PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 523-527 (1976)

Trophic Effect of Cholecystokinin-Pancreozymin on Pancreatic Acinar Cells from Rats of Different Ages[1] (39583)

F. BRANTS AND J. MORISSET

G.I. Research Unit, Department of Biology, Faculty of Sciences, Sherbrooke University, Sherbrooke, P.Q., Canada, J1K 2R1

In recent years, the gastrointestinal hormones gastrin and cholecystokinin-pancreozymin (CCK-PZ) have been looked upon as potential growth factors of the digestive tract glands. A survey on the spectrum of growth-related responses affected by these hormones shows that chronic *in vivo* administration of large amounts of pentagastrin produces oxyntic gland hyperplasia (1). Lichtenberger and Johnson (2) conclude from their studies on the ontogenic development of the small intestine, that some aspects are dependant on weaning and gastrin may be the mediator.

The hormonal control of pancreatic growth could be controlled by gastrin and CCK-PZ. Indeed, in hypophysectomized rats, pentagastrin treatment stimulates pancreatic hyperplasia in addition to hypertrophy (3). In normal rats, large doses of pentagastrin also result in pancreatic acinar cell hypertrophy (4).

Since CCK-PZ and gastrin share the same five C-terminal amino acids which comprise the active center of the gastrin molecule, one would thus expect the duodenal hormone to have comparable trophic effects on the pancreatic tissue. Recent data from Johnson and Guthrie (5) indicate a certain specificity of the trophic effect of CCK-PZ for the pancreatic tissue since CCK-PZ octapeptide administration was associated with increases in DNA synthesis in the pancreas but not in the oxyntic gland or duodenum. Mainz *et al.* (6) established clearly that CCK-PZ administration at a dose of 20 IVY units kg, ip twice daily for 5 days, was associated with increases in cell mass and cell numbers. In similar studies, Barrowman and Mayston (7) also established the trophic effect of CCK-PZ on the exocrine pancreas.

Since the trophic effect of an hormone implies the regulation of a number of growth-related processes in the target tissue, such as stimulation of protein, RNA, and DNA synthesis, these major effects should be seen at any time during the normal growth of an animal. Because all the previous studies on the pancreas were performed on adult animals, we decided to evaluate the trophic effect of CCK-PZ at different times during postnatal development of the rat exocrine pancreas.

Materials and methods. Sprague-Dawley rats from our own colony were used throughout these studies. The day of parturition was set as Day 1 and the number of rats per litter was fixed at 10 at that time. Half of them served as controls and were injected ip with saline while the other received CCK-PZ (20 IVY units/kg, twice a day) for 5 days before sacrifice. After an overnight fast, the rats were weighed and decapitated at the age of 11, 26, 47, 82 days (adult).

The whole pancreas was excised, trimmed of fat, weighed, and then homogenized in 4 *M* urea (75 mg/ml). Samples of the homogenate were precipitated in 2.1 *N* perchloric acid (PCA) for RNA and DNA determinations. Samples of 800 μl were also centrifuged for 1 hr at 235,000*g* using a SW-50-1 rotor in a Beckman L2-65B; the supernatant was saved for amylase, lipase, chymotrypsin (Chti), and soluble proteins assays.

Amylase activity was assayed according to Bernfeld (8), lipase according to Charbonneau and Morisset (9), and chymotrypsin according to Hummel (10). Activities were expressed as total units in the gland and units/100 μg of DNA, a unit being defined as micromoles of products liberated per minute.

RNA and DNA were extracted as described by Mainz *et al.* (6); DNA was deter-

[1] This work was supported by a grant (MA-5644) from the Medical Research Council of Canada.

mined by the diphenylamine method using calf thymus DNA as a standard (11). RNA was determined by the orcinol method using yeast RNA as a standard (12), while protein was determined according to Lowry *et al.* (13).

Results. Figure 1 shows the effects of chronic administration of saline and CCK-PZ on body weight, pancreatic weight, pancreatic weight in terms of gram body weight, and pancreatic weight in terms of 100 μg of DNA.

