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Somatostatin Inhibition of Glucagon-Stimulated Adenosine 3′-5′-Monophosphate Accumulation in Isolated Hepatocytes[1] (39547)

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Somatostatin, or somatotrapin release inhibiting factor is a small polypeptide found in the hypothalamus (1) and in the pancreatic islets of Langerhans (2). This small polypeptide has been shown to inhibit *in vivo* secretions of growth hormone (1, 3, 4) and thyroid stimulating hormone (5, 6) from the anterior pituitary gland and insulin and glucagon from the endocrine pancreas (7-9). Studies with somatostatin *in vitro* have produced similar results with inhibition of secretions of growth hormone, thyroid stimulating hormone (10), insulin, and glucagon (11-13). Somatostatin not only affects endocrine gland function but has also been shown to inhibit glucagon stimulated gluconeogenesis and glycogenolysis in isolated rat hepatocytes (11). The mechanism(s) for these inhibitory effects is at present unknown. The nucleotide adenosine 3′,5′-cyclic monophosphate (cyclic AMP) has been implicated in the secretion of hormones as well as having a role in hepatic glycogenolysis and gluconeogenesis. In the present study, we have investigated the effect of somatostatin on glucagon-stimulated cyclic AMP accumulation in isolated rat hepatocytes in an effort to elucidate the mechanism(s) by which somatostatin acts at the cellular level.

Materials and Methods. Male, well-fed Sprague-Dawley rats (150-200 g) were used throughout these studies. Hepatocytes were prepared by the collagenase perfusion technique (14). Approximately 50-70 mg of cells were incubated in 3.0 ml of Umbreit-Ringer 25 m*M* bicarbonate buffer (15) maintained at 37° and 90 oscillations/min. Hepatocytes with or without somatostatin were preincubated for 5 min before the addition of glucagon. After addition of glucagon the incubation was continued for a further 5 min, after which time 1 ml of 20% trichloracetic acid was added, and the cells were homogenized for 15 sec using a Brinkman Polytron. The homogenate was centrifuged, and the cyclic AMP content of the supernatant was quantitated using the previously described method of Moxley and Allen (16).

Results. The effect of various concentrations of somatostatin on basal and glucagon-stimulated hepatocyte cyclic AMP accumulation are shown in Fig. 1. Somatostatin at concentrations of 0.50 to 8.0 μg/ml caused significant inhibition of cyclic AMP accumulation in hepatocytes stimulated with either 10^{-8} or 10^{-7} *M* glucagon. However, in the complete absence of glucagon, somatostatin at concentrations of 0.50 to 2.0 μg/ml caused a small but significant increase in cyclic AMP accumulation. A similar finding was also observed for hepatocytes exposed to a low concentration of glucagon, 10^{-12} *M*, and a somatostatin concentration of 2.0 μg/ml.

Discussion. These studies indicate that somatostatin can inhibit glucagon-stimulated cyclic AMP accumulation in hepatocytes. The rather paradoxical finding of somatostatin increasing cyclic AMP levels in hepatocytes not exposed to glucagon or as in one case when glucagon concentration was low, 10^{-12} *M*, are difficult to interpret. However, such findings would suggest that somatostatin is behaving as a partial agonist. Evidence in support of this concept is based on a structural relationship between somatostatin and glucagon, both of which have the same four amino acids, Thr-Phe-Thr-Ser occurring in the same sequence.

Studies by other groups (10, 17) have shown that somatostatin inhibits basal and prostaglandin E_1 or thyrotropin releasing hormone-stimulated cyclic AMP accumulation in rat pituitary glands. In isolated rat islets of Langerhans, Efendic *et al.* (18) has

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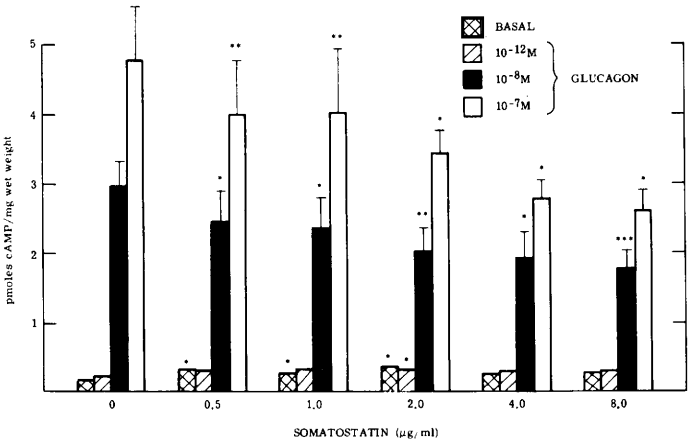

FIG. 1. The effect of increasing concentrations of somatostatin on basal or glucagon-stimulated cyclic AMP accumulation in isolated rat hepatocytes. The bars represent the mean ± SEM for six to eight observations from four rats. Statistical analysis was done by Student's *t* test using paired differences. * Represents $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$.

