Responses of Patients with Insulinomas to Stimulators and Inhibitors of Insulin Release That Have Been Linked with Cyclic Adenosine Monophosphate

PER-ERIC LINS AND SUAD EFENDIĆ

SUMMARY

The study comprises nine consecutive patients who had pancreatic beta-cell tumors and spontaneous hypoglycemia, seven of whom had verified, benign insulinomas and one a malignant insulinoma with metastases to the liver. The ninth patient, who refused surgery, was followed for three years without displaying any signs of malignancy. Among the patients with benign insulinomas, three showed exaggerated, two normal, and two unusual insulin responses to glucose. One such patient as well as the one with a malignant tumor did not respond to glucose. In general, the insulin responses to glucagon were similar to those to glucose. Somatostatin reduced basal insulin levels as well as the responses to glucose and glucagon only in subjects with normal or exaggerated responses to these agents. Epinephrine behaved like somatostatin when insulin release was stimulated by glucose, but it was a weak inhibitor of the glucagon-stimulated response. The subject with the malignant insulinoma did not respond to either glucose or somatostatin. The remission of the disease, which followed streptozotocin treatment, was accompanied by restoration of the normal responsiveness to the above agents.

Glucagon stimulates insulin release by increasing the intracellular level of cyclic adenosine monophosphate (AMP). The effects of glucose, somatostatin, and epinephrine are supposed to be mediated, at least partially, by way of the adenylcyclase-cyclic AMP system. Therefore, the present data suggest that the variability of the beta-cell responsiveness to glucose and glucagon in patients with insulinoma reflects the responsiveness of the adenylcyclase-cyclic AMP system of the beta-cell tumor, which may be exaggerated, normal, or absent. DIABETES *28*:190–195, March 1979.

Of the two insulinagogues, glucagon and glucose, the former most likely enhances insulin release by stimulating adenylcyclase, leading to increased intracellular accumulation of cyclic adenosine monophosphate (AMP).[1] The precise mode of action of glucose on insulin release is not known, but a series of findings indicate that cyclic AMP plays an important role.[2–4] Furthermore, the two naturally occurring insulin release–inhibiting hormones somatostatin[5–7] and epinephrine[8–10] seem to exert their actions, at least partially, by decreasing the intracellular level of the cyclic nucleotide.

In the present study, we tried to explore the significance of cyclic AMP for the regulation of insulin release from insulinomas by investigating the effects of the above agents, known to interfere with cyclic-AMP formation, in eight patients with benign insulinomas and one with a malignant insulinoma.

MATERIAL AND METHODS

Nine consecutive patients with hyperinsulinism and spontaneous hypoglycemia were studied, six women (no. 1, 3–5, 8, and 9) and three men (no. 2, 6, and 7) (Table 1). They presented classic symptoms in connection with marked hypoglycemia. In all the subjects, elevated basal insulin levels were recorded. In one woman (no. 9), the tumor was malignant with multiple metastases to the liver, and she was treated with streptozotocin. Typical benign beta-cell adenomas were removed in seven patients (no. 1, 2, and 4–8). They were followed postoperatively for at least two years, and no signs indicating recurrence of the disease were noted.

One patient (no. 3) refused both radiologic examination and surgical exploration; despite that, she was included in the study, since the clinical picture and laboratory investigation strongly supported the diagnosis. Thus, the patient experienced repeated hypoglycemic attacks characterized by low blood glucose and high plasma-insulin values, e.g. glucose level during one attack was 1.9 mmol/L and the respective insulin level 56 μU/ml. She has now been followed for three years and has not shown any signs

From the Department of Endocrinology, Karolinska Hospital, S-104 01 Stockholm, Sweden.
Address reprint requests to Dr. Suad Efendić, Department of Endocrinology, Karolinska Hospital, S-104 01 Stockholm, Sweden.
Accepted for publication 7 November 1978.