Body weights were not affected by CCK-PZ treatment for all the ages studied. Pancreatic weights remained unaffected at 11 days but were significantly increased by 20.5% at 26 days, by 23.2% at 47 days, and by 24.8% in the adults. Data on pancreatic weight expressed in terms of grams body weight indicate that CCK-PZ is associated with hyperplasia and or hypertrophy at all ages, but the effect is less pronounced at 11 days. However, the expression of pancreatic weights in terms of 100 μg of DNA, which is indicative of cell mass, shows that CCK-PZ is efficient only at 26 days. The high increases in amylase, chymotrypsin, and soluble protein concentrations can explain the increase in cell mass which prevails even if DNA is also being stimulated.

As the DNA content is generally considered indicative of cell numbers, CCK-PZ is associated with significant increases of 16.4% in the 47 days and adult rats (Fig. 2). The hyperplasic effect was less important at 26 days (8.3%) but still significant while no effect was seen at 11 days. Since the body weight did not change in the course of treatment, the expression of DNA per gram body weight can represent the number of cells per pancreas. Data thus show that CCK-PZ increased significantly the number of pancreatic cells by 12.4% at 11 days, by 18% at 47 days and by 15% in the adults

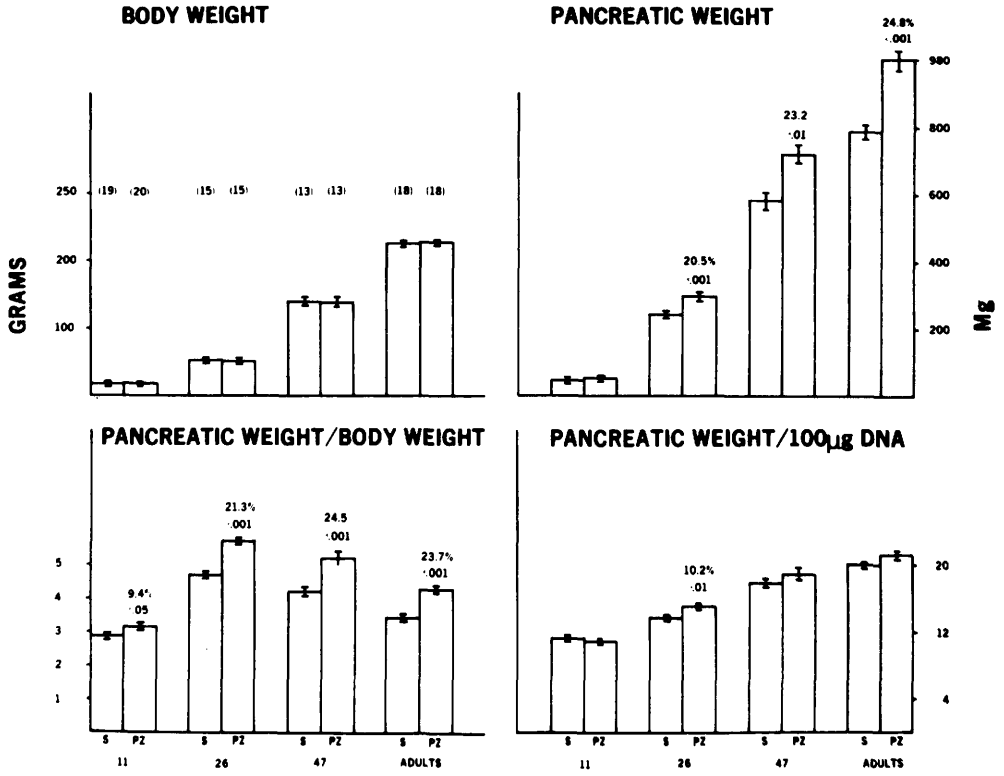

FIG. 1. Effects of chronic administration of cholecystokinin-pancreozymin on body weight, pancreatic weight, pancreatic weight/body weight, and pancreatic weight/100 μg of DNA. (*n*): Number of animals in each group; S: saline group; PZ: cholecystokinin-pancreozymin group. Saline and CCK-PZ (20 IVY units/kg twice a day were injected ip for 5 days.

