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Effects of Reserpine Treatment on Dietary Adaptation of the Rat Exocrine Pancreas

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ABSTRACT. Chronic reserpine treatment (500 μg/kg) of the rat results in generalized exocrinopathy, impaired pancreatic secretion, and decreased pancreatic amylase. These characteristics are similar to those in cystic fibrosis and are the basis for use of this experimental model for cystic fibrosis. Pancreatic enzymes adapt to diet, but it is not known whether chronic reserpine treatment affects this response. Due to the malnutrition induced by this treatment, another dose of reserpine was required that would alter pancreatic function but not induce malnutrition in order to evaluate dietary adaptation. Male rats (100–120 g) were injected subcutaneously daily for 7 days with 1) no injection (control); 2) 1.0 ml/kg vehicle or sham (pair fed-sham); or 3) reserpine: 500, 50, or 5 μg/kg. Food consumption was comparable among control and reserpine-treated (50 and 5 μg/kg) rats and significantly greater (200%) than pair fed-sham and 500 μg/kg reserpine-treated rats. Pancreatic amylase, however, was significantly lower in all reserpine-treated rats (500 μg/kg, 74%; 50 μg/kg, 56%; 5 μg/kg, 52%) than in control rats. To evaluate dietary adaptation, control and reserpine-treated (5 μg/kg) rats were fed high carbohydrate, high fat or high protein diets. Both groups adapted to these diets with the greatest amylase, lipase, and trypsin activities in high carbohydrate-, high fat-, and high protein-fed rats, respectively. Reserpine-treated rats fed high carbohydrate, however, had significantly lower (64%) amylase activity than high carbohydrate-fed control rats. Although reserpine-treated rats can adapt pancreatic enzymes to diet, the adaptation of amylase to carbohydrate is impaired. (*Pediatr Res* 23: 176–180, 1988)

Abbreviations

PFS, pair-fed sham
C, control
R500, reserpine-treated with 500 μg/kg
R50, reserpine-treated with 50 μg/kg
R5, reserpine-treated with 5 μg/kg
HC, high carbohydrate
HF, high fat
HP, high protein
ANOVA, analysis of variance
LSD, least significant difference

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Rats chronically treated with reserpine (500 μg/kg) exhibit altered glycoprotein synthesis and secretion in the cells and ducts of salivary glands (1, 2), lungs (3), and intestine (4, 5). Further, an electron-dense substance is observed obstructing the pancreatic acinar lumen (6), and some zymogen granules appear as less dense, mucin-containing vesicles (7). Abnormalities in the salivary gland include alterations in secretory volume, electrolyte and total protein concentrations (8, 9), and impaired epithelial Cl^- transport (10). Pancreatic morphological abnormalities (6, 11, 12), impaired secretion (12–14), and decreased pancreatic amylase content (13, 14) occur in the reserpine-treated rat. These alterations are similar to those occurring in the human disease cystic fibrosis and are the basis for the use of this experimental animal model for cystic fibrosis (1, 6, 8).

The underlying mechanisms of these effects of reserpine remain unknown. Even though reserpine depletes the brain and peripheral tissues of catecholamines, this does not appear to be the cause of these exocrine effects (16, 17). Reserpine may exert its effect directly on the exocrine cell or indirectly on cellular metabolism. *In vitro* reserpine treatment of colonic cells increases mucus production to the same extent as *in vivo* reserpine treatment (5, 16). Similarly, *in vitro* reserpine treatment of pancreatic acinar cells decreases pancreatic amylase activity and total protein synthesis to the same extent as *in vivo* reserpine treatment (17). The similarity of *in vitro* and *in vivo* reserpine treatment in these cells supports a direct cellular effect.

Chronic reserpine treatment also decreases food consumption (14, 15) and growth in rats and mice (4, 13–15), resulting in malnutrition (14). Pancreatic protein and amylase activity are decreased similarly in chronically reserpine-treated and PFS rats (restricted to the food consumption of the chronically reserpine-treated rats), but pancreatic amylase secretion is significantly less in acini isolated from chronically reserpine-treated rats compared to acini from PFS rats. These results indicate that some effects of reserpine, but not all, may be due to the induced malnutrition (14).