TABLE 1
General characteristics of patients

Patient no.	Age (yr)	Sex	% of ideal* body wt	Whipple's† triad	Duration of symptoms	Histology	K-value‡
1	70	f	95	Yes	7 yr	Insulinoma	6.9
2	20	m	119	Yes	1 yr	Insulinoma	3.8
3	60	f	84	Yes	9 yr	Not explored	4.7
4	32	f	74	Yes	2 yr	Insulinoma	5.5
5	59	f	77	Yes	4 yr	Insulinoma	—
6	56	m	100	Yes	4 yr	Insulinoma	0.3
7	38	m	99	Yes	6 mo	Insulinoma	1.7
8	65	f	115	Yes	1 day	Insulinoma	2.2
9	43	f	90	Yes	2 mo	Metastatic carcinoma	—

* Metropolitan Insurance Company.
† Attacks with hypoglycemic symptoms in the fasting state; blood glucose below 2.5 mmol/L; relief with glucose administration.
‡ Glucose disappearance rate (see METHODS).

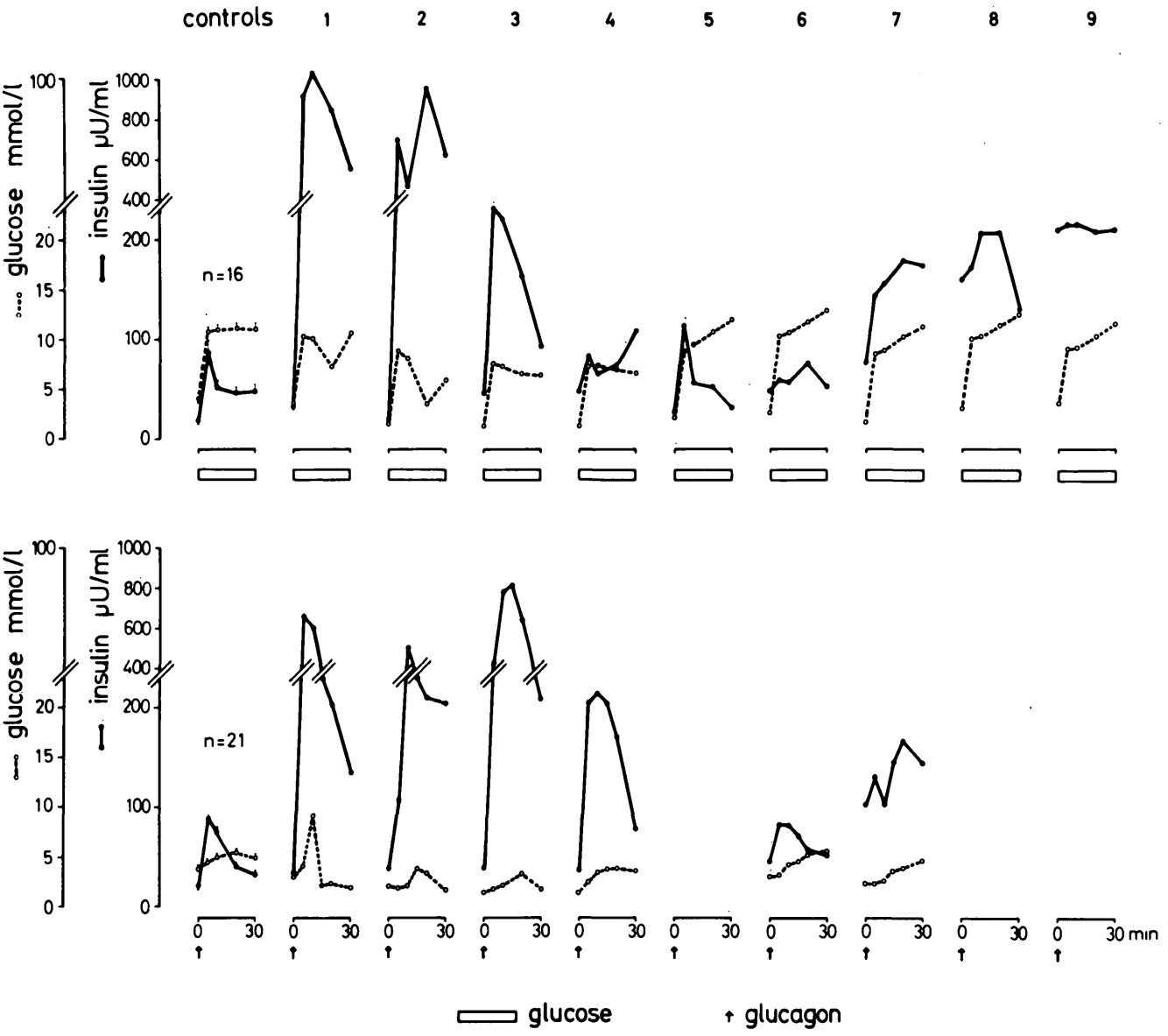

FIGURE 1. Effects of glucose (upper panel) and glucagon (lower panel) on insulin release (solid line) and blood glucose level (broken line) in control subjects (mean ± SEM) and in patients with insulinomas. For experimental conditions, see text.

