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STIMULATION OF GLUCONEOGENESIS BY SOMATOSTATIN IN RAT KIDNEY CORTEX SLICES*

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SUMMARY

The effect of somatostatin on gluconeogenesis was studied in kidney cortex slices. Addition of somatostatin (2μg) stimulated gluconeogenesis from lactate, pyruvate and glutamine by 42%, 50% and 68% respectively. Stimulation of glucose synthesis from lactate by somatostatin was found to be linear with time and dose dependent between 0.1 and 20μg. Somatostatin-stimulated gluconeogenesis was inhibited by phentolamine (10μM) but not by propranolol (10μM) suggesting that somatostatin action is mediated by α-adrenergic stimuli.

Somatostatin, a tetradecapeptide isolated initially from the hypothalamus (1) and subsequently identified also in D-cells of the islets of Langerhans (2) has been demonstrated to inhibit the secretion of growth hormone(1), insulin (3,4) and glucagon (5). In isolated liver parenchymal cells it specifically inhibits glucagon-induced glycogenolysis and gluconeogenesis but is found to be ineffective on epinephrine-stimulated changes (4). Further studies demonstrated that activation of adenyl cyclase and cyclic AMP formation caused by glucagon in liver preparations could be reversed by somatostatin (6,7). However, somatostatin by itself was found to be ineffective in causing any alteration on glycogenolysis or gluconeogenesis in the liver. Although the liver has been considered the primary organ concerned in the

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control of glucose homeostasis, it is well documented that kidney cortex also plays a significant role in gluconeogenesis under a variety of conditions (8-10). Therefore, the present study was carried out to investigate whether somatostatin plays any direct role on gluconeogenesis in the kidney cortex.

MATERIALS AND METHODS

Male albino rats (200-250g) of Sprague-Dawley strain which were fed ad libitum were used in this study. The animals were killed by stunning and decapitation. Kidney pairs were quickly removed and cortex slices prepared (9). Gluconeogenic activity was determined by measuring the glucose production from various substrates. Incubations were carried out in 50ml Erlenmeyer flasks containing 4.0ml Umbriet's Ringer bicarbonate buffer, 1 kidney cortex slice (equivalent to 2-3mg dry wt.) and 10mM substrate. The flasks were incubated in a metabolic shaker at 37° under the atmosphere of O_2:CO_2 (95:5). All the incubations were carried out for 1 hour unless otherwise specified. Somatostatin was dissolved in sodium phosphate buffer containing 3% normal guinea pig serum. Phentolamine and propranolol were dissolved in distilled water. The incubations were terminated by removing the cortex slices, and immersing the tubes containing the medium in a boiling water bath. The clear supernatant was analyzed for glucose by the glucose oxidase method (11). Kidney cortex slices were dried overnight at 90°C and the dry weights were used to express the glucose production. Somatostatin was purchased from Boehringer Mannheim, Biochemicals, Indianapolis, Indiana. Phentolamine (Regitine HCl) was supplied by Ciba-Geigy Corporation, Summit, New Jersey. DL-Propranolol HCl was the product of Ayerst Laboratories, Inc., New York, New York. All substrates used were obtained from Sigma Chemicals Company, St. Louis, Missouri.

RESULTS

Renal cortex is a unique tissue to investigate the control of gluconeogenesis, since this has an extremely low glycogen content. Therefore, the net glucose synthesized from noncarbohydrate precursors will give a direct index of the gluconeogenic activity. In the present study the glucose production from various substrates was determined to investigate the influence of somatostatin on gluconeogenesis in kidney cortex slices. The effect of somatostatin on net glucose production from lactate, pyruvate and glutamine is summarized in Table 1. In the absence of substrate the glucose synthesis was 26.8 ± 2.9

TABLE 1

Effect of Somatostatin on Gluconeogenesis in Kidney Cortex Slices.