Although chronic reserpine treatment alters pancreatic function, it is not known whether this treatment also affects the adaptation of pancreatic enzymes to diet. Pancreatic secretions always contain amylase, lipase, and the proteases trypsin and chymotrypsin; but the exact composition varies according to dietary intake. A change in diet leads to an alteration in substrate available for hydrolytic enzymes and to a synchronous adaptive response by the pancreas. Amylase content in pancreas and pancreatic juice increases 2- to 3-fold when dietary starch or glucose increases from 20 to 75% (w/w) (18, 19). This adaptation results in an increase in the synthetic rate (4- to 5-fold) of amylase (19, 20) and the amount of mRNA (1.8- to 2.0-fold) (21). Lipase content increases 2-fold with a high fat diet (corn oil or lard) (22). However, lipase activity is only increased when the fat content of food is between 47 and 75% of total calories (23). Chymotrypsinogen content and synthesis is highest with an 82% casein diet, while trypsinogen is highest with a 45% casein diet (24). However, the mRNAs coding for these proteases increase

3.6- to 3.9-fold with a high protein diet (25). The adaptive response of these hydrolytic enzymes to their respective substrates begins within 24 h and reaches a maximum in 5–7 days (18, 26–30).

Herein we examined whether chronic reserpine treatment alters this dietary adaptation of the pancreas. Because reserpine treatment decreases food consumption that can limit the study of dietary effects, the first objective was to determine whether another dose of reserpine would affect pancreatic function similarly to the 500-μg/kg dose without decreasing food consumption. The effects on the secretory responsiveness of isolated pancreatic acini to the secretagogue carbachol of a dose of reserpine that decreased amylase content without affecting food consumption were examined. Using a dose of reserpine that decreased pancreatic amylase content and secretory responsiveness without altering food consumption, the adaptive response of pancreatic enzymes to diet was evaluated in reserpine-treated (5 μg/kg) rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats (100–120 g) were housed individually in hanging cages at 25°C with a 12-h light/dark cycle. Rats had *ad libitum* access to water and Wayne Blox rodent food (Wayne Pet Food, Continental Grain Co., Chicago, IL) or purified diet, unless otherwise specified. To examine the reserpine dose response, rats were weight-matched into five groups and received subcutaneous injections daily for 7 days with 1) no injection (C); 2) 1.0 ml/kg 2% propylene glycol-8% ethanol-5% glacial acetic acid (PFS); or 3) reserpine: R500, R50, or R5. PFS rats were fed at 1630 h daily the amount of food consumed in the previous 24 h by the R500 rats. Reserpine (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.05 ml glacial acetic acid and diluted with 2% propylene glycol-8% ethanol. The pH of the reserpine and vehicle solutions was adjusted to 4.5 with $NaHCO_3$. To study dietary adaptation, rats were weight-matched into three groups fed HC, HF, or HP purified diets (Table 1). Each dietary group was subdivided into two treatment groups: C and R5. Food consumption and body weights were determined daily. Rats in the fed state were killed on day 8 of treatment between 0900–1030 h. The pancreata were removed and frozen at −80°C.

Pancreata were homogenized as described previously (14) in phosphate-buffered saline and centrifuged at 16,000 × g, and 4°C for 30 min. An aliquot of the supernatant was removed for proteolytic analysis, and soybean trypsin inhibitor (final concentration 0.1%) was added to the remaining supernatant. The supernatant was analyzed for amylase (EC 3.2.1.1) by the Phadebas method (31) using certified amylase standards, for protein by the Lowry method using bovine serum albumin as the standard (32), and for lipase (EC 3.1.1.3) by a titrimetric method (33). For proteolytic analyses, the supernatant was activated as described previously (34) with 4% enterokinase at 25°C in a pH 8.1 Tris buffer containing 0.02 M Ca^{2+} for maximal enzyme activity. The activated supernatant was then analyzed for chymotrypsin (EC 3.4.21.1) with glutaryl-phenylalanine para-nitroanilide (35) and for trypsin (EC 2.3.21.4) with benzoyl-arginine para-nitroanilide (36).

