PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 161, 538–542 (1979)

Effects of Glucagon on Pancreatic Content and Secretion of Amylase in Mice (40592)

TADAO MANABE AND MICHAEL L. STEER[1]

Department of Surgery, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts 02215

Treatment of acute pancreatitis with glucagon has been proposed on the basis of experimental observations that glucagon depresses pancreatic secretion of water, electrolytes, and enzymes in experimental animals and man. The value, if any, of glucagon in the treatment of acute pancreatitis remains controversial, and the mechanisms by which glucagon alters pancreatic exocrine function have not been adequately defined. It is not clear for example, if glucagon's effects on the pancreas are directly or indirectly mediated. We have utilized the mouse as an experimental model with which to study and compare effects of *in vivo* and *in vitro* administration of glucagon. In this communication, we report studies which show that glucagon's effects depend on its method of administration. *In vitro* exposure of the pancreas to glucagon results in stimulation of enzyme secretion while *in vivo* administration of glucagon appears to inhibit enzyme synthesis. These studies suggest that glucagon has both indirect and direct effects on the exocrine pancreas.

Materials and methods. All studies were performed using CD-1 mice, weighing 20 to 25 g obtained from Charles River Laboratories and maintained on normal laboratory chow. Glucagon was obtained from Sigma. Other reagents were of the highest purity commercially available and were obtained from sources previously reported by Steer and Glazer (1).

Effects of glucagon in vitro. Mice were fasted for 18 hr and sacrificed by decapitation. The pancreas was quickly removed and immediately placed in Krebs–Henseleit bicarbonate buffer (m*M* concentrations are: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.56; $MgCl_2$, 1.13; $NaHCO_3$, 25; NaH_2PO_4, 1.15; and glucose, 5.6) equilibrated with 95% O_2–5% CO_2 at 37°C. Each pancreas was cut into four to six fragments which were pooled, washed with the above buffer, and placed in a 25-ml flask containing 3 ml of buffer at 37°C. Each vessel contained approximately 1½ pancreata. The vessel was gassed for 10 sec with 95% O_2–5% CO_2, tightly closed, and preincubated at 37°C with shaking (100/min) for 30 min. After preincubation, the buffer was replaced with 3 ml of fresh buffer containing glucagon and/or the C-terminal synthetic octapeptide of CCK-PZ (Sincalide), gassed, closed, and incubated with shaking for an additional 30 min at 37°C. After this incubation, the tissue was removed, blotted, weighed, and homogenized in 6 ml of phosphate buffer (0.02 *M*, pH 6.9 containing 4 m*M* NaCl). The incubation solutions as well as the homogenized samples were centrifuged to clarity (5000*g*, 15 min), and the supernatants assayed for protein and α-amylase activity.

Effects of glucagon administration in vivo. After an 18-h fast, glucagon was administered subcutaneously at 0, 8, and 16 hr. The glucagon doses were either 0.2 or 2.0 μg per gram body weight, and control animals received an identical volume of the vehicle (0.9% saline) instead of glucagon. Fasting was continued throughout this period. Mice were sacrificed by decapitation at 2, 4, 8, and 24 hr after the initial dose of glucagon. Fasting was continued throughout this period. The pancreas was quickly removed and utilized for *in vitro* secretion studies as described above.

Protein and enzyme assays. Protein was measured according to the method of Lowry *et al.* (2) using bovine serum albumin as the standard. α-Amylase was measured spectrophotometrically according to the method of Bernfeld (3). One unit of α-amylase activity was defined as that which liberates 1 mg equivalent of maltose from soluble starch in 3 min at 30°C.

Analysis of data. Statistical analysis was performed using Student's *t* test as described by Colton (4). Significant changes are defined as those with *P* values of less than 0.05.

[1] Address reprint requests to: Michael L. Steer, M.D., Beth Israel Hospital, 330 Brookline Avenue, Boston, Mass. 02215.