Substrates	Net Glucose Production (μmoles/g dry wt/hr)		% Increase	P
	Control	Somatostatin (2μg)		
Lactate	148.4 ± 4.5 (40)	212.3 ± 22.5 (7)	42	< 0.001
Pyruvate	123.0 ± 7.7 (6)	184.0 ± 14.4 (4)	50	< 0.01
Glutamine	73.5 ± 6.4 (4)	123.7 ± 16.0 (4)	68	< 0.05

One slice of kidney cortex (2-3mg dry wt) was incubated in 4ml Umbreit Ringer bicarbonate buffer in the presence or absence of substrate (10mM) for 60 min at 37°C. Net glucose production was calculated by subtracting the glucose production in the absence of substrate from that in the presence of substrate. Glucose production in the absence of substrate was 26.8 ± 2.9 μmoles/g dry wt/hr. Each value given is mean ± SEM, with number of observations in parentheses.

μmoles/g dry wt./hr. In the presence of various substrates the glucose production ranged from 100-175 μmoles/g dry wt./hr. It is therefore presumed that net glucose synthesis in the presence of these substrates represents gluconeogenesis. Addition of 2μg of somatostatin significantly stimulated glucose synthesis from lactate, pyruvate and glutamine (Table 1).

In most experiments lactate (10mM) was used as the substrate since it was found to be a better gluconeogenic precursor than other substrates studied. Fig. 1 illustrates the effect of two concentrations of somatostatin (1 and 10μg) on gluconeogenic activity at 30, 60 and 90 minutes. The glucose synthesis was dose dependent and nearly linear with time. The increase in glucose production in presence of 1μg somatostatin was 70% in 30 minutes, 40% in 60 minutes and 32% in 90 minutes. In the presence of 10μg somatostatin the increase in glucose production was 105% in 30 minutes, 72% in 60 minutes and 55% in 90 minutes.

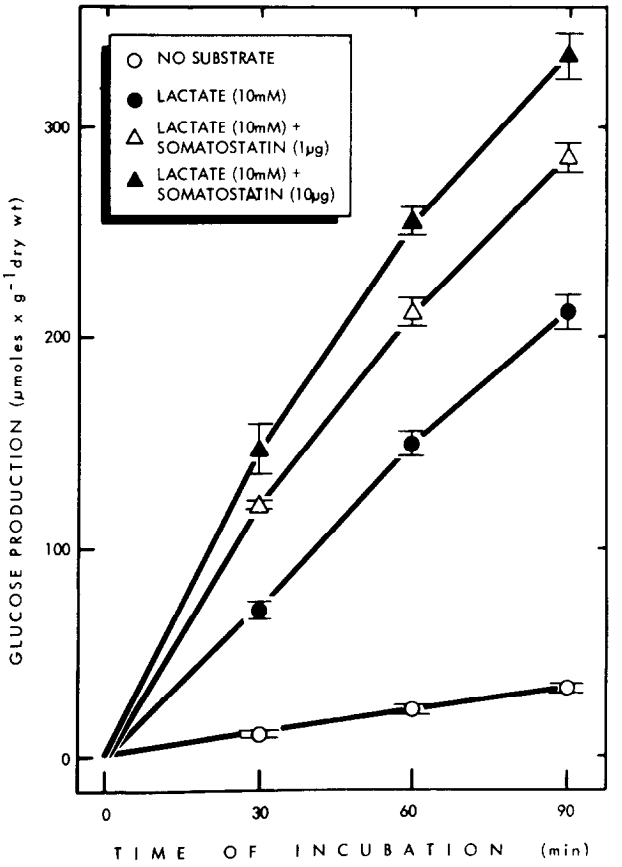

Figure 1. Effect of somatostatin concentration and incubation time on glucose production from lactate (10mM) in kidney cortex slices. Incubations were carried out with 1 or 10 µg of somatostatin for 30, 60 and 90 min. Details of the incubation are given in Materials and Methods. Each value is mean ± SEM of 5 or more observations.

Dose dependence of somatostatin-stimulated gluconeogenesis was further confirmed by using concentrations ranging from 0.1 to 20 µg (Fig. 2). Concentration as low as 0.1 µg produced a statistically significant increase in gluconeogenesis ($P < 0.05$). The stimulatory response was dose dependent for all the concentrations studied.