Table 1. *Composition of purified diets**

Component	HC (% cal)	HF (% cal)	HP (% cal)
Casein	20.7	20.7	67.0
DL-Methionine	0.3	0.3	0.3
AIN mineral mixture	0.3	0.3	0.3
AIN vitamin mixture	1.0	1.0	1.0
Choline bitartrate†			
Cellulose‡			
Corn oil	10.4	67.0	10.4
Cornstarch	67.0	10.4	20.7

* Modified from Snook (42).
† 0.2% by weight.
‡ 5% by weight in HC and HP; 35% by weight in HF.

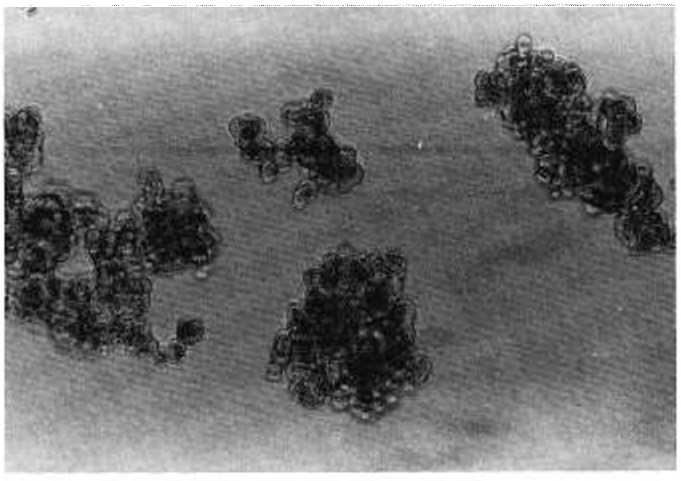

Fig. 1. Dispersed pancreatic acini isolated from control rat. (Phase contrast light micrograph, ×400.)

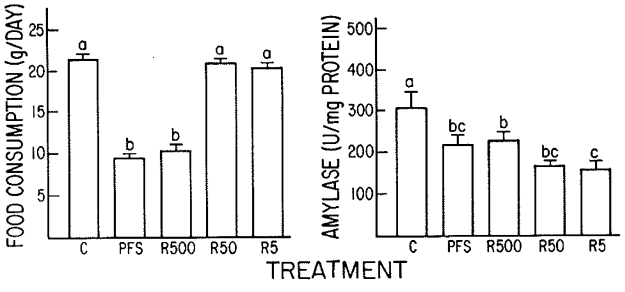

Fig. 2. Effects of various reserpine doses on food consumption and pancreatic amylase. Rats were fed Wayne rodent food, weight-matched into groups, and treated daily subcutaneously with injections as follows: 1) C, no injection and fed *ad libitum* (n = 15); 2) PFS, 1.0 ml/kg 2% propylene glycol-8% ethanol-5% glacial acetic acid and fed the amount of food consumed by the R500 group (n = 14); and 3) R500, 500 μg/kg reserpine (n = 9); R50, 50 μg/kg reserpine (n = 9), or R5, 5 μg/kg reserpine (n = 9) and fed *ad libitum*. After 7 days, rats were killed and pancreata removed. Values are mean ± SEM. ^{abc}Values for a given parameter not sharing a superscript are significantly different ($p < 0.05$) by ANOVA and LSD.