shown that somatostatin inhibits glucose-stimulated accumulation of cyclic AMP. In each of these studies a decrease in cyclic AMP levels was concomitant with a decrease in secretion of hormone from the endocrine gland. In earlier studies from this laboratory (11) glucagon-stimulated glycogenolysis and gluconeogenesis could be inhibited by concentrations of somatostatin (0.2 to 2.0 μg/ml) similar to those used in the present study. Although cyclic AMP has been implicated in glycogenolysis and gluconeogenesis (19, 20), the exact interrelationship between cyclic AMP and these two processes is still unknown. The work of Pilkis *et al.* (21) has shown that the addition of glucagon or of cyclic AMP to isolated rat hepatocytes increased lactate incorporation into glucose. Both the cyclic AMP and glucagon effects on gluconeogenesis were dependent on the presence of extracellular calcium ion; when there was a deficiency of calcium; gluconeogenesis was inhibited. This same group of workers also showed that glucagon-stimulated accumulation of cyclic AMP was independent of extracellular calcium. These findings would perhaps suggest that calcium plays a more important role in gluconeogenesis than does cyclic AMP. Investigators using isolated rat islets of Langerhans (12) and perfused pancreas (22) have shown that the inhibitory effects of somatostatin on glucose-stimulated insulin secretion could be overcome by increasing the extracellular calcium ion concentration.

Data presented in these present studies support our earlier findings (11) that somatostatin is capable of direct action on hepatocytes and strengthen the concept that somatostatin is capable of direct action on other tissues resulting in alterations of normal cellular functions. However, whether somatostatin affects cellular function by inhibiting cyclic AMP accumulation or by altering translocation of calcium within cells still remains to be elucidated.

Summary. The cyclic form of somatotropin release inhibitor factor over a concentration range from 0.50 to 8.0 μg/ml was found to inhibit glucagon-stimulated cyclic AMP accumulation in isolated rat hepatocytes.

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1. Brazeau, P., Vale, W., Burgun, R., Ling, N., Butcher, M., Rivier, J., and Guillemin,R., Science **179,** 77 (1973).
2. Goldsmith, P. C., Rose, J. C., Arimura, A., and Ganong, W. F., Endocrinology **97,** 1061 (1975).
3. Siler, T. M., VandenBerg, G., and Yen, S. S. C., J. Clin. Endocrinol. Metab. **37,** 632 (1973).

4. Hall, R., Schally, A., Evered, D., Kastin, A., Mortimer, C., Tunbridge, W., Besser, G., Coy, D., Goldie, D., McNeilly, A., Phenekos, O., and Weightman, D., Lancet **2,** 581 (1973).
5. Siler, T., Yen, S. S. C., Vale, W., and Guillemin, R., J. Clin. Endocrinol. Metab. **38,** 742 (1974).
6. Vale, W., Brazeau, P., Rivier, C., Rivier, J., Grant, G., Burgun, R., and Guillemin, R., Fed. Proc. **32,** 211 (1973).
7. Koerker, D. J., Ruch, W., Chideckel, E., Palmer, J., Goodner, C. J., Ensinck, J., and Gale, C. C., Science **184,** 482 (1974).
8. Alberti, K. G. M. M., Juel Christensen, N., Engkjaer Christensen, S., Prange-Hansen, A., Iversen, J., Lundbaek, K., Seyer-Hansen, K., and Orskov, H., Lancet **2,** 1299 (1973).
9. Gerich, J. E., Lorenzi, M., Hane, S., Gustafson, G.,Guillemin, R., and Forsham, P., Metabolism **24,** 175 (1975).
10. Borgeat, P., Labrie, F., Drovin, J., Belonger, A., Immer, H., Sestanj, K., Nelson, V., Gotz, M., Schally, A. V., Coy, D. H., and Coy, E. J., Biochem. Biophys. Res. Commun. **56,** 1052 (1974).
11. Oliver, J. R., and Wagle, S. R., Biochem. Biophys. Res. Commun. **62,** 772 (1975).
12. Taminato, T., Seino, Y., Goto, Y., and Imura, H., Biochem. Biophys. Res. Commun. **66,** 928 (1975).
13. Gerich, J. J. E., Lovinger, E., and Gradsey, G. M., Endocrinology **96,** 749 (1975).
14. Ingebretsen, W. R., and Wagle S. R., Biochem. Biophys. Res. Commun. **47,** 403 (1972).
15. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., "Manometraic Techniques," p. 150. Burgess, Minneapolis (1964).
16. Moxley, M. A., and Allen, D. O., Horm. Metab. Res. **7,** 330 (1975).
17. Kaneko, T., Oka, H., Munemura, M., Suzuki, S., Yasuda, H., and Oda, T., Biochem. Biophys. Res. Commun. **61,** 53 (1974).
18. Efendic, S., Grill, V., and Luft, R., FEBS Lett. **55,** 131 (1975).
19. Jefferson, L. S., Exton, J. H., Butcher, R. W., Sutherland, W. E., and Park, C. R., J. Biol. Chem. **243,** 1031 (1968).
20. Exton, J. H., Lewis, S. B., Ho, R. J., and Park, C. R., *in* "Advances in Cyclic Nucleotide Research" (P. Greengard and G. Robison, eds.), Vol. 1, p. 91. Raven Press, New York (1972).
21. Pilkis, S. J., Claus, T., Johnson, R. A., and Park, C. R., J. Biol. Chem. **250,** 6328 (1972).
22. Curry, D. C., and Bennett, L. L., Biochem, Biophys. Res. Commun. **60,** 1015 (1974).

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