Biochemical Changes in the Exocrine Pancreas of Rats Fed Caffeine (42901)

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Abstract. **Coffee consumption has been associated with pancreatic disorders, but the mechanisms involved remain to be elucidated. This investigation examines the effects of caffeine consumption on the structure and function of the exocrine pancreas. Groups of rats, fed *ad libitum* commercial laboratory diet, were given drinking water which contained either caffeine (0.09 mg/ml) or nothing at all. The rats were allowed drink *ad libitum* and were killed 6 weeks later. Final body and pancreatic weights were not significantly different between the groups at the end of the experimental period. Although no ultrastructural effects of caffeine on the pancreas were observed, amylase and trypsinogen activity was 35% higher in pancreatic homogenates from caffeine-fed rats compared with controls. In addition, levels of immunoreactive cationic trypsin(ogen) were 41% higher than control levels in pancreases from the caffeine-fed rats. Also, the circulating levels of amylase and immunoreactive cationic trypsin(ogen) in serum were lower in the caffeine group compared with controls. When dispersed pancreatic acini isolated from the caffeine-fed rats were incubated *in vitro* with increasing concentrations of CCK-8 or nicotine, the rate of release of amylase, trypsinogen, and chymotrypsinogen was lower than in the control rats. This effect did not appear to be due to inhibition of protein synthesis, as determined by [^{3}H]leucine incorporation into acinar protein. These data suggest that prolonged intake of caffeine at common dietary levels inhibits pancreatic enzyme secretion.** [P.S.E.B.M. 1989, Vol 191]

Exocrine pancreatic secretion is known to be influenced by a number of hormonal and environmental factors. Of the environmental factors, cigarette smoking and nicotine have received much attention as important risk factors in the pathogenesis of pancreatitis and pancreatic carcinoma (1–3). A number of investigators have shown that both cigarette smoking and nicotine can stimulate secretion of pancreatic enzymes (4-6 and references cited therein). In recent years, however, controversy has arisen over the association between coffee drinking and a higher incidence of pancreatic adenocarcinoma (7–9). Although MacMahon *et al.* (9) reported that this association was much greater than an association between pancreatic cancer and cigarette smoking, Istvan and Matarazzo (10) found a high correlation between tobacco use and coffee drinking, making it difficult to separate the contribution of these parameters in epidemiologic studies. Since chronic stimulation of the exocrine pancreas, as observed in cigarette smokers, may lead to pancreatic carcinoma (cf. 11), the potential role of coffee consumption or caffeine in pancreatic pathophysiology needs investigation.

Caffeine is one of the most widely used drugs in the world (12, 13), yet little is known about its effects on the structural and functional properties of the pancreas. Andriulli *et al.* (14) could not demonstrate an association between coffee consumption and serum immunoreactive cationic trypsinogen levels following a secretin test given to normal individuals. Coffey *et al.* (11), however, found higher exocrine pancreatic secretion of trypsin in the interdigestive period, following a single administration of caffeine, coffee, decaffeinated coffee, or other substances in coffee. This investigation examines the ultrastructural changes as well as the responsiveness of the exocrine pancreas to various secretagogues in rats fed caffeine in their drinking water for 6 weeks.

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Materials and Methods

Animal and Diets. Two groups of adult, male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA) weighing initially 195 g were fed *ad libitum* a commercial laboratory chow (Wayne Rodent Blox;

Wayne Pet Food, Chicago, IL). In the caffeine-fed group, caffeine was added to the drinking water (0.09 mg/ml). In preliminary studies this level of caffeine was well tolerated and did not induce the overt behavior changes we observed when rats were fed 0.12 mg/ml of caffeine. Water was freely available to both groups of rats. Food consumption and water intake were monitored every other day, and animals were weighed weekly throughout the experimental period. Since this level of caffeine intake did not affect food intake, it was not necessary to include a pair-fed group in this study.

After the 6-week dietary period, rats were weighed and anesthetized with ether. Blood was drawn by cardiac puncture and allowed to coagulate on ice. Serum was recovered by centrifugation and stored at −80°C. The pancreas was quickly removed, washed in cold saline, trimmed of fat, blotted dry, and weighed. An aliquot (0.1–0.2 g) of the pancreas was frozen and stored at −80°C. The rest of the pancreas was immediately used for isolation of acini.