0037-9727/79/090538-05$01.00/0

Results. The effects of glucagon in vitro. Glucagon was noted to directly stimulate basal pancreatic amylase and protein secretion (Table I). The effect of 3.3 μg/ml glucagon was slight, and only a change in amylase secretion was observed. With 33.3 μg/ml glucagon, however, a 40 to 60% stimulation of basal protein and amylase secretion was noted (Table I, Fig. 1). This concentration of glucagon also stimulated amylase secretion from glands already stimulated with the C-terminal octapeptide of CCK-PZ (Sincalide). Even with maximally stimulating concentrations of Sincalide, addition of 33.3 μg/ml glucagon resulted in an additional 20% stimulation of amylase secretion (Fig. 1). Glucagon and Sincalide-induced secretion was additive at each Sincalide concentration so that their sum was the same as that noted when both agents were present (Fig. 1). Higher concentrations of glucagon were not tested.

The effects of in vivo administration of glucagon. Administration of the low dose of glucagon (0.2 μg per gram body weight) at 8-hr intervals for three times significantly reduced the pancreatic content of amylase (Table II). In the presence of a maximally stimulating concentration of Sincalide, amylase and protein secretion over 30 min *in vitro* was not different from controls. The fraction of the pancreatic amylase which was discharged over this period was increased, but this change did not reach statistical significance. In contrast, administration of a higher dose of glucagon (2.0 μg per gram body weight) resulted in a large reduction in pancreatic amylase content. The *in vitro* secretion of protein and amylase in the presence of Sincalide was also reduced, but this reduction was not proportional to the reduction in am-

TABLE I. EFFECTS OF *IN VITRO* GLUCAGON ON BASAL PANCREATIC PROTEIN AND AMYLASE SECRETION[a]

	Protein secretion (mg/g tissue/30 min)	Amylase secretion (U/g tissue/30 min)	Fractional amylase (secretion/30 min)
Control ($n = 6$)	2.45 ± 0.10	622 ± 27	0.068 ± .003
Glucagon 3.3 μg/ml ($n = 6$)	2.74 ± 0.18	723 ± 22*	0.078 ± .003
Glucagon 33.3 μg/ml ($n = 8$)	4.33 ± 0.19***	1053 ± 41***	0.112 ± .004***

[a] Fragments of mouse pancreas were incubated in the presence or absence of glucagon, and amylase and protein secretion measured. The total pancreatic content of amylase in each group was the same. Results represent mean ± SD, and n denotes the number of replicate measurements. The statistical significance of changes from the control values are noted by the asterisk with * signifying a P value of <0.05 and *** a P value of <0.001. Fractional amylase secretion refers to the ratio of amylase secreted after 30 min to total amylase content of sample.

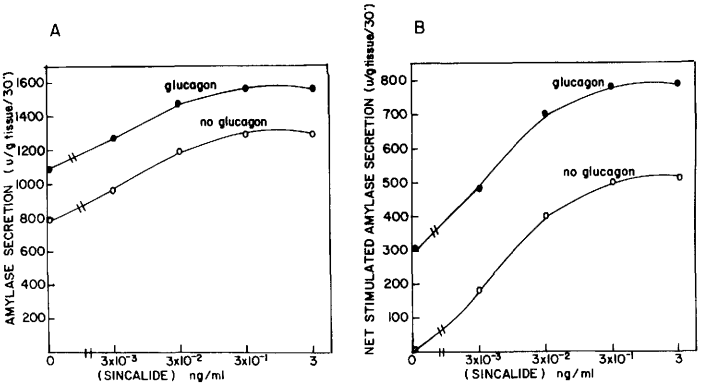

FIG. 1. Effect of *in vitro* glucagon on pancreatic secretion. Pancreatic fragments were incubated with varying concentrations of Sincalide in the presence or absence of glucagon (33.3 μg/ml and amylase secretion over 30 min measured as described in the text. Data represent mean of duplicate measurements which differed by less than 5%. Panel A shows values for total amylase secretion. Panel B shows values for amylase secretion after subtracting basal secretory rate and is referred to as "net stimulated amylase secretion." Values represent mean of duplicate determinations. The figure shown is representative of three such experiments.