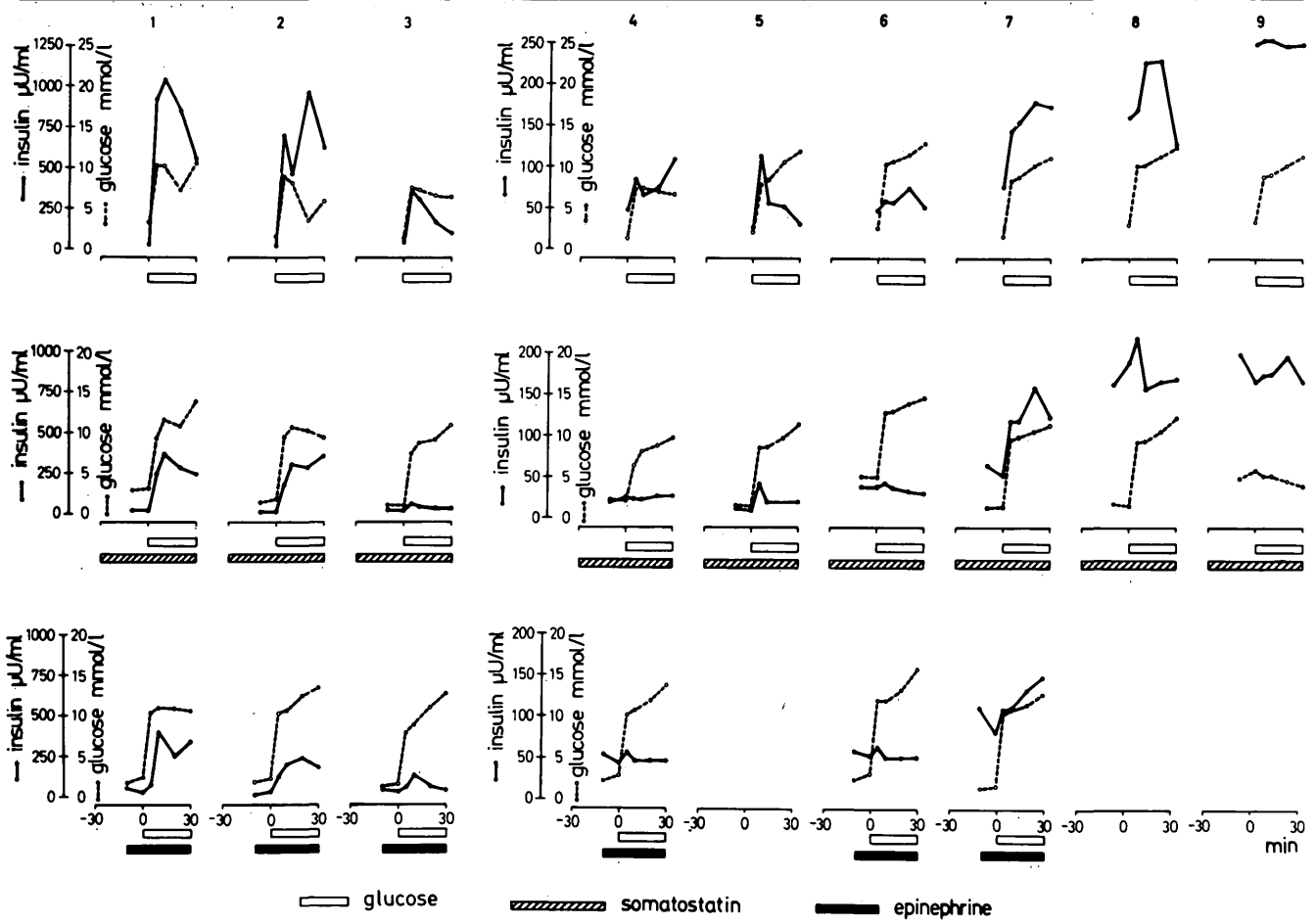

FIGURE 2. Effects of somatostatin (middle panel) and epinephrine (lower panel) on glucose (upper panel)-induced changes in plasma insulin (solid line) and blood glucose (broken line) levels in patients with insulinomas. For experimental conditions, see text.