Isolation of Acini. Dispersed pancreatic acini were isolated from 18-hr fasted rats with slight modification (6) of the procedure described by Williams *et al.* (15). Routinely, acini isolated from 1 g of pancreas were suspended in 10 ml of oxygenated Hepes-buffered Ringer solution (HR buffer) and equilibrated at 37°C for 30 min. Acini were then recovered by a brief centrifugation, resuspended in 40–45 ml of oxygenated HR buffer and immediately divided into 1-ml aliquots. Trypan blue exclusion was used as an assessment of cellular viability, and only preparations containing over 95% viable cells were used.

Incubation Conditions. One-milliliter aliquots of cell suspension were incubated further at 37°C in the absence and presence of cholecystokinin-octapeptide (CCK-8), secretin, carbachol, and nicotine as described in the figure legends. The reactions were terminated by centrifugation at 2000 *g* for 10 min, and the supernatant was assayed for amylase (16), trypsinogen (17), chymotrypsinogen (17), and LDH as previously described (18). The 2000 *g* pellet (cells) was resuspended in dispersing buffer, sonicated, and recentrifuged (19). The resultant supernatant was assayed for amylase, trypsinogen, and chymotrypsinogen as described above. The pellet containing the cells was then homogenized in 1 ml of 0.2 *M* $HClO_4$, and DNA, RNA, and protein were extracted as described previously (18).

In experiments in which the rate of acinar protein synthesis was determined, acini were suspended in HR buffer devoid of unlabeled leucine. The reaction was initiated with 1 μCi of [^{3}H]leucine, terminated by the addition of 1 m*M* cycloheximide, and followed by an immediate centrifugation. Other details of this procedure have been described (18).

Processing of Pancreases. Pancreatic tissue, 0.1–0.2 g, was homogenized in 2 ml of 0.2 *M* Tris-HCl (pH 8.0) containing 0.05 *M* $CaCl_2$ in a tissuemizer. DNA, RNA, and proteins were extracted from $HClO_4$ precipitates of an aliquot of the homogenate according to the method of Wannemacher *et al.* (20). DNA and RNA content were measured according to the method of Burton (21) and Ceriotti (22), respectively. Protein was determined according to the method of Bradford (23). The remaining pancreatic homogenate was centrifuged at 10,000 *g* for 30 min at 2°C, and the supernatant was assayed for amylase, trypsinogen, chymotrypsinogen, and immunoreactive cationic trypsin(ogen).

Radioimmunoassay of Cationic Trypsinogen. Cationic trypsinogen content in serum and pancreatic homogenate was determined by specific radioimmunoassay as previously described (24). A dilution of 1:250,000 of specific antiserum was used. Samples were diluted in radioimmunoassay buffer and assayed in three appropriate dilutions with duplicate determinations for each dilution, using purified rat cationic trypsin as standard.

^{3}H-Pulse-Labeled Acini. The acini were pulse labeled by incubating with 54 n*M* [^{3}H]leucine in leucine-free HR buffer for 3 min at 37°C. The acini were then washed twice in HR buffer containing 4 m*M* unlabeled leucine and subsequently incubated in HR buffer containing essential amino acid in the absence and presence of 100 p*M* CCK-8 or 6 m*M* nicotine. Incubations were terminated by the addition of 1 m*M* cycloheximide, followed by immediate centrifugation. The cells and the medium were processed for liquid scintillation counting as described (18).

Electron Microscopy. Pancreatic aliquots from both control and caffeine-fed rats were fixed in 2% glutaraldehyde in cacodylate buffer. Tissue was then cut into 1-cm^3 cubes and further fixed in glutaraldehyde, washed in cacodylate buffer, and postfixed in 1% osmium tetroxide. The tissue was then serially dehydrated in a graded alcohol, infiltrated with propylene oxide, and embedded in Spurr's low viscosity resin. One-micrometer-thick sections were cut and stained with toluidine blue to localize the area of interest. Thin sections were cut with a diamond knife and sections were examined and photographed in a Zeiss EM 109 electron microscope.