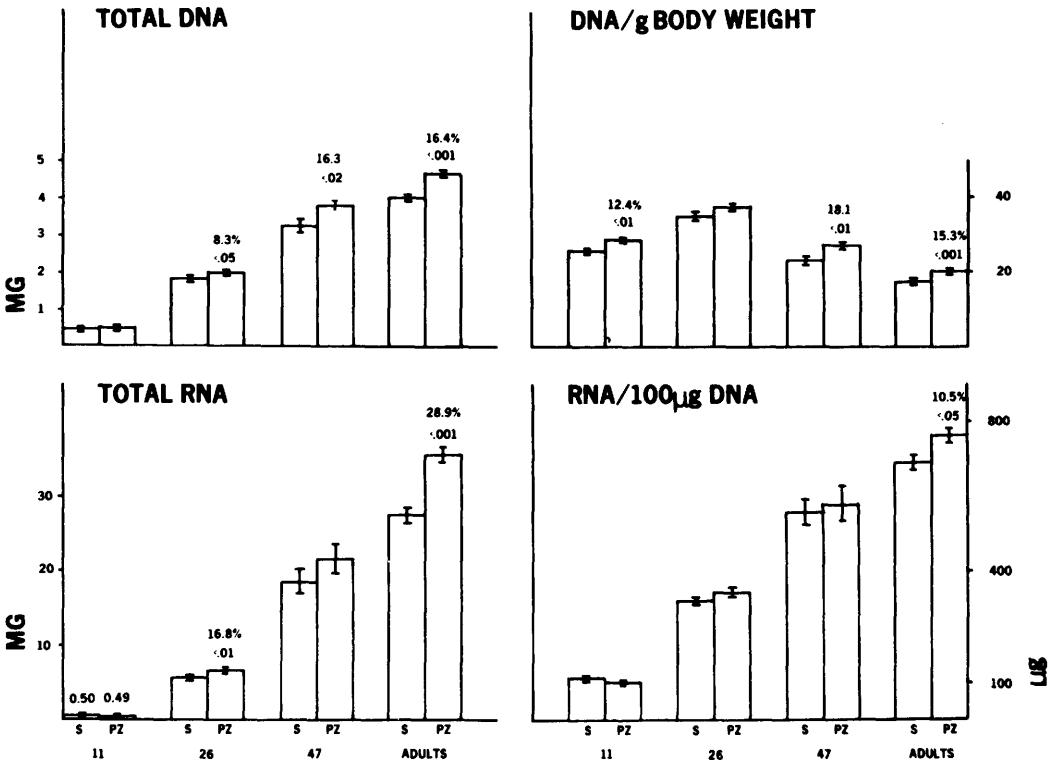

FIG. 2. Effects of chronic administration of cholecystokinin-pancreozymin on total pancreatic DNA content, DNA per gram body weight, total RNA, and its concentration. Treatment and symbols are as in Fig. 1.

while no change was observed at 26 days. Total RNA contents were significantly augmented at 26 days (16.8%) and in the adults (28.9%) while its concentration increased only in the adults (10.5%).

Figures 3 and 4 show that pancreatic amylase content was strikingly increased at 26 (54%), 47 (43%), and 82 days (32%). Parallel increases were observed for chymotrypsin at all ages as well as for the soluble proteins except at 11 days. However, the lipase content was modified only in the adult. The concentration of amylase was increased only at 26 days (41%) while that of chymotrypsin showed an average augmentation of 50% at Days 26, 47, and 82. The soluble protein concentration was significantly modified by 17% at Days 26 and 82. On the contrary, lipase concentration remained unaffected at any time.

Discussion. These studies along with those previously published (5-7) confirm that the pancreas possesses growth capabilities that can be activated by the duodenal hormone cholecystokinin-pancreozymin in adults rats. Our data also indicate that CCK-PZ can provoke hyperplasia in younger animals, those at the age of puberty (47 days).

However, when the treatment was given immediately after weaning (26 days) and on animals still being nursed by their mothers (11 days), hyperplasia following CCK-PZ was moderate. Indeed, the phenomenon is not clear cut since, in these animals, only one of the two criteria indicative of hyperplasia was positive. At 11 days, total DNA was not affected while DNA per gram body weight was significantly increased by 12.4%. In the 26-day-old rats, however, total DNA was elevated by 8.3% ($P < 0.05$) while DNA per gram body weight remained unchanged. This slight trophic effect of CCK-PZ observed at 11 and 26 days could be explained by the fact that at these ages, the pancreatic tissue is already in a state of intensive division as shown by Sesso *et al.* (14). Indeed, their studies indicate