of malignancy. Control experiments, i.e. glucagon (n = 21) and glucose loadings (n = 16), were performed in healthy nonobese volunteers (26 men and 8 women). Their weights ranged from 90 to 110% of the ideal body weight (Metropolitan Insurance Company). All of them had normal K-values[11] (>1.0), their blood glucose (millimoles per liter) was 3.9 ± 0.4 (mean ± SD) and plasma insulin (microunits per milliliter) 19 ± 3 (mean ± SD) after an overnight fast.

All experiments were performed in the morning after an overnight fast and with the subjects resting in a supine position. As a rule, at least 3 days elapsed between two consecutive tests. The patients were informed in detail about the nature and purpose of the studies.

Experimental designs. Glucose infusion test (GIT) was performed as described previously[12] but with one modification: 250 mg of glucose per kilogram body weight was injected rapidly, followed by a 30-min infusion of 10 mg of glucose per kilogram per minute (in the original GIT, these doses were 500 and 20 mg, respectively). The glucose load was decreased in order to prevent hypoglycemic attacks.

In the glucagon experiments, 1 mg of the hormone (Lilly Co., Indianapolis, Ind.) was given rapidly intravenously. This hormone preparation contained only 400 pg somatostatin per milligram of glucagon.

Somatostatin (linear form) was given as a priming i.v. injection at 30 min before GIT and glucagon administrailon, followed by i.v. infusion of the peptide for 60 minutes. The

TABLE 2
Effect of somatostatin infusion on basal insulin and glucose levels

Patient no.	Experiment	Insulin (μU/ml)/Glucose (mmol/L) in plasma*				
		−40	−30	−25	−10	0 min
1	1	35/3.3	33/3.3	25/2.9	22/2.9	23/3.2
	2	29/2.8	29/4.0	22/2.9	19/2.7	20/2.6
2	1	142/2.4	134/2.0	61/1.8	12/1.4	9/1.8
	2	250/1.6	290/1.5	181/1.3	38/1.3	17/1.2
3	1	47/1.5	44/1.3	34/1.2	21/1.0	19/1.0
	2	34/1.4	32/1.3	25/1.3	20/1.4	19/1.6
4	1	36/2.7	33/3.4	27/2.2	22/2.2	26/2.1
	2	35/2.7	36/2.4	33/2.4	32/2.0	34/1.9
5	1	25/1.7	26/1.6	24/1.8	13/1.5	10/1.4
	2	—	—	—	—	—
6	1	44/5.7	43/5.7	40/5.8	42/5.7	37/5.0
	2	44/2.3	50/2.3	44/2.3	44/2.1	40/1.9
7	1	126/1.7	144/1.6	106/1.5	66/1.3	52/1.4
	2	380/1.8	83/2.1	59/1.9	72/2.0	78/1.7
8	1	211/2.0	172/2.0	180/2.0	162/1.8	189/1.6
	2	—	—	—	—	—
9	1	172/5.5	192/4.2	173/3.1	189/4.9	156/5.9
	2	—	—	—	—	—

* Somatostatin infusion was started at −30 min (see METHODS); at least 3 days elapsed between experiments 1 and 2.