Serum Assays. Caffeine concentrations in serum were determined according to the high performance liquid chromatography method of Scott *et al.* (25), following the chloroform/isopropanol extraction of serum. Use of caffeine standards indicated at least a 90% recovery of caffeine in serum by this procedure. Trypsin-inhibitory capacity of α_1-protease inhibitor in serum was determined following incubation of known amounts of trypsin with serum as previously described (26).

Statistical Analysis. Where applicable, the experiments were statistically evaluated by Student's *t* test for nonpaired values, with $P < 0.05$ considered significant.

Results

In the present experiment, caffeine consumption for 6 weeks had no apparent effect on final body weights (Table I). Pancreatic weight, as well as its DNA, RNA, and protein content was also not significantly affected by this level of caffeine intake (Table I), calculated to be 3.68 mg/day/rat over the experimental period. In addition, electron microscopic examination of the pancreas only detected a slight increase in the number of zymogen granules in the caffeine-fed group (data not shown).

In pancreatic homogenates, amylase and trypsinogen activities were found to be about 35% higher in tissue from the caffeine-fed rats in comparison to controls, although only the trypsinogen values achieved statistical significance (Table II). In addition, the levels of immunoreactive cationic trypsin(ogen) were 41% higher in pancreatic tissue from caffeine-fed rats, whereas chymotrypsin activity in the pancreas was similar between the two groups. Amylase and immunoreactive cationic trypsin(ogen) levels in serum from the caffeine-fed rats were 82 and 62%, respectively, of control levels (Table III). It was also observed that caffeine feeding did not significantly affect the trypsin inhibitory capacity of serum and therefore did not affect the protease inhibitor ratio in serum.

In one series of experiments, the responsiveness of isolated, dispersed pancreatic acini to various secretagogues was examined. In dispersed acini isolated from the caffeine-fed rats, the rate of secretion of amylase, trypsinogen, and chymotrypsinogen was significantly lower than that in control acini in response to CCK-8 and nicotine, although basal release of the enzymes was the same in both groups (Fig. 1). In the caffeine-fed rats, amylase secretion was approximately 55% of control values at the three concentrations of CCK-8 employed. In addition, at the 50 p*M* CCK-8 dose, trypsinogen and chymotrypsinogen release was approximately 30% of control values and was about 60% of control levels following the 500 p*M* CCK dose. In contrast, at all doses of nicotine, trypsinogen and chymotrypsinogen release was consistently lower in the caffeine-fed group than in controls, whereas amylase secretion was most affected at the 6 m*M* nicotine dose (Fig. 1). Enzyme release from dispersed acini from the caffeine-fed rats was also significantly lower than that in the controls in response to secretin (1 μM) and carbachol (1 μM) stimulation (data not shown). None of these differences in enzyme release could be attributed to an increased LDH secretion (data not shown).

In dispersed pancreatic acini, in the presence of 100 p*M* CCK-8, the secretion of pulse-labeled proteins was about 35% lower in the caffeine group compared with controls (Fig. 2), suggesting that caffeine feeding also inhibited the CCK-8-induced secretion of newly synthesized proteins. No difference, however, was observed in secretions of pulse-labeled proteins from both groups in the presence of 6 m*M* nicotine.

In another series of experiments, acini were incubated in the presence of [^{3}H]leucine to examine the effects of caffeine on acinar protein synthesis. Although [^{3}H]leucine incorporation into acinar protein was not different in both groups of rats under basal conditions, leucine incorporation was significantly higher in acini from the caffeine-fed rats in comparison to controls in the presence of CCK-8 (100 p*M*), secretion (1 μM) and carbachol (1 μM) (Fig. 3). From examination of tricho-

Table I. Effects of 6-Week Caffeine Consumption on Body and Pancreatic Weights, Pancreatic Protein, and Nucleic Acid Content and Caffeine Consumption[a]

	Control (n = 10)	Caffeine-fed (n = 10)
Final body weights (g)	358 ± 12	369 ± 11
Pancreatic weight (g)	0.855 ± 0.025	0.911 ± 0.061
DNA (mg)	1.15 ± 0.16	1.04 ± 0.13
RNA (mg)	4.2 ± 0.7	4.7 ± 1.1
Protein (mg)	28.5 ± 4.1	26.7 ± 3.9
Caffeine intake (mg/day)	—	3.68 ± 0.19

[a] Data expressed as mean ± SE.