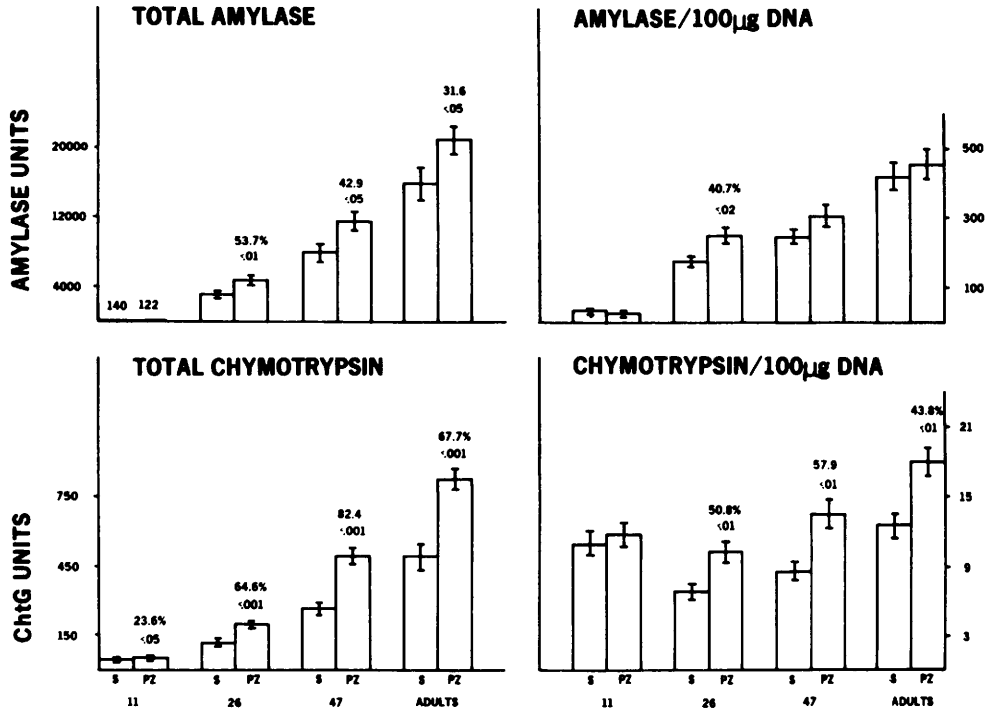

FIG. 3. Effects of chronic administration of cholecystokinin-pancreozymin on total pancreatic content and concentration of amylase and chymotrypsin. Treatment and symbols are as in Fig. 1.

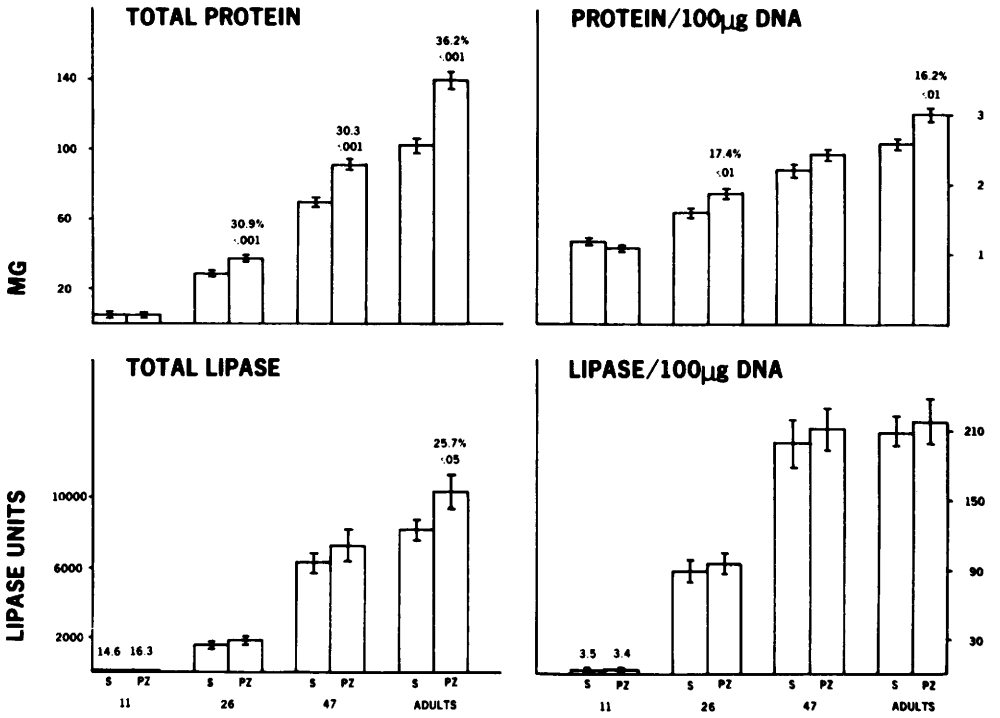

FIG. 4. Effects of chronic administration of cholecystokinin-pancreozemin on total pancreatic content and concentration of soluble protein (supernatant of 235,000*g*) and lipase. Treatment and symbols are as in Fig. 1.

that the mitotic indices are maximum between birth and 23 days; our data would thus suggest that the pancreatic tissue cannot maximally respond to trophic stimuli when the acinar cells are already in a high proliferative state.

