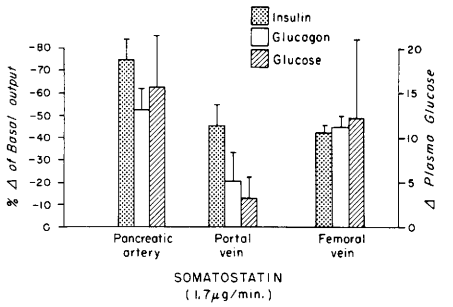

FIG. 3. Comparison of the effects upon IRI and IRG outputs and plasma glucose of infusions of somatostatin (1.7 μg/min for 30 min) administered via pancreatic artery, portal vein, or femoral vein. Data are shown as mean ± SEM.

oral vein ($n = 4$) elicited a decrease of IRI output ($\%\Delta = -44 \pm 11$, $P < 0.02$) and a comparable decrease of IRG output ($\%\Delta = -45 \pm 5$, $P < 0.001$). Although there was a slight decline of plasma glucose levels during this somatostatin infusion, the trend was not statistically significant. No statistically significant alterations of PDV blood flow were observed during any infusion of somatostatin.

Discussion. Numerous investigations using *in vitro* preparations have shown that somatostatin is a potent inhibitor of insulin and glucagon secretion (1–3, 11). Those reports have led, therefore, to the widely accepted concept that this peptide acts directly upon the pancreatic endocrine cells. Similar conclusions have been drawn from the effect of somatostatin *in vivo* due to its rapid inhibition of pancreatic endocrine function (12–14). In this study, infusions of somatostatin into the pancreatic artery of anesthetized dogs promptly decreased both IRI and IRG outputs, an effect that was reversed rapidly at the termination of the infusions. Thus, our present results are in basic agreement with the conclusion that somatostatin affects the islet A- and B-cells directly. Nevertheless, our observation of decrements of IRI and IRG outputs when somatostatin was given via a femoral vein or the hepatic portal vein suggest that, in addition to a direct effect upon the endocrine pancreas, somatostatin may inhibit islet hormone release through an indirect mechanism as well.

Blood flow to the canine pancreas is approximately 2% of total cardiac output (15). Therefore, we expected that when somatostatin at a dose of 1.7 μg/min was infused via a femoral vein or the hepatic portal vein, only 2% of that amount would reach the pancreas (i.e., approximately 0.034 μg/min). Since a dose of 0.17 μg/min given directly into the pancreatic artery had failed to decrease IRI and IRG outputs significantly, no inhibitory effect was expected following infusions of somatostatin at 1.7 μg/min via the other sites. However, when the above mentioned dose of somatostatin was given into a femoral vein, both IRI and IRG outputs were inhibited significantly. An identical infusion into the hepatic portal vein also elicited a significant decrement of IRI output that was comparable to the effect observed when somatostatin was administered via a femoral vein. Therefore, peripheral and portal infusions of somatostatin were more potent inhibitors of insulin secretion than could be explained by reasonable blood flow calculations. It is difficult to draw conclusions regarding the intraportal action of somatostatin on IRG output, since the effects of this infusion were to produce a decrease of glucagon secretion that was neither significantly different from basal levels nor significantly different from the decrease observed by infusions of somatostatin into a femoral vein.

The unexpected inhibition of IRI and IRG outputs during peripheral infusions of somatostatin and the effect of this peptide on IRI output when administered intraportally do not seem consistent with potential artifacts such as a somatostatin-mediated decrease of pancreatic blood flow or the reflux of this peptide into the pancreas. Although it has been reported that administration of somatostatin to humans reduced splanchnic blood flow (16, 17) leading to a potential decrease of islet hormone output, no statistically significant change of pancreaticoduodenal vein blood flow was observed during the administration of this peptide to dogs. Reflux of somatostatin into the pancreas during infusions into the hepatic portal vein seem unlikely, since the monitored blood flow was always in the other direction. Further, this type of artifact cannot explain the effectiveness of somatostatin when given via a femoral vein. Thus, because the concentration of somatostatin reaching the pancreas during infusions into a femoral vein or the hepatic

portal vein is presumably far less than that required to elicit a direct inhibitory effect, it seems reasonable to postulate that this peptide also alters islet hormone secretion by as yet unknown mechanisms.