Table III. Effects of 6-Week Caffeine Consumption on Serum Parameters[a]

	Control (n = 10)	Caffeine-Fed (n = 10)
Amylase (units/ml)	12.7 ± 1.3	10.4 ± 0.9
Immunoreactive cationic trypsin(ogen) (ng/ml)	5.3 ± 0.8	3.3 ± 0.5
Trypsin-inhibitor capacity (% inhibition/20 μl)	78.9 ± 2.0	73.2 ± 1.2
Caffeine (mg/dl)	—	1.35 ± 0.11 (8)

[a] Data expressed as mean ± SE.

Table II. Effects of 6-Week Caffeine Consumption on Pancreatic Enzymes[a]

	Control (n = 10)	Caffeine-fed (n = 10)
Amylase (units/g)	32,047 ± 2,874	41,104 ± 4,676
Trypsinogen (μmol/min/g)	53,946 ± 3,778	72,319 ± 4,422*
Chymotrypsinogen (μmol/min/g)	22,604 ± 1,573	24,314 ± 1,172
Immunoreactive cationic trypsin (ogen) (μg/g)	638 ± 51	905 ± 65*

[a] Mean ± SE. * $P < 0.05$ from control.

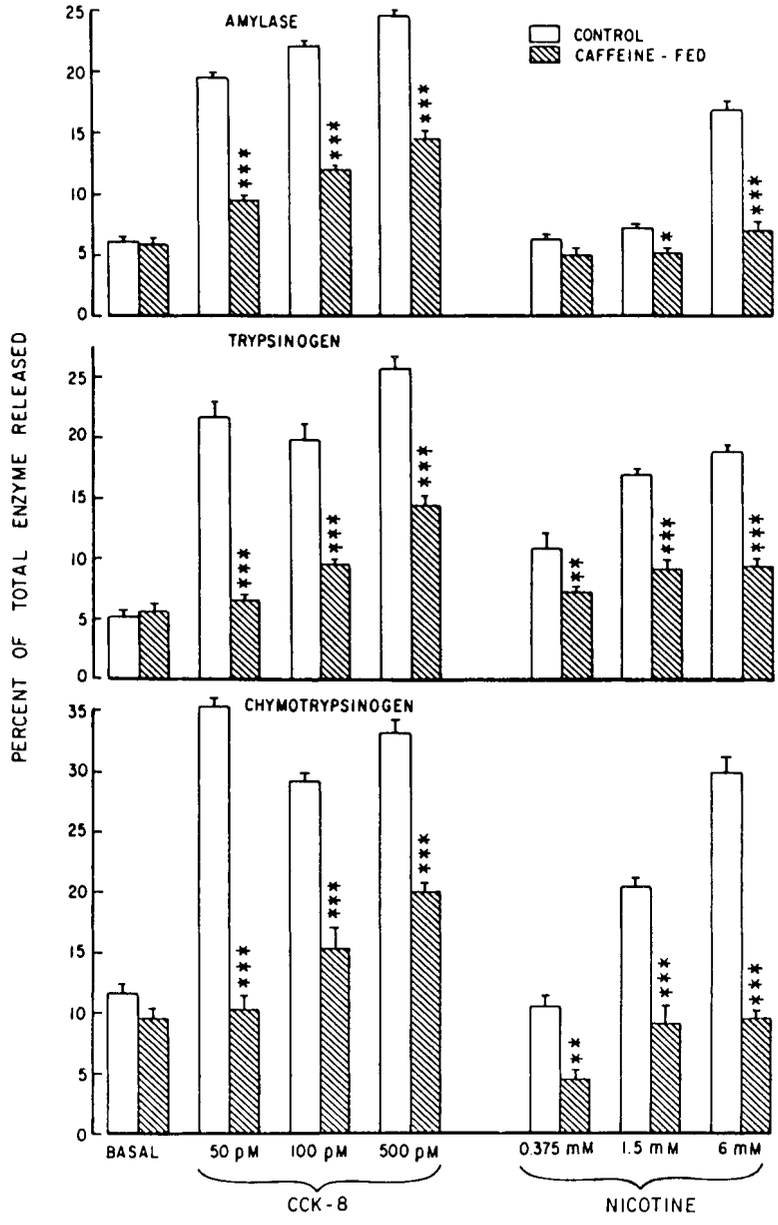

Figure 1. Effects of CCK-8 and nicotine on enzyme secretion from dispersed pancreatic acini isolated from caffeine-fed and control rats. Each value represents the mean ± SE of quadruplicate determinations from three experiments. * $P < 0.05$ from corresponding control; ** $P < 0.01$; *** $P < 0.001$.

loracetic acid-soluble radioactivity, it did not appear that these differences in [^{3}H]leucine incorporation were due to differences in the rate of uptake of this amino acid by acini. Although the leucine pool size was not determined in these experiments, concentrations of leucine in dispersed acini from the two groups of rats were similar (795 nmol/mg protein in control acini vs 728 nmol/mg protein in the caffeine group).

