Effects of Malnutrition and Chronic Reserpine Treatment on Pancreatic Exocrine Function¹

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ABSTRACT. The chronically reserpine-treated rat, an experimental model for cystic fibrosis, exhibits generalized exocrinopathy, impaired pancreatic secretion, and decreased pancreatic amylase. Although chronic reserpine treatment induces malnutrition by decreasing food consumption and growth, the effects of this malnutrition per se on the exocrine pancreas have not been considered. In this study, the effects of chronic reserpine treatment and malnutrition on the exocrine pancreas were determined using pair-fed controls. Male, Sprague-Dawley rats were treated daily subcutaneously for 5 to 7 days with: 1) no injection (control), 2) 1.0 ml/kg vehicle or sham (control-sham, pair fed-sham), or 3) 0.5 mg/kg reserpine (chronically reserpine-treated). Both chronic reserpine-treatment and pair-feeding significantly decreased food consumption (40%), body weight (51 and 59%), total pancreatic amylase (49 and 56%) and specific amylase activity (62 and 61%), pancreatic protein (65 and 75%), and pancreatic weights (62 and 65%) compared to controls. These decreases, however, were comparable between the chronically reserpine-treated and pair fed-sham rats. In contrast, the secretory response to the biologically active cholecystokinin analog cholecystokinin octapeptide was significantly attenuated in isolated pancreatic acini prepared from reserpine-treated rats compared to that from either control or pair-fed sham rats. Malnutrition decreased pancreatic amylase activity and protein comparably to reserpine treatment, but only partially attenuated the secretory response to cholecystokinin octapeptide. Based on the results of this study, pair-fed controls should be used to distinguish between the effects of reserpine alone and the induced malnutrition on pancreatic exocrine function in studies of this experimental model of cystic fibrosis. (*Pediatr Res* 20: 1236-1239, 1986)

ANOVA, analysis of variance
LSD, least significant difference

CF is the most prevalent lethal genetic disease in the Caucasian race occurring in 1:2000 live births. CF is an autosomal recessive disorder, but the exact nature and mechanism of the defect remains unknown. CF is characterized by chronic pulmonary disease and exocrine malfunction of the pancreas, sweat glands, salivary glands, and the mucus-producing glands of the gastrointestinal tract and respiratory system. It has been difficult to study the pancreatic defects in this disease because of the lack of available human tissue and the lack of an animal model. However, in 1975, Martinez and colleagues (1, 2) proposed the chronically reserpine-treated rat as an experimental animal model for CF. Reserpine-treated rats exhibit altered glycoprotein synthesis and secretion in the cells and ductus of salivary (1, 4), tracheobronchial (5, 6), pancreatic exocrine glands (7), and the intestine (8, 9). In addition they manifest CF-like abnormalities in salivary gland secretions including alterations in volume, electrolyte, and total protein concentrations (2, 3, 10, 11), and they show morphological abnormalities in the pancreas (12, 14), impaired pancreatic acinar secretion (13, 15), and decreased pancreatic acinar amylase content (16). These characteristics are similar to those in patients with CF.

The exact mechanisms by which chronic reserpine treatment exerts these effects are not known. Reserpine's depletion of catecholamine at nerve endings does not appear the cause of these effects (2). Reserpine may act directly on the exocrine cell membrane or may exert a toxic effect on cellular metabolism which leads to alterations in enzyme synthesis, storage, or secretion (4, 17). Reserpine treatment of colonic cells *in vitro* increases mucus production similarly to that seen with *in vivo* treatment (18), supporting a direct cellular mechanism of reserpine action.

Although chronic reserpine treatment alters food consumption and growth in rats and mice, the effects of these alterations on the exocrine pancreas have not been considered (19, 20). Decreased food consumption (malnutrition) or fasting in the rat also can result in decreased pancreatic amylase content (21, 25). Consequently, this study was undertaken to determine the effects of malnutrition and chronic reserpine treatment on the rat exocrine pancreas using pair fed-sham controls.

