Vol. 115, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 15, 1983 s 743-748

A RISE IN CYCLIC AMP WITH A LACK OF GLYCOGENOLYSIS BY SECRETIN IN THE ISOLATED PERFUSED RAT LIVER AND ITS INHIBITION BY EPINEPHRINE

KEIICHI YAMATANI, NORIHIRO SATO, KENJI TAKAHASHI,
MASAO HARA AND HIDEO SASAKI

Third Department of Internal Medicine, Yamagata University,
School of Medicine, Yamagata 990-23, Japan

Received August 11, 1983

The effects of secretin on glucose output and cyclic AMP from the isolated perfused rat liver were investigated. Secretin 0.1 U/ml increased cyclic AMP in the effluent without an increase in glucose output. Glucose output induced by epinephrine 10^{-8}M was not affected by secretin 0.1 U/ml administered simultaneously, whereas the increase in cyclic AMP produced by secretin 0.1 U/ml was inhibited by epinephrine 10^{-8}M. The increase in cyclic AMP produced by glucagon 10^{-10}M was not affected by epinephrine 10^{-8}M. These results suggest that secretin does not affect glycogenolysis in the liver and secretin activates adenylate cyclase through a different receptor from glucagon in the liver.

Secretin is similar to glucagon in its amino acid sequence and some of its biological effects (1). Although several investigators have reported secretin-induced adenylate cyclase in the cell membrane (2, 3), the effect of secretin on glucose metabolism in the liver is still uncertain. In this report we examine the effects of secretin on glucose output and cyclic AMP from the isolated perfusee rat liver.

MATERIALS AND METHODS

The isolated rat liver perfusion was performed according to the method of Sugano et al (4). Briefly, the livers were isolated from male Wistar rats weighing 200 to 250 g fed ad lib and perfused noncyclically at a rate of 30 ml/min with hemoglobin-free Krebs-Henseleit bicarbonate buffer gassed with a humidified mixture of 95% O_2 - 5% CO_2. The livers were maintained at 32°C and the effluent was collected every one minute. After the liver were pre-perfused for 30 min, pork secretin (Eisai, Tokyo, Japan), pork glucagon (Novo A.S., Copenhagen, Denmark) and L-epinephrine (Daiichi, Tokyo, Japan) were administered alone or together through a side arm for 10 min. Cyclic AMP was assayed by RIA kit (Yamasa Shoyu, Choshi, Japan). Glucose was measured by the glucose oxidase method. Cyclic AMP and glucose were corrected by wet weight of the liver at the end of the experiment. Statistical analysis was done with Student's t test.

RESULTS

Cyclic AMP and glucose in the effluent from the isolated perfused rat liver induced by secretin, epinephrine and glucagon. Basal cyclic AMP in the

0006-291X/83 $1.50

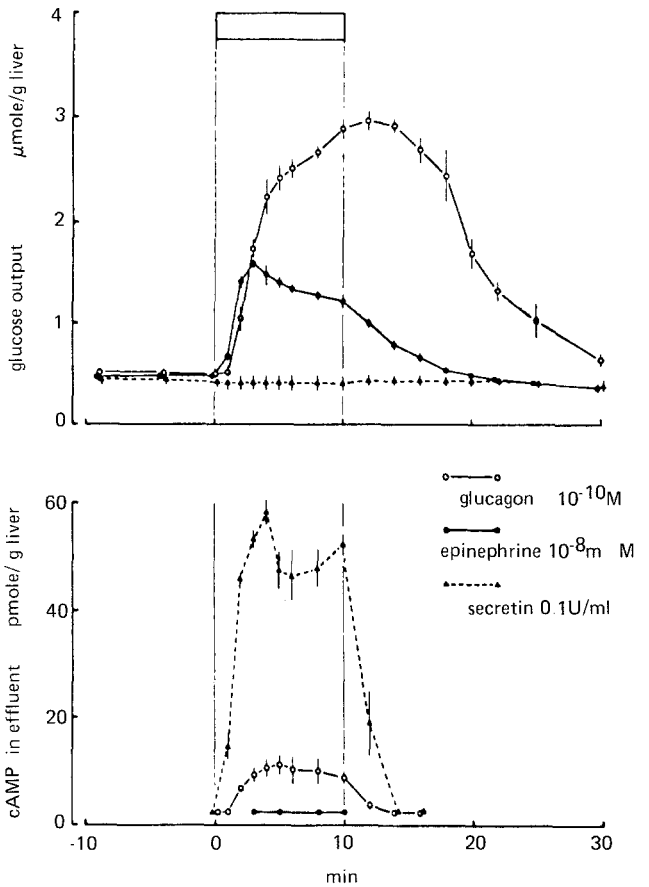

Figure 1 Glucose output (upper panel) and cyclic AMP in the effluent (lower panel) from the isolated perfused rat liver when secretin 0.1 U/ml (▲), epinephrine10^{-8}M (●) or glucagon 10^{-10}M (○) was administered for 10 min. Each point shows mean ± standard error.