To study secretory responsiveness, pancreatic acini were isolated from C, PFS, R500, and R5 rats by a modified procedure of Bruzzone and coworkers (37). These modifications included a longer digestion (15 min) with collagenase in a shaking water bath at 37°C and 200 cycles/min, separation of exocrine from endocrine tissue through 3% Ficoll (Sigma Chemical Co., St. Louis, MO) 5% calf serum (GIBCO, Grand Island, NY) gradient, and use of 10^{-5} M carbachol (Sigma Chemical Co.) as the secretagogue. Pancreatic acini isolated from a control rat by this procedure showed dispersion of the isolated acini with exposure of numerous basal membranes by light microscopy (400×, Fig. 1). Trypan blue exclusion was similar among acini from all groups (>90%). Isolated acini were preincubated for 30 min at 37°C, allowed to settle via gravity, and were then incubated for 0 or 30 min in the presence of (stimulated) or in the absence of (basal) carbachol. Samples of the medium and cells were removed at 0 and 30 min and were centrifuged at 15,600 × g for 20 s. The media supernatant and cellular pellet from 0- and 30-min samples were analyzed for amylase activity. Amylase release was expressed as the percentage of amylase release (percent of the

Table 2. *Effects of various reserpine doses on body wt, pancreas wt, and lipase activity**

Treatment	Body wt (g)	Pancreatic Wt (g)	Pancreatic Protein (mg)	Pancreatic Lipase (U/mg protein)
Control	175 ± 2†[a]	0.89 ± 0.02[a]	108 ± 4[a]	50 ± 4
PFS	108 ± 2[c]	0.59 ± 0.02[b]	83 ± 5[b]	43 ± 3
R500	103 ± 7[c]	0.63 ± 0.04[b]	89 ± 6[b]	44 ± 6
R50	162 ± 3[b]	0.94 ± 0.04[a]	124 ± 12[a]	34 ± 4
R5	166 ± 5[ab]	0.90 ± 0.04[a]	112 ± 9[a]	48 ± 3

* Values are mean ± SEM. Rats were fed Wayne rodent food, weight-matched into groups, and treated daily with subcutaneous injections as follows: 1) C, no injection and fed *ad libitum* ($n = 15$); 2) PFS, 1.0 ml/kg 2% propylene glycol-8% ethanol-5% glacial acetic acid and fed the amount of food consumed by the R500 group ($n = 14$); and 3) R500, 500 μg/kg reserpine ($n = 9$); R50, 50 μg/kg reserpine ($n = 9$); or R5, 5 μg/kg reserpine ($n = 9$) and fed *ad libitum*.

† [abc] Values for a given parameter not sharing a superscript are significantly different ($p < 0.05$) by ANOVA and LSD.

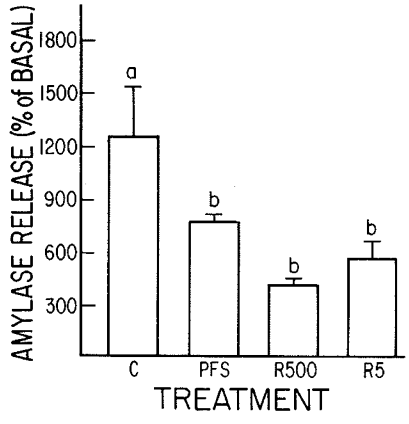

Fig. 3. Effects of different doses of reserpine on pancreatic amylase secretion. Rats were fed Wayne rodent food, weight-matched into groups, and treated with daily subcutaneous injections as described in Figure 2. Values are mean ± SEM of six experiments for PFS and R5, five experiments for C, and four experiments for R500. [ab] Values for a given parameter not sharing a superscript are significantly different ($p < 0.05$) by ANOVA and LSD.

total initial content) in the presence of secretagogue for 30 min to basal amylase release in the absence of secretagogue for 30 min.

Data (mean ± SEM) were analyzed by one-way ANOVA and LSD (38) for the dose response and secretion studies. Two-way ANOVA and LSD (38) were used to analyze the data from the dietary adaptation studies to compare the independent effects of diet and treatment, as well as the interaction between diet and treatment.