Abbreviations

CCK₈, cholecystokinin octapeptide
CF, cystic fibrosis
C, control
CS, control sham
PFS, pair-fed sham
CRT, chronic reserpine treated
KHB, Krebs Henseleit bicarbonate buffer
HR, HEPES-buffered Ringer's solution

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MATERIALS AND METHODS

Male, Sprague-Dawley rats (100-180 g) were housed individually in hanging cages at 25° C with a 12 h light/dark cycle. Rats were fed Wayne rodent food (Wayne Pet Food Div., Continental Grain Co., Chicago, IL) as indicated below and water *ad libitum*. Rats were weight-matched into four groups: C, CS, PFS, and

CRT and were injected subcutaneously daily for 5 to 7 days as follows: 1) no injection (C); 2) 1.0 ml/kg 2% propylene glycol-8% ethanol-5% glacial acetic acid (CS and PFS); or 3) 0.5 mg/kg reserpine (CRT). Reserpine (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.05 ml glacial acetic acid and diluted with 2% propylene glycol and 8% ethanol. The pH was adjusted to 4.5 with 1 N NaOH. C, CS, and CRT rats were fed *ad libitum*. PFS rats were fed at 1630 h daily the amount of chow consumed in the previous 24 h by the CRT rats. Food consumption and body weights were determined daily.

To measure pancreatic enzyme content, rats were killed on day 8 between 0900 and 1030 h. The pancreata were removed and frozen at -80°C . Pancreata were homogenized in phosphate-buffered saline-1% trypsin inhibitor and centrifuged at $16,000 \times g$, 4°C , 30 min. The supernatant was analyzed for amylase (E.C. 3.2.1.1) by the Phadebas method using the Phadebas standard reference serum (26), for protein by the Lowry method using bovine serum albumin as the standard (27), for DNA by a microfluorometric method (28), and for lipase (E.C. 3.1.1.3) by a titrimetric method (29).

To determine pancreatic secretion, rats were killed on day 6 following an overnight fast. Fasting overnight reduced variability of secretion among rats; however, rats treated for 7 days with reserpine had a limited tolerance for fasting. Therefore, rats were killed after 5 days of reserpine-treatment rather than 7 days to ensure viability of the isolated acini. Isolated pancreatic acini were prepared as previously described (30, 31). Following injection of pancreatic tissue with 10 ml KHB containing 300–350 U collagenase (Cooper Biomedical, Malvern, PA) and 18 mg hyaluronidase (Sigma Chemical, St. Louis, MO), acini were separated from cellular debris by sedimentation in KHB buffer containing 4% bovine serum albumin (30). Acini were then preincubated in HEPES-buffered Ringer's solution supplemented with 0.01% soybean trypsin inhibitor (HR buffer) for 60 min at 37°C (30). Viability was monitored by trypan blue exclusion and was always greater than 95%.

Before use in secretion experiments, acini were resuspended in 40 to 50 ml fresh HR buffer. Amylase activity in the supernatant and cell pellets was assayed as described to obtain total amylase content. Aliquots of suspensions of acini were then incubated at 37°C for 30 min with or without various concentrations of CCK₈ (30). Amylase release during this incubation was expressed as the percent of acinar amylase released in the presence of CCK₈ compared to the release in the absence of CCK₈ (control).

Data were analyzed by ANOVA and LSD (32). Differences were significant when $p < 0.05$. Data are expressed as mean $\pm$ SEM.

RESULTS

After 2 days, the CRT rats became hypodynamic and exhibited palpebral ptosis and diarrhea, while C and PFS rats exhibited normal behavior. CRT and PFS rats consumed significantly ($p < 0.001$) less food (40%) than the C group (Table 1). Pancreatic weights significantly decreased ($p < 0.001$) in the reserpine-treated (65%) and PFS (62%) rats (Table 1). The ratio of pancreatic weight to body weight significantly increased ($p < 0.001$) only in the reserpine-treated rats. C and CS rats gained 44% of initial body weight in 8 days while the CRT and PFS rats lost 26 and 20% of initial weight (Fig. 1). C and CS rats had significantly ($p < 0.001$) greater final weights than PFS (59%) and reserpine-treated (51%) rats. Further, the final body weight of PFS rats was significantly greater than that of reserpine-treated rats.