TABLE II. EFFECTS OF *IN VITRO* ADMINISTRATION OF GLUCAGON[a]

	Amylase content (U/g tissue)	Protein secretion (mg/g tissue/30 min)	Amylase secretion (U/g tissue/30 min)	Fractional amylase (secretion/30 min)
Control (n = 10)	9273 ± 248	3.98 ± 0.18	1460 ± 90	0.158 ± .009
Glucagon 0.2 μg/g × 3 (n = 10)	7727 ± 352***	3.93 ± 0.28	1439 ± 89	0.183 ± .010
Glucagon 2.0 μg/g × 3 (n = 8)	3609 ± 347***	2.50 ± 0.21***	775 ± 66***	0.217 ± .008***

[a] Glucagon was administered at 0, 8, and 16 hr by subcutaneous injection. At 24 h the pancreas was removed and secretion measured *in vitro* in the presence of 0.3 ng/ml Sincalide over a 30-min period at 37°C. Results represent mean ± SD, and n denotes the number of replicate measurements. The statistical significance of changes from the control values are noted by the asterisk with *** signifying a P value of <0.001. Fractional amylase secretion refers to the ratio of amylase secreted after 30 min to the total amylase content of the sample.

ylase content, and the fraction of the pancreatic amylase which was discharged over this 30-min period actually increased.

The time course of the effects of *in vivo* administration of glucagon on pancreatic amylase content and on the *in vitro* secretion of amylase in the presence of Sincalide is shown in Fig. 2. A significant decrease in amylase content and *in vitro* secretion in the presence of Sincalide was noted throughout the course of the experiment. Two hours after the first injection of glucagon, amylase content had decreased 13% while secretion was 6% less than the control value. By 4 hr the decrements were 45 and 26%, respectively. Eight hours after the first injection (i.e., just prior to the second injection) of glucagon, amylase content and *in vitro* secretion were less markedly reduced from the control values. After the second and third injections of glucagon, however, amylase content and *in vitro* secretion were further reduced so that these values were only 35 and 56% of the control values, respectively. The fractional amylase output was increased at each time point.

Discussion. Previous studies (5–7) have shown that glucagon administration to experimental animals results in pancreatic acinar cell degranulation and diminished enzyme content. The observations of Jarett (8) and Adler (9) that glucagon inhibits pancreatic protein synthesis suggested that the degranulation and diminished enzyme content might reflect glucagon-induced inhibition of enzyme synthesis. The reports of Dyck and co-workers (10, 11), Zajitchuk *et al.* (12), and Shaw and Heath (13) indicated that glucagon causes a rapid reduction in the rate of pancreatic enzyme secretion as well as fluid and electrolyte secretion in humans and experimental animals, and this phenomenon could not be attributed to glucagon-induced inhibition of enzyme synthesis since it occurs very shortly after glucagon is administered. Because of the current interest in glucagon as a therapeutic agent for pancreatitis, we have reexamined the effects of glucagon on pancreatic exocrine function and compared the effects of *in vitro* and *in vivo* glucagon administration. The mouse pancreas was chosen for these studies since, because of their size and cost, large numbers of animals could be easily studied and the statistical significance of observed effects readily demonstrated. In addition, mouse pancreatic fragments can be quickly prepared and *in vitro* secretory studies easily performed.

We have found that glucagon is a potent pancreatic secretagogue *in vitro*. At a concentration of 33.3 μg/ml, glucagon stimulates amylase secretion at a rate which is approximately 50% of that achieved with a maximally stimulating concentration of the C-terminal octapeptide of CCK-PZ (Sincalide). In addition to stimulating the basal secretory rate (Table I), glucagon further stimulates the gland in the presence of a maximally stimulating concentration of Sincalide and, in this case, the effects of the two secretagogues are additive (Fig. 1). This suggests that glucagon and Sincalide interact with the acinar cell membrane at separate sites (i.e., receptors) and that the cellular mechanisms (i.e., "second messengers") mediating secretion induced by these agents differ.