Although the precise nature of this presumed indirect effect of somatostatin upon the endocrine pancreas is unclear, several explanations could account for the inhibition observed during this study. It is possible that, during a peripheral infusion, dihydrosomatostatin is converted to a more potent form. Preliminary work in this laboratory suggests that, when administered via a femoral vein, cyclic somatostatin is a more potent inhibitor of insulin secretion than dihydrosomatostatin, but that both forms of somatostatin exert comparable inhibitory effects upon glucagon secretion. However, previous studies by others show that dihydrosomatostatin and the oxidized or cyclic somatostatin are equipotent as inhibitors of insulin and glucagon release both *in vivo* and *in vitro* (18, 19). Conversion to an analog of somatostatin seems extremely unlikely due to the chemical changes required. Further, somatostatin analogs generally exhibit less biologic activity than the native peptide in terms of their inhibition of insulin and glucagon secretion (20, 21). Another factor that argues against a change of peptide form is the rapid metabolic clearance of somatostatin from the general circulation (22).

As alternative explanations for the presumed indirect action of somatostatin upon IRI and IRG output, we hypothesize that this effect may be related to a decrease of endogenous stimulatory signals for islet hormone release or to the initiation of an inhibitory neural-pancreatic reflex. Several studies have shown recently that somatostatin suppresses the circulating levels of gastrointestinal hormones including gastrin (1), secretin (23), and gastric inhibitory polypeptide (24). Since several intestinal hormones are known to potentiate the release of insulin and glucagon (25, 26), it seems reasonable to postulate that the present results may have been due to a decrease of circulating gut factors leading to a suppression of islet hormone secretion. Recent reports (27, 28) have also suggested that somatostatin release from the pancreatic islets and gastric antrum may be regulated, in part, by autonomic neural signals. Previously, we observed that phentolamine, an α-adrenergic blocker, markedly reduces the effectiveness of somatostatin as an inhibitor of hormone secretion from the pancreas of dogs (6–8). Those findings, therefore, suggest that somatostatin interacts with autonomic neural signals to the islets. In view of the extensive data concerning neural control of the endocrine pancreas (25, 29, 30) and the fact that somatostatin exists within certain central and peripheral neurons (4, 31), it is additionally possible that somatostatin acts upon a center within the central nervous system that, in turn, inhibits the release of islet hormones. At present, these hypotheses seem most consistent with our experimental findings.

Summary. The inhibitory action of somatostatin upon the endocrine pancreas of dogs was studied as a function of dose and site of administration. Hormone output was measured directly in the pancreaticoduodenal vein. Infusions of somatostatin into the pancreatic artery show that a predictable dose-response relationship exists for this controller of islet function. The high relative potency of somatostatin when administered via a femoral vein or the hepatic portal vein suggests that indirect mechanisms of action are responsible, in part, for the effect of this peptide upon insulin and glucagon output from the pancreas *in vivo*. It is postulated that, in its indirect effects, somatostatin may inhibit islet hormone secretion by decreasing the circulating levels of several gastrointestinal hormones known to potentiate insulin and glucagon release, or it may elicit an inhibitory neural-pancreatic reflex.