Total pancreatic amylase-activity (U) decreased significantly ($p < 0.05$) in the reserpine-treated (49%) and PFS (56%) rats compared to the C group (Fig. 2). However, total amylase-activity was comparable in the reserpine-treated and PFS rats. Specific amylase activity (U/mg pancreatic protein) also significantly

Table 1. Effects of reserpine treatment on food consumption and pancreas*

Treatment	Food consumption (g/day)	Pancreas wt (g)	Ratio of pancreatic wt (g) to body wt (g) ($\times 10^{-3}$)
C	21.7 ± 0.6^a	0.92 ± 0.02^a	5.21 ± 0.099^{bc}
CS	21.5 ± 0.5^a	0.83 ± 0.02^b	4.80 ± 0.142^c
PFS	8.8 ± 0.4^b	0.60 ± 0.02^c	5.71 ± 0.113^b
Reserpine	8.8 ± 1.0^b	0.57 ± 0.04^c	6.37 ± 0.363^a

* Values are mean $\pm$ SEM of 8 rats. Rats were fed rat food, weight-matched into groups and injected daily subcutaneously with 1) no injection and fed *ad libitum* (C), 2) 1.0 ml/kg 2% propylene glycol-8% ethanol-5% acetic acid and fed *ad libitum* (CS), 3) 0.5 mg/kg reserpine and fed *ad libitum* (reserpine), and 4) 1.0 ml/kg sham and fed the amount of food consumed by the reserpine-treated group (PFS). After 7 days, rats were killed and pancreata removed.

^{abc} Values for a given parameter not sharing a superscript are significantly different ($p < 0.001$) by ANOVA and LSD.

Fig. 1. Effects of chronic reserpine treatment on growth of rats. Rats were fed rat food, weight-matched into groups, and treated daily subcutaneously with injections as follows: 1) C, no injection and fed *ad libitum*, 2) CS, 1.0 ml/kg 2% propylene glycol-8% ethanol-5% glacial acetic acid and fed *ad libitum*, 3) CRT, 0.5 mg/kg reserpine and fed *ad libitum*, and 4) PFS, 1.0 ml/kg sham and fed the amount of food consumed by the reserpine-treated group. Initial (I) and final (F) body weights are shown as mean $\pm$ SEM for eight rats. ^{abc} Values are significantly different than corresponding control values ($P < 0.05$) by ANOVA and LSD.

Fig. 2. Effects of chronic reserpine treatment on pancreatic enzymes. Rats were fed rat food, weight-matched into groups, and treated with daily injections subcutaneously as described in Figure 1. Values are mean $\pm$ SEM of eight rats. ^{abc} Values for a given parameter not sharing a superscript are significantly different ($P < 0.05$) by ANOVA and LSD.

Table 2. Effects of reserpine treatment on pancreatic exocrine function*

Treatment	DNA (μ g)	Pancreatic protein (mg)	Amylase (U/mg protein)
C	64 $\pm$ 7 ^a	108 $\pm$ 5 ^a	341 $\pm$ 44 ^a
CS	51 $\pm$ 2 ^a	101 $\pm$ 4 ^{ab}	293 $\pm$ 44 ^{ab}
PFS	48 $\pm$ 5 ^a	92 $\pm$ 7 ^{bc}	208 $\pm$ 18 ^b
Reserpine	59 $\pm$ 10 ^a	81 $\pm$ 5 ^c	210 $\pm$ 21 ^b

* Values are mean $\pm$ SEM of 8 rats. Rats were fed rat food, weight-matched into groups and injected daily subcutaneously as described in Table 1.

^{abc} Values for a given parameter not sharing a superscript are significantly different ($p < 0.05$) by ANOVA and LSD.