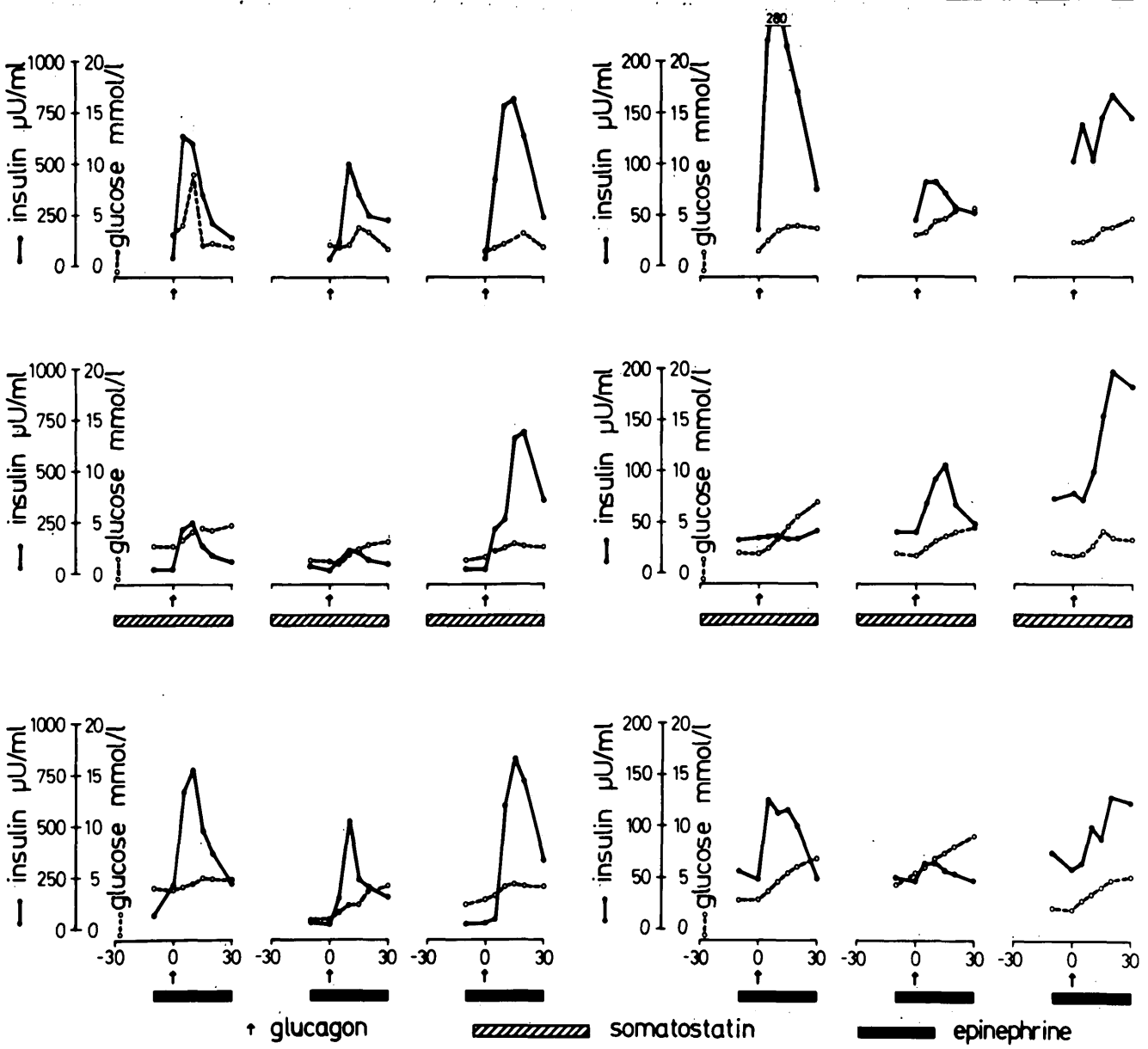

FIGURE 3. Effects of somatostatin (middle panel) and epinephrine (lower panel) on glucagon (upper panel)-induced changes in plasma insulin (solid line) and blood glucose (broken line) levels in patients with insulinomas. For experimental conditions, see text.

priming dose used was 3 μg/kg and the dose during infusion 66 ng/kg/min.

In the epinephrine experiments, the GIT and glucagon tests were combined with epinephrine infusion at a dose of 90 ng/kg/min, given 10 min before and during the GIT and the glucagon administration.

Blood samples were collected in heparinized tubes at the time intervals shown in the figures. Glucose was analyzed in whole blood by the glucose-oxidase method. Plasma insulin was determined by a two-antibody radioimmunoassay.[13]

RESULTS

Effects of glucose and glucagon (Figure 1). In three patients with benign insulinomas (Cases 1–3), glucose administration was followed by insulin release and this was almost ten times higher than that of normal subjects. In two patients (no. 4 and 5), the pattern of insulin response resembled that of normals, whereas in three other patients glucose was either without effect (no. 6) or the insulin response had an unusual pattern (no. 7 and 8). Finally, glucose had no stimulatory effect on insulin release in the patient with malignant insulinoma (no. 9).