In order to examine the involvement of adrenergic stimuli, if any, on somatostatin action in the kidney cortex, specific antagonists of α and β receptors were used to examine the

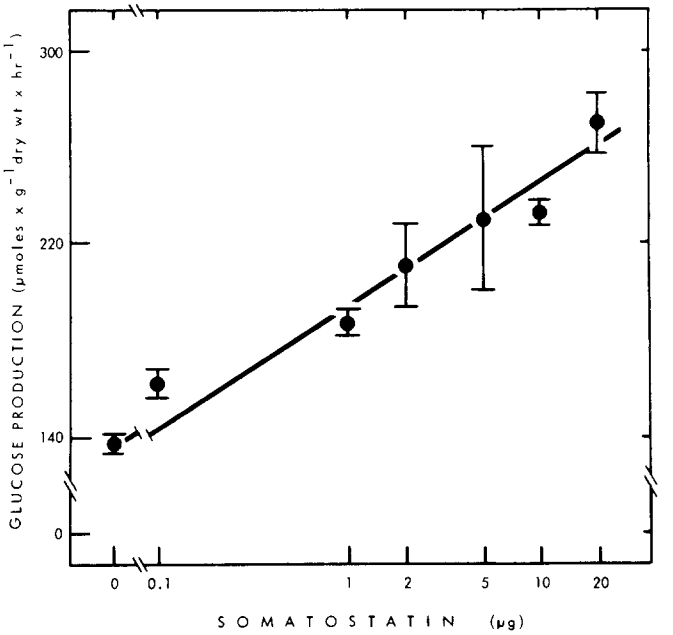

Figure 2. Dose response curve for the effect of somatostatin on net glucose production from lactate (10mM) in kidney cortex slices. Details of the incubation are given in Materials and Methods. Each value is mean ± SEM of 5 or more observations.

blockade of somatostatin-induced glucose production (Fig. 3). The somatostatin-mediated (1 or 10 μg) increase in glucose production was completely blocked by α-adrenergic antagonist phentolamine (10 μM), whereas no blockade was observed with same concentration of the β-adrenergic antagonist, propranolol. Phentolamine or propranolol alone did not cause any alteration in glucose production.

DISCUSSION

Data presented (Table 1, Fig. 1,2) in this communication clearly demonstrate that somatostatin stimulates gluconeogenesis in kidney cortex slices. A direct effect of this tetradecapeptide in bringing about metabolic changes in any tissue has not yet been documented in literature. However, it has been shown to

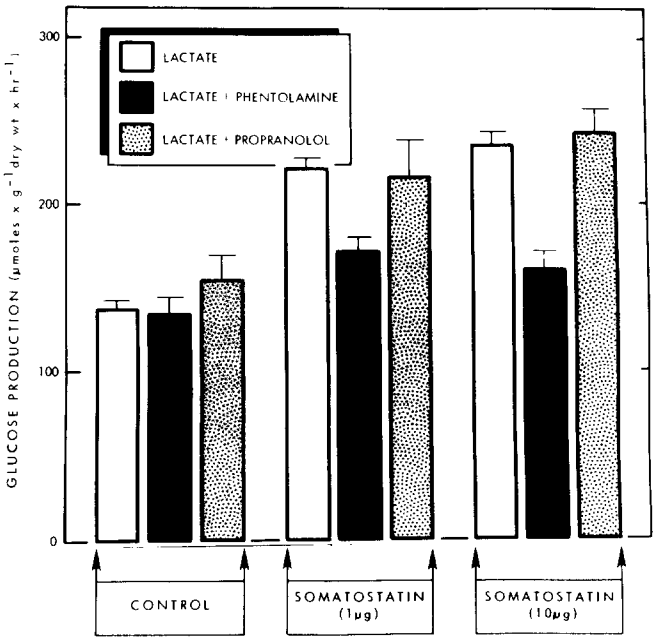

Figure 3. Effects of phentolamine and propranolol (α and β-adrenergic antagonist respectively) on somatostatin-stimulated gluconeogenesis from lactate (10mM) in kidney cortex slices. Adrenergic blockers were added at 10 μM concentration. Somatostatin concentrations were 1 and 10 μg. Details of the incubation are given in Materials and Methods. Each value is mean ± SEM of net glucose production of 5 or more observations.

be capable of reversing glucagon-stimulated glycogenolysis, gluconeogenesis and cyclic AMP production in liver preparations (6,7).