When glucagon is administered *in vivo*, a time- and dose-dependent lowering of pancreatic amylase content is observed. In addition, a parallel change in the *in vitro* secretion of amylase is found which, most likely, results

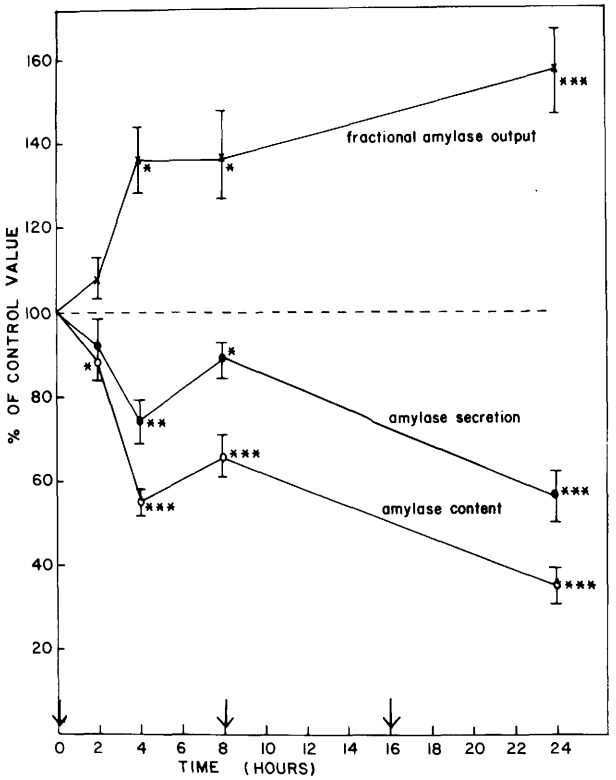

FIG. 2. Time course of effects of *in vivo* administration of glucagon. Glucagon (2.0 μg per gram body weight) or a comparable volume of 0.9% saline was injected subcutaneously at times indicated by the arrows. At various times, mice from glucagon-treated or saline-treated (control) group were sacrificed and the pancreas removed. Total amylase content and amylase secretion over 30 min in the presence of 0.3 ng/ml Sincalide were measured as described in the text. Fractional amylase output refers to the fraction of total amylase content that was secreted over 30 min. Data for glucagon-treated mice are expressed as the percentage of values obtained for mice given saline (control). Values represent mean ± SD of 8 to 12 replicate samples each measured in duplicate. Statistical significance of changes from control values are denoted by asterisks ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

from the diminished enzyme content since the fraction of amylase which is present and that is secreted does not fall. In fact, after *in vivo* glucagon administration, the fractional secretion of amylase actually increases (Table II, Fig. 2). A likely explanation of this finding is that, after glucagon administration and presumably after inhibition of enzyme synthesis, a larger fraction of the digestive enzymes is of the "older" variety and is present in mature zymogen granules near the lumenal membrane rather than present in other areas of the acinar cell. Thus, a larger fraction of the total amylase content would, under these conditions, be available for secretion.

The reported studies suggest that glucagon has at least two and possibly three independent effects on pancreatic acinar cell function and that the response to glucagon depends on the method of glucagon administration. First, as demonstrated by our *in vitro* studies, glucagon can act directly on the pancreas and, in this case, it stimulates enzyme secretion. Second, as noted by our *in vivo* studies, glucagon administration lowers pancreatic enzyme content probably by inhibiting enzyme synthesis. It is not clear whether this is a direct or indirect effect of glucagon on the pancreas. Finally, as demonstrated in previous studies, *in vivo* glucagon administration causes a short-term inhibition of pancreatic secretion. Our studies suggest that this latter phenomenon is an indirect effect of glucagon on the pancreas since, as noted above, glucagon *directly stimulates* secretion.