The insulin response to glucagon resembled that to glucose. Thus, in the three subjects showing enhanced insulin response to glucose (no. 1–3), glucagon was also a potent releaser of insulin. On the other hand, patients 6 and 7 showed a considerably lower and atypical insulin response to glucagon, as was the case with glucose. Finally, the insulin response to glucagon was somewhat enhanced in spite of a normal insulin response to glucose in patient 4.

Effects of somatostatin and epinephrine on glucose-induced insulin release (Figure 2). Somatostatin suppressed glucose-stimulated insulin release in all the patients with benign insulinomas and with exaggerated or normal insulin responses to glucose (no. 1–5). In patients 7 and 8, who had an unusual insulin response to glucose, this response was not inhibited by somatostatin.

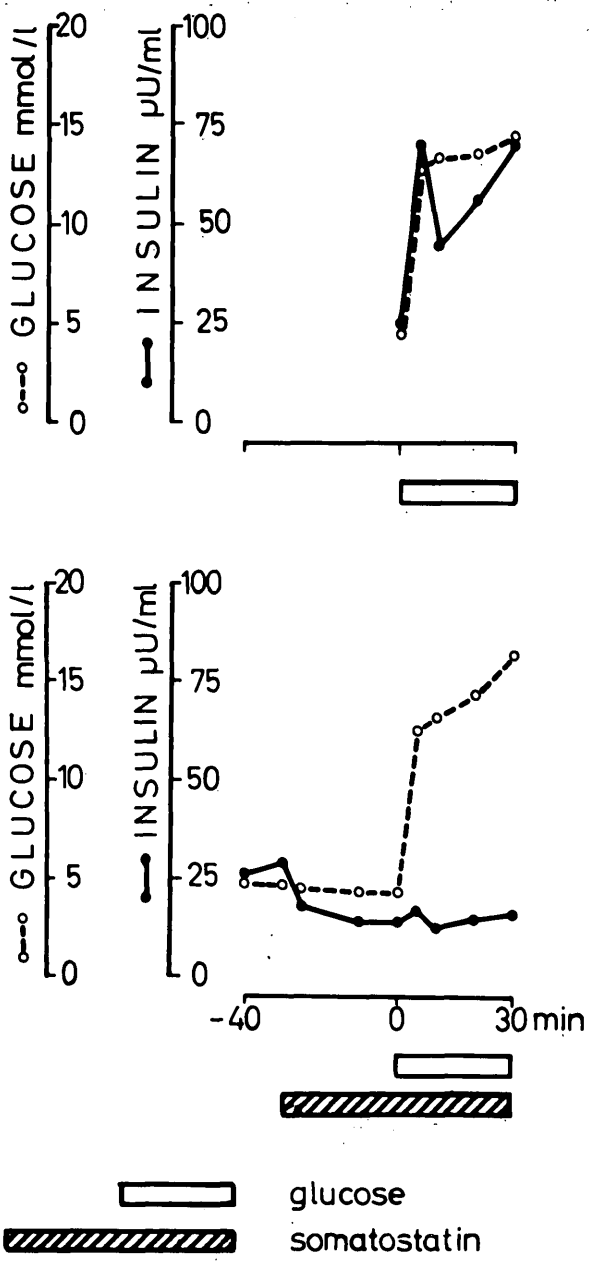

FIGURE 4. Effects of glucose on plasma insulin (solid line) and blood glucose (broken line) levels in the absence (upper panel) and presence (lower panel) of somatostatin in one patient with malignant insulinoma during remission that followed streptozotocin.

The inhibitory effect of epinephrine resembled that of somatostatin.

Effects of somatostatin and epinephrine on glucagon-induced insulin release (Figure 3). Somatostatin readily inhibited glucagon-induced insulin release in the patients with exaggerated or normal insulin response to glucose (no. 1, 2, and 4). Again, somatostatin did not inhibit glucagon-stimulated insulin release in the patient who did not respond to glucose (no. 6) and in the patients with an unusual response to glucose (no. 7).