The finding that somatostatin-induced enhancement in gluconeogenesis is blocked by the α-adrenergic antagonist phentolamine, and not blocked by the β-adrenergic antagonist propranolol strongly suggests that the somatostatin action could be via α-adrenergic stimuli. Guder and Rupprecht (12) using kidney tubule fragments from fasted rats, have demonstrated that the norepinephrine stimulation of gluconeogenesis is mediated by α-adrenergic response. Recently, MacDonald and Saggerson (13) reported that renal gluconeogenesis might be increased by α and not by β-adrenergic stimuli and that the stimulation is probably

independent of alterations in 3':5' cyclic AMP or 3':5' cyclic GMP. Assuming that α-adrenergic receptors are located on the plasma membrane of the cells and that α-agonists are unlikely to work through any direct interaction with the enzymes of gluconeogenesis, a second messenger may be postulated in transmitting the α-adrenergic stimulus. Since Ca^{2+} has been implicated in the mediation of α-adrenergic action in various systems (14-16) and that the α-adrenergic stimulation of gluconeogenesis requires extracellular Ca^{2+} (17), an increase in Ca^{2+} flux may be speculated as the causative factor in the somatostatin-stimulated gluconeogenesis in the kidney cortex.

To our knowledge this is the first report demonstrating a direct effect of somatostatin on gluconeogenesis in kidney cortex. Thus, the kidney may be a target organ for this hypothalamic peptide.

REFERENCES

1. Vale, W., Brazeau, P., Rivier, C., Brown, M., Boss, B., Rivier, J., Burgus, R., Ling, N. and Guillemin, R. (1975). Recent Progr. Hormone Res. 31, 365-397.
2. Hokfelt, T., Efendic, S., Hellerstrom, C., Johansson, O., Luft, R. and Arimura, A. (1975). Acta Endocrinol. 80 (Suppl. 200) 1-25.
3. Curry, D., Bennet, L.L. and Li, C.H. (1974). Biochem. Biophys. Res. Comm. 58, 885-892.
4. Oliver, J.R. and Wagle, S.R. (1975). Biochem. Biophys., Res. Comm. 62, 772-777.
5. Koerker, D., Ruch, W., Chideckel, E., Palmer, J., Gowdner, C., Ensink, J. and Gale, C. (1974). Science 184, 482-485.
6. Oliver, J.R., Long, K., Wagle, S.R. and Allen, D.O. (1976). Proc. Soc. Expt. Biol. Med. 153, 367-370.
7. Ignarro, L.J. and Gross, R.A. (1978). Fed. Proc. 37, 339.
8. Wagle, S.R., Saskin, R.K., Jacoby, A. and Ashmore, J. (1966). Life Sci. 5, 655-663.
9. Lupiáñez, J.A., Faus, M.J., Muñoz-Clares, R. and Sánchez-Medina, F. (1976). FEBS Lett. 61, 277-281.
10. Faus, M.J., Lupiáñez, J.A., Vargas, A. and Sánchez-Medina, F. (1978). Biochem. J. 174, 461-467.
11. Hugget, A. and Nixon, S.N. (1957). Lancet 2, 368.
12. Guder, W.G. and Rupprecht, A. (1975). Eur. J. Biochem. 52, 283-289.
13. MacDonald, D.W.R. and Saggerson, E.D. (1977). Biochem. J. 168, 33-42.

14. Kaminsky, N.C., Baradus, A.E., Hardman, J.G., Jones, D.J., Ball, J.H., Sutherland, E.W. and Liddle, G.W. (1970). J. Clin. Invest. 49, 2387-2395.
15. Goldberg, N.D., O'Dea, R.F. and Haddox, M.K. (1973). Adv. Cyclic Nucleotide Res. 3, 155-223.
16. Selinger, Z., Batzri, S., Eimerl, S. and Schramm, M. (1973). J. Biol. Chem. 248, 369-372.
17. Tolbert, M.E.M. and Fain, J.N. (1974). J. Biol. Chem. 249, 1162-1166.