The early reports of Knight and co-workers (14, 15) suggested that glucagon therapy might be beneficial in acute pancreatitis, but

more recent double-blind studies (16–18) showed that glucagon did not alter the course or mortality rate of acute pancreatitis. In this communication we have demonstrated that glucagon may have multiple effects on normal pancreatic exocrine function, but an understanding of the effects of glucagon during pancreatitis will require additional studies. Our recent observation that glucagon pretreatment but not glucagon treatment decreases the severity and mortality rate of diet-induced acute pancreatitis in mice (19) may indicate that the effects of glucagon on pancreatic function depend on the state of the pancreas at the time of glucagon administration.

Summary. Glucagon administration to intact animals has been reported to inhibit pancreatic exocrine secretion and pancreatic protein synthesis. In this study, the effects of *in vitro* and *in vivo* glucagon on mouse exocrine pancreatic function have been evaluated. When the *in vitro* mouse pancreas was exposed to glucagon, a concentration-dependent stimulation of amylase secretion was noted. Glucagon-stimulated secretion and cholecystokinin/pancreozymin-stimulated secretion were noted to be additive. The *in vivo* administration of glucagon was found to lower pancreatic amylase content in a time and concentration dependent fashion. The *in vitro* secretion of digestive enzymes after *in vivo* administration of glucagon also decreased, but this is believed to be a reflection of diminished pancreatic content since fractional amylase secretion was noted to rise. These observations indicate that glucagon's effects on the mouse exocrine pancreas depend on its method of administration and suggest that glucagon has both indirect and direct effects on mouse pancreatic function.

This work was supported in part by Grant AM 20302 from the National Institute of Arthritis, Metabolism and Digestive Diseases. M.L.S. is the recipient of Career Development Award NS 00160 from the National Institute of Neurological and Communicative Disorders and Stroke.

1. Steer, M. L., and Glazer, G., Amer. J. Physiol. **231,** 1860 (1976).
2. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).
3. Bernfeld, P., *in* "Methods in Enzymology," Vol. 1, p. 144. Academic Press, New York (1955).
4. Colton, T., "Statistics in Medicine," Little, Brown, Boston (1974).
5. Lazarus, S. S., and Volk, B. W., Endocrinology **63,** 359 (1958).
6. Jarett, L., and Lacy, P. E., Endocrinology **70,** 867 (1962).
7. Cameron, J. M., and Melrose, A. G., Brit. J. Exp. Pathol. **43,** 384 (1962).
8. Jarett, L., Proc. Soc. Exp. Biol. **114,** 550 (1963).
9. Adler, G., Cell Tiss. Res. **182,** 193 (1977).
10. Dyck, W. P., Rudick, J., Hoexter, B., and Janowitz, H. D., Gastroenterology **56,** 531 (1969).
11. Dyck, W. P., Texter, E. C., Jr., Lasater, J. M., and Hightower, N. C., Jr., Gastroenterology **58,** 532 (1970).
12. Zajitchuk, R., Amato, J. J., Paloyan, E., and Baker, R. J., Surg. Forum **18,** 410 (1967).
13. Shaw, H. M., and Heath, T. J., Canad. J. Physiol. Pharmacol. **51,** 1 (1973).
14. Knight, M. J., Condon, J. R., and Day, J. L., Lancet **1,** 1097 (1972).
15. Knight, M. J., Condon, J. R., and Smith, R., Brit. Med. J. **2,** 440 (1971).
16. Durr, H. K., Maroske, D., Zelder, O., and Bode, J. Ch., Gut **19,** 175 (1978).
17. Olazabal, O., and Fuller, R., Gastroenterology **74,** 489 (1978).
18. MRC Working Party, Lancet **2,** 632 (1977).
19. Manabe, T., and Steer, M. L., Gastroenterology **76,** 529 (1979).

Received March 2, 1979. P.S.E.B.M. 1979, Vol. 161.