Epinephrine did not inhibit the effect of glucagon on insulin release in the patients with exaggerated response to glucose and glucagon (no. 1 and 2). It decreased the insulin response in patient 4, showing moderate response to glucagon and normal response to glucose. Finally, epinephrine did not inhibit the effect of glucagon in patients 6 and 7, who showed impaired or unusual response to glucose.

Effect of somatostatin on basal insulin release. The peptide decreased (and almost normalized) the basal insulin levels (≤22 μU/ml) in the patients with exaggerated or normal response to glucose (no. 1–5) (Figures 2 and 3 and Table 2). On the other hand, somatostatin did not normalize the basal insulin levels in the patients with impaired or unusual response to glucose (no. 6–8); nor did it influence basal insulin release in the patient with malignant insulinoma.

Effect of glucose and somatostatin on insulin release in a patient with malignant insulinoma during remission following streptozotocin treatment (Figure 4). Glucose induced normal insulin response, which was readily inhibited by somatostatin.

DISCUSSION

Although all the subjects with insulinomas were not submitted to all the tests, three different patterns of insulin responses, could be distinguished: (a) Exaggerated insulin responses to both glucose and glucagon, which were readily inhibited by somatostatin, whereas epinephrine inhibited only the effect of glucose; (b) normal or somewhat enhanced responses to glucose or glucagon, which were clearly inhibited by somatostatin or epinephrine; (c) impaired or unusual insulin responses to glucose or glucagon, which were not influenced noticeably by somatostatin or epinephrine. Furthermore, somatostatin normalized basal insulin release in subjects belonging to groups (a) and (b).

In other words, the two patients of group (b) with normal insulin responses to glucose behaved like normals regarding responses to stimulation and inhibition of insulin release. They differed from normals regarding basal insulin release which was always elevated, at least in relation to blood glucose. In the three patients of group (a) who had exaggerated insulin responses to glucose, the regulation of insulin release again resembled that of normal subjects but with maximally stimulated insulin release. In normals, somatostatin, used in the same dose as in the present study, markedly inhibited glucose as well as glucagon-induced insulin release, while epinephrine was a strong inhibitor of the effect of glucose and a weak inhibitor of that of glucagon.[14,15] In contrast, in the three patients of group (c) and in the patient with malignant insulinoma the responses to stimulation or inhibition were deranged or absent.

Insulin responses to glucagon[1] and, possibly, to glucose[2–4] are mediated by the adenylcyclase–cyclic AMP system in the beta cells. In this context, our results might indicate that this system is hyperresponsive in some insulinomas, normal in others, and deranged in a third group. The importance of the responsiveness of this system for insulin release in insulinoma patients is further supported by the findings that agents known to decrease insulin release by way of suppression of cyclic AMP—somatostatin and epinephrine—did so only when the system was functioning at least normally, i.e. in patients who showed normal or exaggerated insulin responses to glucose and glucagon. It should be stressed, however, that the above conclusions are based on indirect evidence. Therefore, the possibility cannot be excluded that the variability of insulin responses in patients with insulinomas may be due to differences in

control points other than cyclic AMP. Thus, the heterogeneity of responses to the four agents tested may also be due to modifications of the sensor systems, possibly receptors. It should be pointed out here that no response to glucose was noted in the subject with a malignant insulin-producing tumor. Moreover, the clinical remission of the disease seemed to be followed, indeed, by restitution of the responsiveness of beta cells to glucose. The question whether this finding might be of some use for the follow-up of streptozotocin-treated patients has to be elucidated.

The practical implication of the above findings is that agents inhibiting cyclic-AMP formation in the beta cell might be of therapeutic value in patients with insulinoma who exhibit a normal or hyperresponsive insulin output after glucose or glucagon. Somatostatin proper is not likely to be of value in long-term treatment of responsive insulinomas, since it is so widely distributed in the body. In this respect, it is of interest that a somatostatin analogue has been produced that predominantly inhibits insulin release.[16-17]

ACKNOWLEDGMENTS

We are grateful to Mrs. Anneli Nyberg and Miss Anita Nylén for their technical assistance and to Mrs. Ulla-Britt Wedin and Miss Britt-Marie Witasp for preparing the manuscript.

The somatostatin used was generously donated by Kabi AB, Stockholm.