RESULTS

Dose response. Food consumption was not altered in the R50 (96%) and R5 (96%) groups but was significantly ($p < 0.001$) decreased in the R500 (46%) and PFS (48%) groups compared to controls (Fig. 2). All reserpine doses significantly ($p < 0.0002$) decreased pancreatic amylase activity: 74% in R500, 56% in R50, and 52% in R5 compared to control (Fig. 2). Final body weights were significantly ($p < 0.001$) reduced in the R500 (58%), R50 (92%), and PFS (62%) groups when compared to control (Table 2). Pancreatic weights and protein were significantly ($p < 0.001$) reduced only in the R500 (71 and 82%) and PFS (66 and 77%) groups (Table 2). Pancreatic lipase was not altered in any of the groups (Table 2). Because there was no significant difference between the 50 and 5 μg/kg doses, the 5 μg/kg dose was used for all subsequent studies. Carbachol-stimulated amylase release as a percentage of basal secretion was significantly ($p < 0.02$) lower in acini from the R500 (35%), R5 (46%), and PFS (64%) rats compared to that from control acini (Fig. 3).

Dietary adaptation. Food consumption was significantly ($p < 0.03$) lower (10%) in the HP-fed rats but was unaffected by reserpine treatment (Table 3). Body weight was not significantly altered by diet or reserpine treatment (Table 3). Pancreatic weight and protein were significantly ($p < 0.001$) higher in the HP-fed rats but were not affected by reserpine treatment (Table 3). Diet significantly affected all pancreatic enzyme activities (U/mg protein) with the greatest amylase in HC-fed rats ($p < 0.001$), the greatest lipase activity in HF-fed rats ($p < 0.001$), the greatest trypsin activity in HP-fed rats ($p < 0.002$), and the lowest chymotrypsin activity in HP-fed rats ($p < 0.01$, Table 3). Only pancreatic amylase activity was significantly ($p < 0.03$) affected by reserpine treatment (Table 3) and was 64% lower in all reserpine-treated groups than controls. There was also a significant ($p < 0.05$) interaction of reserpine and diet on amylase activity (Table 3). Even though pancreatic amylase did adapt to

Table 3. *Effects of chronic reserpine treatment and dietary composition in rat**

Diet	Food consumption (g/day)	Body wt (g)	Pancreatic Wt (g)	Pancreatic Protein (mg)	Pancreatic Amylase (U/mg protein)	Pancreatic Lipase (U/mg protein)	Pancreatic Trypsin (U/mg protein)	Pancreatic Chymotrypsin (U/mg protein)
HC								
C	18 ± 1	151 ± 7	0.76 ± 0.03†[b]	89 ± 2[b]	300 ± 31[a]	43 ± 5	199 ± 11[ab]	5.3 ± 0.7
R5	19 ± 1	150 ± 5	0.78 ± 0.03[b]	92 ± 5[b]	193 ± 43[b]	51 ± 7	177 ± 4[bc]	5.4 ± 0.6
HF								
C	18 ± 1	155 ± 5	0.79 ± 0.07[b]	83 ± 3[b]	36 ± 4[c]	109 ± 9	185 ± 12[bc]	5.5 ± 0.6
R5	17 ± 1	150 ± 5	0.73 ± 0.03[b]	80 ± 4[b]	31 ± 4[c]	76 ± 16	151 ± 7[c]	5.0 ± 0.8
HP								
C	16 ± 1	144 ± 4	0.97 ± 0.05[a]	121 ± 7[a]	80 ± 9[c]	62 ± 12	233 ± 25[a]	3.7 ± 0.3
R5	16 ± 1	139 ± 7	0.98 ± 0.05[a]	131 ± 9[a]	69 ± 12[c]	56 ± 9	232 ± 33[a]	3.7 ± 0.1
ANOVA (p)								
Diet	0.03	NS	0.001	0.001	0.001	0.001	0.002	0.01
Treatment	NS	NS	NS	NS	0.03	NS	NS	NS
Diet × treatment	NS	NS	NS	NS	0.05	NS	NS	NS

* Values are mean ± SEM of six rats. Rats were weight-matched into groups, fed their respective diets *ad libitum* for 7 days, and treated daily with subcutaneous injections as follows: 1) C, no injection and 2) R5, 5 μg/kg reserpine.

† [abc] Values for a given parameter not sharing a superscript are significantly different ($p < 0.05$) by ANOVA and LSD. NS, nonsignificant effect ($p > 0.05$).