Discussion

Caffeine is perhaps the most widely consumed drug in the world, with estimates that up to 92% of adults consume caffeine in some form during any period of time (12). In 1972, it was estimated that the average daily per capita consumption of caffeine in the United States was 200 mg, with individual intakes ranging up to about 800 mg/day at the 99.9 percentile (13). In the present study, the average daily consumption of caffeine was 3.68 mg/rat or about 0.03% of the diet. This level is well within the typical range of moderate caffeine intake in the United States (13).

Numerous studies have investigated the pharmacologic effects of caffeine, and it appears that caffeine may affect every major organ system in the body. Caffeine has been reported to affect the central nervous system, the heart and vasculature, gastric secretions,

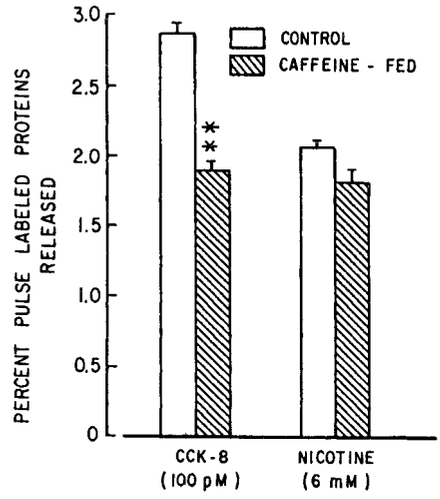

Figure 2. Effects of CCK-8 and nicotine on ^{3}H-pulse-labeled protein secretion from dispersed pancreatic acini isolated from caffeine-fed and control rats. Values represent mean ± SE for five observations from three experiments. Incubations were performed at 37°C for 30 min following the 3-min pulse with [^{3}H]leucine. $^{**}P < 0.05$ from corresponding control.