Fig. 3. Effect of CCK₈ on amylase release. Acini were incubated for 30 min with or without the indicated concentrations of CCK₈. Values are expressed as the percent of acinar amylase released in the presence of CCK₈ compared to the respective release in the absence of CCK₈ (C) and are mean $\pm$ SEM of four or five experiments. ^{abcde} Values not sharing a superscript differed significantly ($P < 0.05$) by 2-way ANOVA and LSD.

decreased ($p < 0.005$) in the reserpine-treated (62%) and PFS (61%) rats compared to the C group, but again was comparable between reserpine-treated and PFS rats (Table 2). Pancreatic protein also decreased comparably in the reserpine-treated (75%) and PFS (85%) rats. Pancreatic lipase was not altered by reserpine treatment or food restriction compared to C rats (Fig. 2). DNA also was comparable among the four groups (Table 2).

CCK₈ exerted a characteristic dose-dependent effect on amylase release in acini prepared from C, PFS, and reserpine-treated rats (Fig. 3). Maximal stimulation of amylase release occurred at 3×10^{-10} M CCK₈ in all three groups. Higher concentrations of CCK₈ were less effective, exhibiting what has been termed a restricted stimulation. The magnitude of the stimulatory effect was greatest in the controls, intermediate in the PFS, and least in the reserpine-treated group. Further, there was a highly significant ($p < 0.0001$, $F_{2,67} = 28.629$) effect of *in vivo* reserpine treatment on the secretory responsiveness of isolated acini as analyzed by 2-way ANOVA and LSD.

DISCUSSION

Reserpine-treatment of rats resulted in decreased food consumption as previously reported in neonatal rats (20) and adult mice (19). The decreased food consumption resulted in lower body weights (19, 20). In the present study, the decreased body

weight cannot be attributed solely to decreased food consumption because the PFS rats lost less weight than the reserpine-treated rats despite comparable food intake. The 40% decrease in food consumption, 49% decrease in body weight, and the 39% decrease in pancreatic weight in reserpine-treated rats demonstrated that reserpine treatment induced malnutrition.

Pancreata were smaller in the reserpine-treated and PFS rats compared to C rats. However, pancreatic weight was greater relative to total body weight in the reserpine-treated rats. In contrast, Perlmutter and Martinez show a significant reduction in pancreas weight of reserpine-treated rats, but the ratio of pancreatic weight to body weight remains comparable to the C rats (15). Chung *et al.* (20), however, report slightly higher pancreatic weights relative to body weight in reserpine-treated rat pups compared with controls. The higher pancreatic weight to body weight ratios suggest a relative sparing of the pancreas in reserpine-treatment.

Our results showed that chronic reserpine-treatment decreased rat pancreatic protein, amylase, and specific amylase activity. Similarly, Joehl and DeJoseph (16) show a significant decrease in the total amylase content of rat pancreatic acini isolated from CRT rats. However, these parameters were comparably decreased in the malnourished PFS rats in the present study. Other studies of malnutrition show either a parallel decrease in pancreatic enzymes (20) or a nonparallel decrease in pancreatic amylase and an increase in pancreatic lipase (23, 24). Pancreatic lipase, however, was not affected by either reserpine-treatment or malnutrition (PFS) in this study resulting in a nonparallel change in pancreatic enzymes. The decreased amylase and total protein observed in both the reserpine-treated and PFS rats suggest that the reserpine-induced alterations in pancreatic enzyme content may be secondary to the malnutrition.

Despite the comparable decrease in amylase content in pancreata from PFS and CRT rats, the secretory response of these acini were distinctly different. CCK₈-mediated amylase-release in acini prepared from PFS rats was significantly greater than the release by CRT acini and significantly less than C acini (Fig. 3). Although chronic reserpine treatment caused a markedly attenuated secretory response over a wide concentration range of CCK₈, it did not shift the dose-response curve. This attenuated responsiveness might be due either to a receptor or postreceptor defect. Irrespective of the underlying mechanisms, these data indicate that the alterations in secretory responsiveness resulting from malnutrition only partly account for those induced by reserpine.

Results from the present study suggest that chronic reserpine leads to a dual defect in the exocrine pancreas. One defect can be attributed to the malnutrition induced by reserpine treatment, while the other is more directly related to the reserpine treatment. Therefore, studies using the reserpine-treated experimental model need to control for the induced malnutrition by using appropriate pair feeding.

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