HC diet in both C and R5 rats, this adaptation was significantly impaired in the R5 rats compared to C rats (Table 3).

DISCUSSION

Malnutrition or fasting in the rat can lead to a nonparallel decrease in pancreatic enzymes (14, 29). These factors, therefore, can limit studies on dietary effects in the chronically reserpine-treated rat. By using lower doses of reserpine these complications were eliminated. Pancreatic amylase was significantly altered by a 50 or 5 μg dose of reserpine, even when there was no decrease in food consumption. These results suggest that the alterations of pancreatic amylase by reserpine are not secondary to malnutrition.

The lower dose of reserpine also decreased pancreatic amylase secretion. This secretion was decreased in the R500, R5, and PFS rats. In a previous study, pancreatic acini from rats treated with 500 μg/kg reserpine secrete significantly less amylase in response to cholecystokinin than do acini from control rats, while acini from PFS rats show intermediate secretion between reserpine-treated and control acini (14). In the present study using 10^{-5} M carbachol, amylase secretion by acini from PFS rats also tended to be intermediate between that of C and R500 or R5 acini.

In agreement with other studies, body weight was not altered by diet in this study (20, 21). Pancreatic weights, however, were significantly greater in rats fed HP diet than those fed HC and HF diets in the present study. In another study, pancreatic weight was greater in rats fed HP than HC diet (40), but one other study showed no alterations in pancreatic weights in rats fed HP and HC diets (21). Herein, pancreatic protein was greater in rats fed HP diet than in rats fed HC and HF diets.

In this study, pancreatic amylase from control and reserpine-treated rats did adapt to HC diet. Amylase activity was 7-fold higher with HC than with HF diet and 3-fold higher than with HP diet. The changes in amylase with diet reported herein are similar to those reported in other studies (22, 23, 27, 28, 30, 39–41). Also, herein, pancreatic lipase from control and reserpine-treated rats adapted to HF diet. Lipase activity was 2-fold higher with HF than with HC diet and 1.6-fold higher than with HP diet. The 2-fold or 100% increase in lipase activity with HF diet compared to an HC diet was comparable to other studies (22).

In both control and reserpine-treated rats, trypsin activity was 1.4-fold higher with HP than with HF diets and 1.2-fold higher than with HC diet in the present study compared with 1.5- to 1.7-fold higher with HP than with HC diets in another study (18). Contrary to other reports, pancreatic chymotrypsin activity was decreased in control and reserpine-treated rats fed HP diet compared to HC and HF diets herein. Other studies show that chymotrypsin is synthesized in direct proportion to the dietary substrate (24) and its activity is 2- to 3-fold higher with HP than with HC diets (18). These differences in the response of chymotrypsin to dietary protein cannot be readily explained.

Even though amylase adapted to diet in reserpine-treated rats, amylase content was still significantly lower in all reserpine-treated rats than in controls. Further, its adaptation to dietary carbohydrate was impaired in reserpine-treated rats compared to controls. This decreased amylase content raises the possibility that reserpine treatment modifies amylase synthesis, degradation, or secretion. Enzyme content can be decreased by decreased synthesis, increased degradation, or increased secretion. Herein we demonstrated that reserpine treatment decreased secretion suggesting that the decreased amylase content was not due to this change in secretion. The most likely mechanisms of this change are a decrease in synthesis or an increase in degradation or both, but the present data do not distinguish among these possibilities. The rates of synthesis may be altered at the transcriptional or translational levels. Further research is required to determine whether reserpine decreases pancreatic amylase content by decreasing its rate of synthesis or increasing its rate of degradation.

Results from the present study indicate that treatment of rats with a lower dose of reserpine can alter pancreatic amylase content without affecting nutritional status. Reserpine does not alter the adaptation of pancreatic lipase, trypsin, and chymotrypsin to diet, but does impair the adaptation of pancreatic amylase to dietary carbohydrate. These results suggest that reserpine may modify the regulation of pancreatic amylase.

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