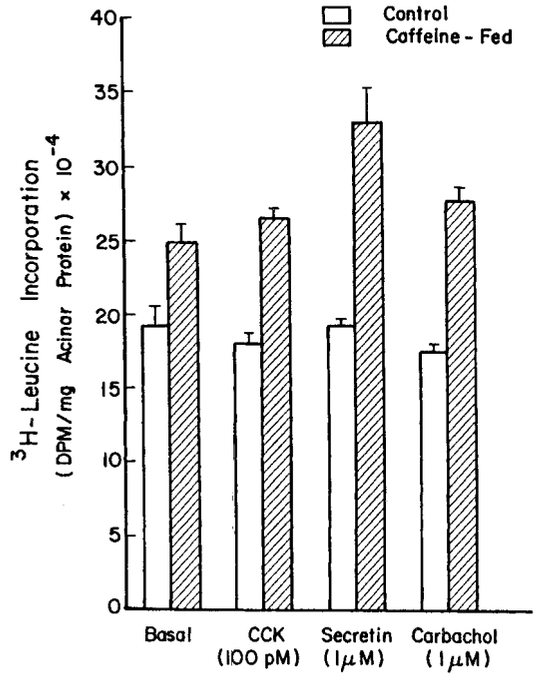

Figure 3. Effects of CCK-8, secretin, and carbachol on [^{3}H]leucine incorporation into acinar protein. Each value represents the mean ± SE for quadruplicate determinations from three experiments. Incubations were performed at 37°C for 30 min. $^{*}P < 0.05$ from corresponding control.

and diuresis (27–30). In addition, after a single oral dose, caffeine was found to be rapidly and widely distributed to all tissues examined (31).

The results from this study indicate that, in rats, moderate caffeine intake affects pancreatic function. Although 6-week caffeine consumption did not affect either the weight of the pancreas or its DNA, RNA, or protein content, amylase and trypsinogen activities, as well as immunoreactive cationic trypsin(ogen) levels, were higher in pancreatic homogenates from caffeine-fed rats than in controls. On the other hand, CCK-8, carbachol, secretin, and nicotine-mediated stimulation of enzyme secretion from pancreatic acini from caffeine-fed rats was lower than in controls. In addition, CCK-8-stimulated release of ^{3}H-pulse labeled protein from pancreatic acini was lower in the caffeine-fed group compared with the controls. In their studies, Andriulli *et al.* (14) observed no increased responsiveness of the exocrine pancreas in coffee drinkers following a secretin test. On the other hand, Coffey *et al.* (11) found that both coffee and caffeine increased the exocrine pancreatic secretion of trypsin in fasting humans and that trypsin secretion was somewhat higher in chronic coffee drinkers than in noncoffee drinkers. Although our data suggest that caffeine does not directly enhance exocrine pancreatic secretion and may inhibit it, the study by Coffey *et al.* (11) clearly indicates that other factors are involved in the *in vivo* response of the exocrine pancreas to coffee and caffeine. Further studies in this area are warranted.

Based on our observations, the next series of experiments explored whether the effects of caffeine on pancreatic enzyme secretion in rats were due to effects on the secretory process or on protein synthesis. [^{3}H] Leucine incorporation into acinar protein was generally higher in the caffeine-fed rats than in the controls. Although the specific activity of leucine was not determined in this experiment, it was found that the concentration of leucine in the pancreas was not affected by 6-week caffeine feeding. These data suggest that caffeine feeding may increase protein synthesis in the pancreas and that the higher enzyme concentrations measured reflect both a higher rate of synthesis and a lower rate of secretion of the enzymes. Since enzyme release in response to various secretagogues was similarly affected by caffeine feeding, it appears that caffeine acts at a site common to all secretagogues, but the exact mechanism for caffeine-induced inhibition of enzyme release is unknown. Presently, three different mechanisms have received the most attention to account for the numerous actions of caffeine. These include (i) inhibition of cAMP degradation through action on the enzyme phosphodiesterase; (ii) alterations in cellular calcium metabolism; and (iii) antagonism of endogenous adenosine (32). Except for the antiasthmatic effects of methylxanthines, inhibition of the cell surface adenosine receptor binding appears to explain caffeine's pharmacologic effects (32 and references cited therein). Whether adenosine is involved in the pancreatic secretory process and its inhibition by caffeine accounts for the decreased enzyme release in caffeine-fed rats observed in this study awaits further investigation.

With the exception of a slightly higher number of zymogen granules in the pancreas, no other significant ultrastructural changes were apparent in the caffeine-fed group. This is similar to observations in autopsy

specimens in which no significant histologic differences were detected in acinar or ductal pancreatic tissue from coffee drinkers in comparison to tissue from noncoffee drinkers (33). The majority of the histologic abnormalities could be associated with cigarette smoking. The data from this study indicate that moderate caffeine intake affects the secretory function of the exocrine pancreas of the rat by diminishing the responsiveness of acinar cells to various secretagogues. Further studies are needed to determine the site where caffeine acts in the secretory process.

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

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