effluent was under detectable levels (approximately 2 pmole cAMP/g liver/min) and basal glucose output was 0.44 ± 0.02 (mean ± SEM) μmole glucose/g liver/min. When secretin 0.1 U/ml was administered for 10 min, cyclic AMP in the effluent was increased to 52.3 ± 1.9 pmole cAMP/g liver/min at 10 min. However, glucose output was not affected by secretin 0.1 U/ml (Fig. 1). Glucose output was increased to 1.60 ± 0.04 μmole glucose/g liver/min at 3 min, but cyclic AMP in the effluent was under detectable levels during the administration of epinephrine 10^{-8}M for 10 min (Fig. 1). Both glucose and cyclic AMP in the effluent were increased by the administration of glucagon 10^{-10}M for 10 min resulting in 2.91 ± 0.10 μmole glucose/g liver/min and 8.1 ± 1.1 pmole cAMP/g

liver/min at 10 min, respectively (Fig. 1). Since glucagon and some other hormones increase cyclic AMP levels both in the perfusate and in the liver (5), the increase in cyclic AMP in the effluent from the isolated perfused rat liver is thought to be reflected in the accumulation of cyclic AMP in the liver. Because of the absence of substrates for gluconeogenesis in the perfusate, the increase of glucose output is mostly due to glycogenolysis in this investigation.

Effects of simultaneous administration of epinephrine with secretin or glucagon. Epinephrine 10^{-8}M did not increase cyclic AMP in the effluent in this investigation (Fig. 1). It is likely that glucose output was increased mainly by α-adrenergic effect of epinephrine 10^{-8}M (6). In order to ascertain the permissive effect of cyclic AMP increased by secretin on glucose output increased by α-adrenergic stimulation, secretin 0.1 U/ml and epinephrine 10^{-8}M were administered for 10 min simultaneously. Glucose output increased by epinephrine 10^{-8}M was not affected by concomitant administration of secretin 0.1 U/ml. However, cyclic AMP in the effluent was 3.0 ± 0.4 pmole cAMP/g liver/min at 10 min when secretin 0.1 U/ml and epinephrine 10^{-8}M were administered together, and was significantly lower than that by secretin 0.1 U/ml alone ($P < 0.001$, Fig. 2). When glucagon 10^{-10}M and epinephrine 10^{-8}M were administered simultaneously, glucose output was higher ($P < 0.05$) than that of glucagon 10^{-10}M alone at 2 and 3 min, and cyclic AMP in the effluent was slightly low (6.4 ± 0.4 pmole cAMP/g liver/min) at 10 min but insignificant (Fig. 3).

DISCUSSION

Cyclic AMP increased by secretin seemed unconnected with glycogenolysis in the liver, because secretin 0.1 U/ml alone did not affect glucose output (Fig. 1), and it also did not have the permissive effect on glucose output increased by α-adrenergic stimulation of epinephrine 10^{-8}M (Fig. 2). Two explanations can be made for a lack of glucose output in spite of a rise in cyclic AMP by secretin. First, there may be a component of cyclic AMP which is not linked to glucose metabolism in the hepatocytes. Second, secretin may

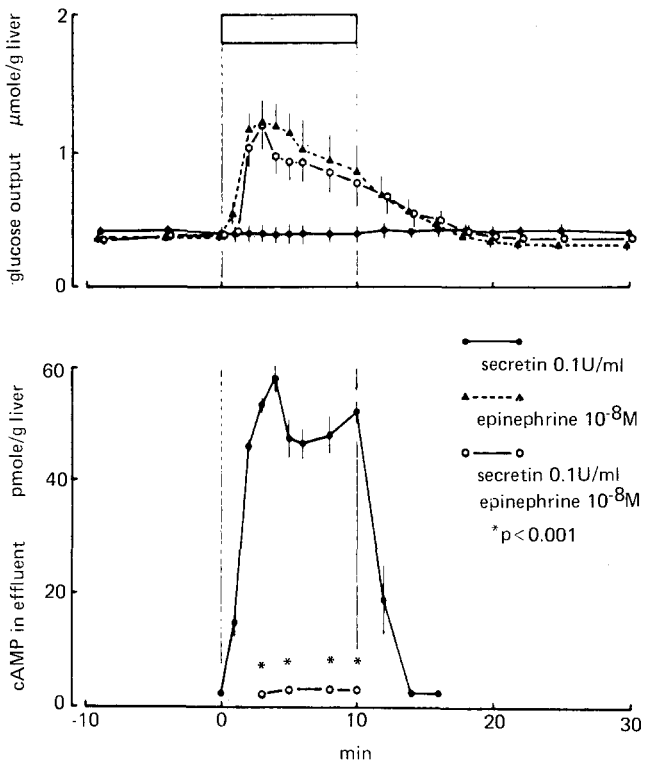

Figure 2 Glucose output (upper panel) and cyclic AMP in the effluent (lower panel) from the isolated perfused rat liver when secretin 0.1 U/ml (●) or epinephrine 10^{-8}M (▲) was administered for 10 min alone or together (○).