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1. Guillemin, R., and Gerich, J. E., Ann. Rev. Med. **27,** 379 (1976).
2. Curry, D. L., Bennett, L. L., and Li, C. H., Biochem. Biophys. Res. Commun. **58,** 885 (1974).
3. Efendic, S., and Luft, R., Acta Endocrinol. **78,** 510 (1975).
4. Hökfelt, T., Efendic, S., Hellerström, C., Johansson, O., Luft, R., and Arimura, A., Acta Endocrinol. **89,** Suppl. 200 (1975).
5. Orci, L., and Unger, R. L., Lancet **11,** 1243 (1975).
6. Smith, P. H., Woods, S. C., and Porte, D., Jr.,

Endocrinology **98,** 1073 (1976).
7. Porte, D., Jr., Smith, P. H., and Ensinck, J. W., Metabolism **25,** Suppl. 1, 1453 (1976).
8. Smith, P. H., Woods, S. C., Ensinck, J. W., and Porte, D., Jr., Metabolism **26,** 841 (1977).
9. Porte, D., Jr., Girardier, L., Seydoux, J., Kanazawa, Y., and Posternak, J., J. Clin. Invest. **52,** 210 (1973).
10. Ensinck, J. W., Shepard, C., Dudl, R. J., and Williams, R. H., J. Clin. Endocrinol. Metabol. **35,** 463 (1972).
11. Fujimoto, W. Y., Endocrinology **87,** 1494 (1975).
12. Alberti, K. G. M. M., Christensen, N. H., Christensen, S. E., Hansen, Aa. P., Iversen, J., Lundbaek, K., Seyer-Hansen, K., and Orskov, H., Lancet **11,** 1299 (1973).
13. Koerker, D. J., Ruch, W., Chideckel, E., Palmer, J., Goodner, C. J., Ensinck, J. W., and Gale, C. C., Science **184,** 482 (1974).
14. Efendic, S., and Luft, R., Acta Endocrinol. **78,** 516 (1975).
15. Sanders, T. M., Werner, R. A., and Bloor, C. M., J. Appl. Physiol. **40,** 927 (1976).
16. Felig, P., Wahren, J., Sherwin, R., and Hendler, R., Diabetes **25,** 1091 (1976).
17. Wahren, J., Efendic, S., Luft, R., Hagenfeldt, L., Björkman, O., and Felig, P., J. Clin. Invest. **59,** 299 (1977).
18. Brown, M., Rivier, J., and Vale, W., Endocrinology **98,** 336 (1976).
19. Efendic, S., Luft, R., and Grill, V., FEBS Letters **42,** 169 (1974).
20. Vale, W., Brazeau, P., Rivier, C., Brown, M., Boss, B., Rivier, J., Burgur, R., Ling, N., and Guillemin, R., Rec. Prog. Horm. Res. **31,** 365 (1975).
21. Brown, M., Rivier, J., and Vale, W., Metabolism 25, Suppl. **1,** 1501 (1976).
22. Ensinck, J. W., Laschansky, E., Chideckel, E., Palmer, J., and Goodner, C. J., Clin. Res. **24,** 155A (1976).
23. Boden, G., Sivitz, M. C., Owen, O. E., Essa-Koumar, N., and Landor, J. H., Science **190,** 163 (1975).
24. Pederson, R. A., Dryburgh, J. R., and Brown, J. C., Can. J. Physiol. Pharmacol. **53,** 1200 (1975).
25. Gerich, J. E., Charles, M. A., and Grodsky, G. M., Ann. Rev. Physiol. **38,** 353 (1976).
26. Brown, J. C., Dryburgh, J. R., Ross, S. A., and Dupre, J., Rec. Progr. Horm. Res. **31,** 487 (1975).
27. Samols, E., Weir, G. C., Patel, Y. C., Loo, S. W., and Gabbay, K. H., Clin. Res. **25,** 499A (1977).
28. Uvnas-Wallensten, K., Efendic, S., and Luft, R., Acta Physiol. Scand. **99,** 126 (1977).
29. Smith, P. H., and Porte, D., Jr., Ann. Rev. Pharm. Toxicol. **16,** 269 (1976).
30. Woods, S. C., and Porte, D., Jr., Physiol. Rev. **54,** 596 (1974).
31. Parsons, J. A., Erlandsen, S. L., Hegre, O. D., McEvoy, R. C., and Elde, R. P., J. Histochem. Cytochem. **24,** 872 (1976).

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