Mainz *et al.* (6) show that CCK-PZ administration in adult rats was associated with both hypertrophy and hyperplasia. Their criteria for the evaluation of hypertrophy were increases in cell mass and in the concentration of RNA and proteins. In our studies, the cell mass (pancreatic weight/100 μg DNA) was not significantly increased but the concentration of RNA (+10.5%, $P < 0.05$), soluble protein (+16.2%, $P < 0.01$), and chymotrypsin (+44%, $P < 0.01$) were observed while those of amylase and lipase remained at the control level. Barrowman and Mayston (7) also obtained augmented concentrations of RNA, proteins, and proteases. These observations have to be pointed out because they seem to indicate that CCK-PZ influences the synthesis of a specific class of digestive enzymes: those responsible for the hydrolysis of proteins. In the 47-day old rats, only the chymotrypsin concentration (58%, $P < 0.01$) was modified by CCK-PZ.

CCK-PZ treatment administered to weaning rats was associated with a well-defined hypertrophy since all the parameters (cell mass, RNA concentration, and that of all enzymes and proteins except lipase) were significantly increased over control level. However, 11-day-old rats did not respond to the hypertrophic effect of the hormone since all the parameters indicative of this effect were not changed. This lack of response from the gland does not indicate that it cannot hypertrophy at this stage of development since the pancreas of trypsin inhibitor-fed rats of this age has undergone both hypertrophy and hyperplasia (15).

These studies have presented the kinetics of the hyperplasic and hypertrophic effects of chronic administrations of CCK-PZ. Hyperplasia is moderate in 11- and 26-day rats while well established in older animals (47 and 82 days). Hypertrophy cannot be detected in suckling rats (11 days) but is most evident at 26 days since the concentrations of all parameters studied, with the exception of lipase, are significantly increased. In the 47-day and adult rats, CCK-PZ provokes a selective augmentation in the concentration of RNA and chymotrypsin. Thus, we can say that the trophic effects of the duodenal hormone do exist but vary in intensity and specificity with the age of the animal.

1. Crean, G. P., Marshall, M. W., and Rumsey, R. D. E., Gastroenterology **57,** 147 (1969).
2. Lichtenberger, L., and Johnson, L. R., Amer. J. Physiol. **227,** 390 (1974).
3. Mayston, P. D., and Barrowman, J. A., Gastroenterology **64,** 391 (1973).
4. Mayston, P. D., and Barrowman, J. A., Quar. J. Exp. Physiol. **56,** 113 (1971).
5. Johnson, L. R., and Guthrie, P., Gastroenterology **70,** 59 (1976).
6. Mainz, D. L., Black, O., and Webster, P. D., J. Clin. Invest. **52,** 2300 (1973).
7. Barrowman, J. A., and Mayston, P. D., J. Physiol. **238,** 73 P (1974).
8. Bernfeld, P., *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), p. 149. Academic Press, New York (1955).
9. Charbonneau, R., and Morisset, J., "Forum Int. Francophone. Rech. Gastroenterol." p. 147. Sherbrooke, P.Q. (1975).
10. Hummel, B. C., Canad. J. Biochem. Physiol. **37,** 1393, (1959).
11. Volkin, E., and Cohn, W. E., Methods Biochem. Anal. **1,** 287 (1954).
12. Schneider, W. C., Methods Enzymol. **3,** 680 (1957).
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).
14. Sesso, A., Abrahamsohn, P. A., and Tsanaclis, A., Acta Physiol. Lat. Amer. **23,** 37, (1973).
15. Melmed, R. N., Et-Aaser, A. A. A., and Holt, S. J., Biochim. Biophys. Acta **421,** 280 (1976).

Received June 7, 1976. P.S.E.B.M. 1976, Vol. 153.