act on another cell than the hepatocyte. The latter is most likely; hence, hepatic bile production increased by secretin was reported (7). On the other hand, the activation of adenylate cyclase by secretin through a different receptor from that of glucagon in the liver cell membrane was reported (2, 3), so it is difficult to deny the former hypothesis. In this investigation, simultaneous administration of epinephrine 10^{-8}M inhibited the increase of cyclic AMP by secretin 0.1 U/ml but not that of glucagon 10^{-10}M (Fig. 2, 3). This indicates that the secretin-linked adenylate cyclase system and the glucagon-linked adenylate cyclase system differed in their interaction with the epinephrine receptor in the isolated perfused rat liver. However, this does not mean that secretin and glucagon act on different cells. Recently, α-adrenergic agonist was found to inhibit glucagon stimulated cyclic AMP accumulation in the liver membrane (8) and the isolated hepatocytes (9). It

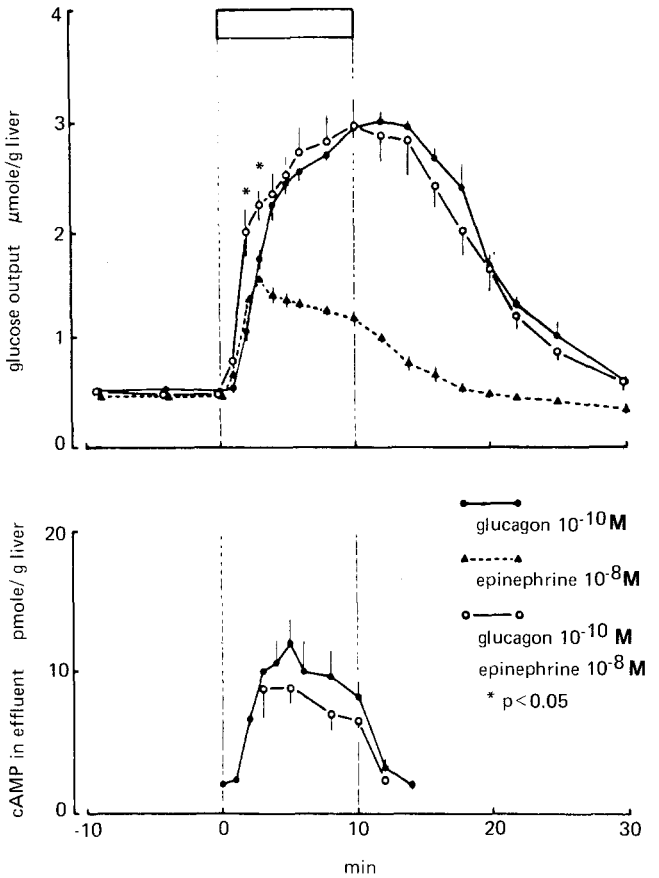

Figure 3 Glucose output (upper panel) and cyclic AMP in the effluent (lower panel) from the isolated perfused rat liver when glucagon 10^{-10}M (●) or epinephrine 10^{-8}M (▲) was administered for 10 min alone or together (○).

is partly due to the difference in the preparation (10) that epinephrine 10^{-8}M did not inhibit the increase of cyclic AMP in the effluent from the isolated perfused rat liver by glucagon 10^{-10}M in this investigation (Fig. 3). So it seems secretin may partly activate adenylate cyclase in a cell other than the hepatocyte, and also it is likely that secretin increases cyclic AMP which is not linked to glycogenolysis in the hepatocytes.

In conclusion, secretin produced a rise in cyclic AMP in the effluent from isolated perfused rat liver but did not affect glycogenolysis in the liver.

REFERENCES

1. Mutt, V. (1980) Gastrointestinal Hormones, pp.85-126, Raven Press, New York.
2. Bataille, P., Freychet, P. and Rosselin, G. (1973) Endocrinology 95,713-721.

3. Desbuquois, B., Laudat, M.H. and Laudat, Ph. (1973) Biochem. Biophys. Res. Comm. 53, 1187-1194.
4. Sugano, T., Suda, K., Shimada M. and Oshino, N. (1978) J. Biochem. 83, 995-1007.
5. Exton, J.M., Robison, G.A., Sutherland, E.M. and Park, C.R. (1971) J. Biol. Chem. 246, 6166-6177.
6. Blackmore, P.F., Dehaye, J.P. and Exton, J.M. (1979) J. Biol. Chem. 254, 6945-6950.
7. Levine, R.A. and Hall, R.C. (1976) Gastroenterology 70, 537-544.
8. Jard, S., Cantau, B. and Jakobs, K.H. (1981) J. Biol. Chem. 256, 2603-2606.
9. Assimacopoulos-Jeannet, F.D., Blackmore, P.F. and Exton, J.M. (1982) J. Biol. Chem. 257, 3759-3765.
10. Okajima, J. and Ui, M. (1982) Arch. Biochem. Biophys. 213, 658-668.