The investigation was supported financially by the Swedish Medical Research Council (grant B-76-19X-04540-03) and the Nordic Insulin Foundation, Gentofte, Denmark.

REFERENCES

[1] Goldfine, I. D., Roth, J., and Birnbaumer, L.: Glucagon receptors in beta-cells: binding of ^{125}I-glucagon and activation of adenylate cyclase. J. Biol. Chem. *247*:1211–18, 1972.

[2] Charles, M. A., Fanska, R., Schmid, F. G., Forsham, P. H., and Grodsky, G. M.: Adenosine 3′,5′-monophosphate in pancreatic islets: glucose-induced insulin release. Science *179*: 569–71, 1973.

[3] Grill, V., and Cerasi, E.: Activation by glucose of adenyl cyclase in pancreatic islets of the rat. FEBS Lett. *33*:311–14, 1973.

[4] Hellman, B., Idahl, L. A., Lernmark, A., and Täljedahl, J. B.: The pancreatic beta-cell recognition of insulin secretogogues. Does cyclic AMP mediate the effect of glucose? Proc. Natl. Acad. Sci. USA *71*:3405–09, 1974.

[5] Efendić, S., Grill, V., and Luft, R.: Inhibition of somatostatin of glucose-induced 3′:5′-monophosphate (cyclic AMP) accumulation and insulin release in isolated pancreatic islets of the rat. FEBS Lett. *55*:131–33, 1975.

[6] Claro, A., Grill, V., Efendić, S., and Luft, R.: Studies on the mechanisms of somatostatin action on insulin release. IV. Effect of somatostatin on cyclic AMP levels and phosphodiesterase activity in isolated rat pancreatic islets. Acta Endocrinol. (Copenhagen) *85*:379–88, 1977.

[7] Hansen, B. L., and Hedeskov, C. J.: Somatostatin inhibition of glucose-stimulated IRI-release and cAMP production in isolated pancreatic islets. Diabetologia *13*:381, 1977. Abstr.

[8] Turtle, J. R., and Kipnis, D. M.: An adrenergic receptor mechanism for the control of cyclic 3′5′ adenosine monophosphate synthesis in tissues. Biochem. Biophys. Res. Commun. *28*:797–802, 1967.

[9] Kuo, W. N., Hodgins, D. S., and Kuo, J. F.: Adenylate cyclase in islets of Langerhans: Isolation of islets and regulation of adenylate cyclase activity by various hormones and agents. J. Biol. Chem. *248*:2705–11, 1973.

[10] Montague, W., and Cook, J. R.: The role of adenosine 3′:5′-cyclic monophosphate in the regulation of insulin release by isolated rat islets of Langerhans. Biochem. J. *122*:115–20, 1971.

[11] Ikkos, D., and Luft, R.: On the intravenous glucose tolerance test. Acta Endocrinol. (Copenhagen) *25*:312–34, 1957.

[12] Cerasi, E., and Luft, R.: The plasma insulin response to glucose infusion in healthy subjects and in diabetes mellitus. Acta Endocrinol. (Copenhagen) *55*:278–304, 1967.

[13] Hales, C. N., and Randle, P. J.: Immunoassay of insulin with insulin-antibody precipitate. Biochem. J. *88*:137–46, 1963.

[14] Efendić, S., Luft, R., and Claro, A.: Studies on the mechanism of somatostatin action on insulin release. II. Comparison of the effects of somatostatin on insulin release induced by glucose, glucagon and tolbutamide. Acta Endocrinol. (Copenhagen) *81*:743–52, 1976.

[15] Efendić, S., Luft, R., and Cerasi, E.: Quantitative determination of the interaction between epinephrine and various insulin releasers in man. Diabetes *27*:319–26, 1978.

[16] Efendić, S., Luft, R., and Sievertsson, H.: Relative effects of somatostatin and two somatostatin analogues on the release of insulin, glucagon and growth hormone. FEBS Lett. *58*:302–05, 1975.

[17] Efendić, S., Lins, P. E., Luft, R., Sievertsson, H., and Westin-Sjödahl, G.: Studies on the mechanism of somatostatin action on insulin release. V. Effect of somatostatin analogues on arginine-induced release of insulin and glucagon from the perfused rat pancreas. Acta Endocrinol. (Copenhagen) *85*:579–86, 1977.